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STUDIES ON VERBENALIN AND VERBENALOL

By

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APPROVED BY Lloyd N. Parks

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INTRODUCTION

In 1908, Bourdier[1] isolated from Verbena officinalis, a glycoside, verbenalin. Since that time, a number of research workers have made many experimental studies, attempting to deduce the chemical structure of the glycoside and of its aglycone, verbenalol.

This present study has approached the problem of structure determination by means of reactions and methods not previously used on these compounds, has also tried to determine why certain methods and reactions previously used were unsuccessful, and has attempted to improve the experimental conditions of the latter so that they might yield valuable structural information.

An attempt has also been made to improve the method of extraction of verbenalin from the crude drug, which, throughout the whole of the present study, has been Verbena stricta.

A third facet of this study has been the syntheses of type compounds approximating the structures proposed for verbenalol by several workers. In this case, comparison of ultra-violet absorption spectra has been used to indicate similarity or lack of similarity between the synthesized compounds and verbenalol.

Infrared absorption spectra of verbenalin, verbenalol and several of their derivatives are reported and analyzed in the current study for the first time. Analysis of such spectra represent most important aid in structure determinations.

Before coming to the experimental portion of this thesis, it is proposed to discuss briefly the methods which have been used to extract verbenalin from various species of Verbena, and then to

discuss at length all studies which have attempted to elaborate the structures of verbenalin and verbenalol.

DISCUSSION

METHODS OF EXTRACTING VERBENALIN

Bourdier[1] obtained verbenalin from Verbena officinalis by extraction of the plant material with boiling 90% ethanol in the presence of calcium carbonate, concentration by means of vacuum distillation, and treatment of this thick extract with anhydrous ethyl acetate, from which crystals were obtained on concentration.

In 1834, Geiger[2] obtained a compound which he named cornin by extracting exhaustively with ether a dried aqueous extract of the root bark of Cornus florida, then recrystallizing his compound from ethanol. Much later, Reichert[3] proved the identity of cornin and the verbenalin which Bourdier[1] had obtained subsequently from Verbena officinalis.

Chatterjee[4,5], working with Verbena stricta, showed that petroleum ether (Skelly B) did not extract the glycoside from the ground dried plant material. Further work showed that extraction was possible by means of prolonged treatment with ether, the impure verbenalin, which precipitated from the ether extract being recrystallized from ethanol and ethyl acetate. Chatterjee[4,5] standardized her method of extraction as follows. Two kilogram portions of drug were exhaustively extracted with ether in a Soxhlet apparatus for approximately 100 hours. The ether extract was concentrated, diluted with acetone and stored in a refrigerator. The crude crystals thus obtained were recrystallized from ethanol and from ethanol-ethyl acetate. A further modification of method eliminated the necessity of concentration of the ether solution by beginning subsequent extractions with

the supernatants from the previous runs, they being already saturated with the glycoside. In this case, the crude precipitate was treated to obtain pure verbenalin.

Young[6] investigated alternative methods of obtaining verbenalin from Verbena stricta, and was forced to conclude exhaustive extraction with ether gave the best yields of glycoside. He concluded that the use of water, chloroform-ethanol, and acetone gave incomplete extraction, and that, while chloroform is a good solvent for verbenalin, yields with it as solvent did not quite measure up to those obtained with ether in the method standardized by Chatterjee[4,5].

An attempt has been made in the current investigation to apply the glycosidal extraction method of Trim and Hill[7] to verbenalin. In this method, Verbena stricta was extracted with boiling water, suspended in which was calcium carbonate to prevent hydrolysis of any extracted glycoside. The filtered aqueous extract was treated with a small quantity of Norite to remove gums, then treated with a much larger quantity of Norite to adsorb the glycoside. After having been washed with water, the Norite pad was eluted with 50% ethanol and the latter solution concentrated to a small volume in the presence of calcium carbonate. The concentrate was extracted exhaustively with ethyl acetate, the fairly pure compound crystallizing from this extract after concentration. Yields obtained by this method, while reasonable, did not match those obtained by the Chatterjee method.

PHYSICAL AND CHEMICAL PROPERTIES OF VERBENALIN

Verbenalin is quite soluble in water, fairly soluble in alcohol, sparingly soluble in ethyl acetate, acetone and chloroform, insoluble in ether, petroleum ether and benzene. It can be crystallized from

ethyl acetate, acetone, or alcohol, m.p. 182.5-183°, $[\alpha]_D^{24} = -172°$ (water); -165.3° (alcohol)[4,5,6].

Verbenalin dissolves quite readily in aqueous alkali with the almost instantaneous formation of a deep yellow color. In concentrated sulfuric acid, it becomes brownish-black. It reduces Tollens' Reagent (ammoniacal silver nitrate) in the cold, and Fehling's Solution when warmed. It is neutral to litmus in aqueous and alcoholic solutions. It does not give a color test with ferric chloride[4,5,6].

Cheymol[8] determined that verbenalin turned golden yellow with nitric acid; green with Frohde's Molybdic Acid Reagent; green in acetic acid solution when treated with bromine in acetic acid. Reichert[3] and Cheymol[8] also determined the presence of one nethoxyl group, an observation confirmed by several later investigators. Cheymol[8] felt the glycoside behaved as a lactone because of the following observations; (a) an aqueous solution of glycoside was neutral to litmus; (b) on the addition of a limited amount of alkali to the aqueous solution, it gradually became neutral again. Although producing no analysis data to support his assertion, Cheymol said the product of this reaction between alkali and verbenalin was the sodium salt of verbenalosidic acid, $C_{17}H_{24}O_{11}$. He did state that 1 g. of the glycoside combined with 0.0644 g. of sodium, producing 1.103 g. of sodium verbenalosidate. From this salt, he obtained verbenalosidic acid, m.p. 210-2° (from ethyl acetate) $[\alpha]_D^{22} = -185.99°$ (in water)[9]. Chatterjee[4] found inconclusive evidence for the presence of a lactone structure when determining the neutralization equivalent of verbenalin. She expressed the opinion that the use of additional alkali indicated

another structure in the molecule (perhaps an enol) capable of combining with sodium hydroxide.

Cheymol detected an olefinic linkage by the reaction of verbenalin with alkaline potassium permanganate and with bromine in acetic acid. With the latter, on addition of excess bromine, the color of the product was emerald green. Since reduction of the glycoside gave tetrahydroverbenalin in which the carbonyl function he had demonstrated in verbenalin was no longer present, he concluded one double bond only was present. Despite the observation that hydrogenation seemed sometimes to involve more than 2 moles uptake, this assumption of one double bond only was not questioned until the work of Asano, Ueno and Tamaki[10]. From the nature of the products they obtained upon hydrogenation of verbenalin, they postulated several double bonds difficultly reducible in the glycoside, and indeed expressed the view they were contained in a furan nucleus. In a bromine titration of verbenalin, Chatterjee[4] found a take-up of one mole of bromine per mole of glycoside. In the current study, the rate of uptake of ozone indicated the presence of but one olefinic linkage.

Much work has been done by several investigators to determine if verbenalin contains an hydroxyl group other than the one through which the glucose moiety is linked. Since the bulk of this endeavour has involved acetylation of verbenalin and attempts to determine if the acetylated glycoside contains four or five acetyl groups, and since the acetylated glycoside has also been used to prove or disprove the presence of a carbonyl function in verbenalin, this work has been recorded in a separate section on Pages 15 and 16. The evidence seems

to indicate the only hydroxyl in the aglycone is the one involved in glycosidic linkage and that the verbenalin molecule possesses a carbonyl function, likely a ketone, either actual or potential.

Bourdier[1] reported positive reactions between verbenalin and phenylhydrazine acetate and hydroxylamine hydrochloride respectively, and negative tests between verbenalin and sodium bisulfite solution and semicarbazide reagent respectively. However, with the exception of the compound obtained in crystalline condition with hydroxylamine, and which contained much more nitrogen than a monooxime, no crystalline derivatives were prepared to prove definitely the presence of the carbonyl function. Cheymol[8] noted a negative test with Schiff's Reagent and a positive Legal's test as well as the odor of iodoform when the glycoside was treated with iodine in an alkaline medium. He further obtained a red compound with phenylhydrazine, and a yellow acetone-soluble compound with 2,4-dinitrophenylhydrazine, although not reporting analysis data for these derivatives. As noted previously, the preparation of and analysis of well-crystallized carbonyl derivatives of acetylated verbenalin by Reichert and Hoffmann[11] and by this worker, seem to indicate at least the potential presence of the carbonyl group in verbenalin.

Whether the carbonyl function of verbenalin is an aldehyde or a ketone has long been a baffling question. Bourdier[1] felt the high reducing activity indicated an aldehyde. However, Cheymol[8] assumed a ketone because of a negative Schiff's test and a positive Legal's test. Hoffmann[12] felt that since attempted oxidation did not yield a carboxylic acid, the carbonyl must be ketonic.

Chatterjee[4] was able also to obtain a positive iodoform test on verbenalin qualitatively. When she applied the iodoform reaction quantitatively, she obtained 37.4% of theory for one mole of iodoform per mole of verbenalin. In addition, she obtained a crystalline halogen-containing acid, m.p. 113.5° (analysis values, 23.07% C and 2.40% H; neutralization equivalent 182.8; 179.8). The similarity of this compound with α-iodocrotonic acid identified as the product of sodium hypoiodite oxidation of verbenalol by Asano, Ueno and Tamaki[10] will be discussed later.

On prolonged reaction with sulfuric acid (2.50% W/V) verbenalin was found by Cheymol[8] to be decarboxylated, the volume of carbon dioxide liberated corresponding to the decomposition of one carboxyl group in the molecule. In the present work, decarboxylation of verbenalin was effected by heating a solution of the glycoside in N/4 sulfuric acid at 95° for 32 hours, during which time, 0.56 mole of carbon dioxide was caught in the barium hydroxide traps. Nothing crystalline was obtained by ether extraction of the residue which had charred rather badly. In a similar experiment, after 45 hours, 1% phosphoric acid had failed to release any carbon dioxide. However, when catalytic amounts of sulfuric acid were added, decarboxylation began immediately and there was obtained slightly more than one mole of carbon dioxide. An attempt to decarboxylate or decarbonylate verbenalin in pyridine solution in which was suspended dry sodium fluoride, was unsuccessful. Because of the likelihood of glucose cleaved from the glycoside introducing further difficulties, it was decided to concentrate on the decarboxylation of the aglycone. This

will be discussed under the properties of verbenalol.

It should be noted that the ten oxygen atoms of verbenalin appear to be involved as follows: five in the glucose residue; one in the hydroxyl through which the glucose moiety is linked; one in a methoxyl group; one in a carbonyl (probably ketone) function which can be demonstrated by the preparation of carbonyl derivatives of the acetylated glycoside; and two in the lactone.

In the present study, verbenalin has been shown to contain one C-methyl group, as has tetrahydroverbenalol.

DERIVATIVES OF VERBENALIN

First attempts to prepare derivatives of verbenalin were made by Bourdier[1], who observed positive reactions between the glycoside in aqueous solution and phenylhydrazine acetate solution, and between the glycoside and alkalinized solution of hydroxylamine hydrochloride. The product of the latter reaction was crystalline, m.p. 155° (decomp.) Bourdier found that verbenalin did not give positive tests with saturated sodium bisulfite solution, semicarbazide or fuchsin solution. Cheymol[8] found upon investigation of the analysis data of Bourdier's "oxime" of verbenalin that it contained about six times as much nitrogen as a monooxime would.

The acetyl derivative of verbenalin, prepared by Reichert and Hoffmann[11], will be mentioned later (Pages 15 and 16). Hoffmann[12] satisfied himself that this was a tetraacetyl derivative, by analysis of the p-bromophenylhydrazone of the acetylated glycoside. In the present work, there has been prepared the 2,4-dinitrophenylhydrazone of acetylated verbenalin (lemon-yellow needles, m.p. 256-8° (decomp.))

and it has been submitted for nitrogen analysis. The nitrogen content of the 2,4-dinitrophenylhydrazone of a pentaacetyl derivative of verbenalin would be 7.20%, while for the corresponding tetraacetyl derivative, it would be 7.61%. The data found (7.15%N) would seem to indicate a pentaacetyl derivative had been formed. Analysis data of carbon and hydrogen values for acetylated verbenalin, on the other hand, more closely favor the tetraacetyl structure. A microacetyl determination of acetylated verbenalin in the Roth-Kuhn apparatus, gave over seven acetyl groups per molecule. However, this anomalous result could be due to alkaline fission of other volatile acids from the acetylated glycoside as well as the error due to the glucose moiety.

Reichert and Hoffmann[11] were able to prepare a crystalline oxime of acetylated verbenalin, m.p. 175-6°. Analysis data again failed to distinguish between a tetraacetyl or pentaacetyl verbenalin oxime. This, of course, was regarded as proof for the presence of a carbonyl function. In an attempt to decide if the carbonyl function were an aldehyde or a ketone, Reichert and Hoffmann[11] tried to prepare a nitrile from the oxime by dehydration in the presence of acetic anhydride. Analysis data seemed to indicate that another acetyl group had entered the molecule and that dehydration had not taken place. They felt this indicated that the carbonyl function was ketonic. This acetyl derivative of the oxime of acetylated verbenalin has m.p. 184°. When Reichert and Hoffmann[11] succeeded in preparing the true oxime of acetylated verbenalin, Cheymol[9] attempted the preparation of an oxime of the non-acetylated glycoside. He

obtained a crystalline colorless compound which decomposed, without melting, at 155°. However, the analysis data did not fit the picture of a true oxime.

Hoffmann[12] did prepare the phenylhydrazone, m.p. 197°, and the p-bromophenylhydrazone, m.p. 215 (decomp.) of acetylated verbenalin. In the present study, we were able to obtain the phenylhydrazone, the oxime and the 2,4-dinitrophenylhydrazone (see P. 88).

Hoffmann[12] prepared a crystalline product by brominating acetylated verbenalin in chloroform solution, but he could not obtain a melting point for the product which gave a distinct test for halogen. The decomposition point of the extremely reactive substance was not constant. The product itself darkened within a few days and occasionally decomposed spontaneously with evolution of a strongly acid gas.

Hoffmann[12] hydrogenated acetylated verbenalin with sodium amalgam in dilute acetic acid solution, obtaining a colorless stable compound for which analysis data did not distinguish between a dihydro- or tetrahydro- derivative of acetylated (tetra) verbenalin. It had m.p. 147°, which indicated to Hoffmann that the carbonyl group as well as the double bond had been reduced. The yield was small.

Chatterjee[4] attempted to prepare various carbonyl derivatives of verbenalin. From semicarbazide, she obtained biurea; from hydroxylamine nothing; and from 2,4-dinitrophenylhydrazine, starting material. Bromination of verbenalin did not yield any crystalline material.

CLEAVAGE OF VERBENALIN

ACID HYDROLYSIS — Cheymol[8] found that when an aqueous solution of

verbenalin was treated with a dilute sulfuric acid (2.50 Gm. acid in 100 c.c. aqueous solution of glycoside), hydrolysis to glucose and aglycone took place (just as was the case in the presence of emulsin), but that on prolonged reaction, a molar proportion of carbon dioxide was liberated. In view of the special type of reaction, i.e. decarboxylation, involved here, discussion of it will be left for a later section.

Chatterjee[4] made a series of studies concerning hydrolysis of verbenalin by acids. She found that sulfuric acid, in both 10% and 1% concentrations, caused aqueous solutions of verbenalin to turn dark yellow, then dark brown, and concluded that no recoverable amounts of verbenalol could be demonstrated. Treatment of the glycoside with glacial acetic acid and a trace of sulfuric acid yielded similar results. Refluxing an aqueous solution of the compound after adding a few drops of concentrated hydrochloric acid gave a gummy residue only after ether extraction. Chatterjee also obtained a gummy residue only when she refluxed the glycoside with hydriodic acid and a trace of red phosphorus. She concluded that hydrolysis of verbenalin with acid to yield recoverable quantities of the aglycone, was not possible.

During the course of the present study, decarboxylation of the glycoside at room temperature and at elevated temperatures by means of 1% phosphoric acid, was attempted. When no decarboxylation resulted until a trace of sulfuric acid was added, it was decided to attempt hydrolysis of the glycoside then by means of phosphoric acid. It was not successful.

An attempt was made to effect hydrolysis of the glycoside to its aglycone by stirring an acid ion-exchange resin, Amberlite IRA-50 with an aqueous solution of verbenalin at room temperature for 18 hours. The glycoside proved to be stable to this treatment.

ALKALINE HYDROLYSIS - Cheymol[8] determined that an aqueous solution of verbenalin was neutral to litmus, and that addition of either sodium hydroxide or barium hydroxide apparently caused the rupture of a lactone, since the alkalinized solution gradually became neutral again if less than one equivalent were added. At the same time, he noted accompanying degradation when he employed barium hydroxide. During the determination of a neutralization equivalent for verbenalin, Chatterjee[4] noted a yellow colour develop in an aqueous solution of the glycoside. Chatterjee was unsuccessful in obtaining crystalline hydrolytic products when she treated solutions of verbenalin with 5% aqueous sodium hydroxide at room temperature, with 2.5% boiling aqueous sodium hydroxide, and with strong ammonia. In each case, severe degradation was noted.

EMULSIN HYDROLYSIS - For the purpose of determining the sugar moiety of verbenalin, Bourdier[1] hydrolyzed the glycoside in aqueous solution by means of emulsin. He identified glucose as the sugar in question and obtained as a second hydrolytic product, a bright yellow amorphous product which he was unable to crystallize. Cheymol[8] was able to prepare the crystalline aglycone by extracting with ether the aqueous solution of verbenalin treated with emulsin. He did not mention the conditions of hydrolysis. Reichert and Hoffmann[11] noted an inevit-

able loss of substance when they attempted hydrolysis of the glycoside. Asano, Ueno and Tamaki[10] accomplished emulsin decomposition of verbenalin to its aglycone in nearly 70% yield by saturating a 2% aqueous solution of verbenalin containing 0.67% emulsin with carbon dioxide, and storing it in a refrigerator for one week, after which ether extraction removed the aglycone. Karrer and Saloman[13] obtained verbenalol in 60% yield and a brown oil which they were not able to crystallize. Chatterjee[4,5] studied enzymatic hydrolysis of verbenalin under varying conditions of temperature (22° to 30°), pH (4.0 to 6.2), and dilutions of solution. She found a maximum yield of aglycone was obtained at 23-25°, a pH of 4.3-4.6, and a dilution of 2 g. of glycoside in 125-150 c.c. of solution. Her yield was about 60% of theory. Young[6] was unable to repeat Chatterjee's work to the extent of obtaining such yields. He did obtain better than 35% of theory by mixing the emulsin with the aqueous solution adequately, and by seeding the ether extract with verbenalol. Since the work of Asano, Ueno, and Tamaki[10] did not become generally available outside Japan until the end of 1950, this writer had carried out experiments designed to improve the yield of aglycone obtainable in hydrolyses set up in the general methods of Chatterjee[4,5] and Young[6]. However, as soon as the Asano method was used, it was realized this was the superior method.

MANNICH METHOD OF HYDROLYSIS - In 1942, Mannich and Siewert[14] developed a milder method of glycosidal cleavage, applying it to g-strophanthidin and obtaining for the first time its undegraded genin. The method of treatment involved solution of the glycoside in cold

acetone containing a small amount of hydrogen chloride. Attempts were made in the present work to adapt this method to the cleavage of verbenalin. Neither the use of concentrated hydrochloric acid nor of dry hydrogen chloride proved successful.

METHANOLYSIS OF VERBENALIN - The purpose of this reaction was to cleave the glycoside and simultaneously etherify the hydroxyl group through which the sugar moiety was formerly attached. Absolute methanol containing dry hydrogen chloride was the medium for the reaction. Nothing crystalline was obtained from this reaction.

Barium methoxide was also used for attempted methanolysis. Nothing crystalline could be prepared from the deeply-colored solution after reaction.

ACETYLATED VERBENALIN

Cheymol[9] prepared an acetyl derivative of verbenalin (acetyl verbenaloside) by the action of acetic anhydride on the glycoside in the presence of fused sodium acetate. He crystallized the derivative from acetone. He also prepared it by treating verbenalin with acetic anhydride and pyridine, precipitating the compound with 5% aqueous sulfuric acid, and recrystallizing it from alcohol. Since analytical data were as satisfactory for a tetraacetyl as for a pentaacetyl derivative, it was impossible to determine if the aglycone portion of the glycoside molecule contained a free acetylable hydroxyl group. Reichert and Hoffmann[11] repeated this work, and attempted to decide whether four or five acetyl groups had been introduced by a Freudenberg[15] acetyl determination and a Kogl[16] microacetyl determination.

Neither method yielded conclusive results. Rabate[17] has shown that the estimation of acetyl in an acetylated glycoside may be complicated by the presence of a carbonyl function. Cheymol[9] attempted to hydrolyze acetylated verbenalin by means of emulsin, but after keeping the solution for one month at 33°, could detect no hydrolysis.

Hoffmann[12] attempted to reach a decision on the number of acetyl groups in the acetylated glycoside by preparing the p-bromophenylhydrazone of acetylated verbenalin and determining its bromine content. The difference in bromine content of the derivative of a tetraacetyl verbenalin and of the derivative of a pentaacetyl verbenalin would be but 0.61% but Hoffmann was satisfied that only four acetyl groups had been introduced into verbenalin and that consequently the aglycone portion of the glycosidal molecule did not contain an acetylable hydroxyl group. Hoffmann repeated the Kogl[16] microacetyl determination, obtained up to four and one-half equivalents of acid, and postulated that the extra acid was due to formic acid split hydrolytically from the acetylated glycoside. His distillate had reducing properties, i.e. it gave a positive Tollens' test. In a control, glucose itself yielded a distillate with reducing properties. Hoffmann[12] also prepared a crystalline phenylhydrazone of acetylated verbenalin to prove the existence in the glycoside of a carbonyl function.

The present writer obtained acetylated verbenalin in more than 70% yield by the acetic anhydride-anhydrous pyridine method. Attempts to hydrolyze the acetylated glycoside by the method of Asano, Ueno and

Tamaki[10] were unsuccessful.

Attempts were made to cleave acetylated verbenalin and to acetylate simultaneously the products of cleavage, by means of a mixture of acetic anhydride, glacial acetic acid and perchloric acid, and a mixture of acetic anhydride, glacial acetic acid and concentrated sulfuric acid. From the former transforming mixture, nothing was obtained. From the latter mixture, there was obtained in good yield glucose α-pentaacetate. Nothing corresponding to the aglycone was obtained.

REDUCTION OF VERBENALIN

Cheymol[8] hydrogenated verbenalin at room temperature with Raney nickel catalyst by the method of Delephine and Horeau[18]. He obtained thus a tetrahydro derivative, $C_{17}H_{28}O_{10}$, in the form of orthorhombic crystals, having a bitter taste, reacting negatively to reducing agents and giving no Legal test. Cheymol[9] reported later obtaining crystals in two forms, rectangular plates or cylindrical, m.p. 160-165° (according to the samples prepared from different fractionations) $[\alpha]_D^{18} = +28.22°$. From the hydrogenated glycoside, Cheymol[9] prepared by treatment with 2.5% sulfuric acid, the hydrogenated aglycone, tetrahydroverbenalol. Similarly, hydrolysis of the hydrogenated glycoside could be effected with emulsin. In the presence of air, Cheymol[9] found the solution became badly discoloured. When the hydrolytic procedure was carried out in vacuo, the liquid was colorless. Tetrahydroverbenalol was extracted from the hydrolysis mixture by ether. Cheymol[9] found it unstable in air like verbenalol,

without reducing action, unlike verbenalol, and having $[\alpha]_D$ = -10.44°. Cheymol stated that the uptake of two moles of hydrogen saturated an olefinic linkage and reduced a carbonyl function to an alcohol.

Hoffmann[12] carried out the hydrogenation of acetylated verbenalin by means of sodium amalgam in dilute acetic acid solution. His compound analyzed as well for a dihydro- as for a tetrahydro-tetraacetyl glycoside. The product was stable and colorless. Since its melting point (147°) was higher by 14° than the starting material, he concluded that not only the olefinic linkage had been saturated but also the carbonyl function had been reduced to an alcohol. His yield was small.

Karrer and Saloman[13] made many attempts to hydrogenate verbenalin catalytically with platinum without satisfactory results. They modified Cheymol's[19] use of platinized Raney nickel by using pressure with Raney nickel catalyst (100° and 15 atmospheres pressure). As their chief product of reduction, but in poor yield, they obtained a well-crystallized tetrahydroverbenalin, $C_{17}H_{28}O_{10}$, m.p. 195-6°, $[\alpha]_D^{20}$ -65° (in water). A second reduction product, $C_{10}H_{16}O_3$, designated desoxyverbanol, apparently arose through reductive cleavage of the methoxyl group and the sugar moiety. Hydrolysis of tetrahydroverbenalin with emulsin gave tetrahydroverbenalol, m.p. 102-3°, crystallizing in large clear plates. Contrary to the experience of Cheymol[19], Karrer and Saloman[13] found that the mixture of tetrahydroverbenalin and emulsin, which they kept at 30-31° for four

days, did not become colored. The tetrahydro derivative of the aglycone gave analysis values for $C_{11}H_{18}O_5$, one methoxyl group, two active hydrogens, $[\alpha]_D^{16} = +8.4^\circ$ in water. From the emulsin hydrolysis of tetrahydroverbenalin, they obtained evidence of a second compound which could not be obtained in the pure state.

In 1942, Asano, Ueno and Tamaki[10] reduced verbenalin catalytically in the presence of palladium carbon and found that the glycoside absorbed no more than one mole of hydrogen, and that very slowly. Products of reaction consisted of a crystalline glycoside, m.p. 198°, and a glutinous substance together with unaltered verbenalin. They further reported (but gave no details) that forced catalytic reduction of their dihydro glycoside produced the glutinous material which was not a tetrahydroverbenalin. Emulsin hydrolysis of dihydroverbenalin in an atmosphere of carbon dioxide produced dihydroverbenalol, $C_{11}H_{16}O_5$, m.p. 65°. They determined that the carbonyl function had been reduced to an alcohol.

Chatterjee[4] attempted the hydrogenation of verbenalin in absolute ethanol at room temperature and atmospheric pressure, with a 5% palladium-charcoal catalyst. She recovered unchanged glycoside and from this concluded that verbenalin contained no isolated labile carbon to carbon unsaturation. On the other hand, hydrogenation with platinum oxide catalyst resulted in a hydrogen uptake approximately equivalent to two moles per mole of verbenalin. She was able to purify a tetrahydroverbenalin, m.p. 173-174.5° from ethyl acetate. Nothing crystalline was obtained from an attempted hydrolysis of the reduced glycoside with hydrochloric acid. However,

hydrolysis with emulsin gave a tetrahydroverbenalol, m.p. 123-124°, in a yield of 60%.

Young[6] carried out the hydrogenation of verbenalin in aqueous solution at room temperature with a catalyst of platinum oxide. The glycoside appeared to take up three moles of hydrogen, the first two in about three hours, the third in another 24 hours. By repeated recrystallizations, Young obtained a product which appeared to be the tetrahydroverbenalin obtained previously by Karrer[13], m.p. 193-7°, $[\alpha]_D^{26} = -75.0°$. There were indications of the presence also of the desoxyverbanol reported by Karrer and Saloman[13].

In the present study, three types of hydrogenation were carried out. The first method was the use of platinum oxide catalyst with an aqueous solution of verbenalin at room temperature. This hydrogenation was allowed to proceed for 2 1/2 hours, at which time slightly more than two moles of hydrogen had been absorbed. There were obtained from this reaction, unchanged verbenalin and a reduced product, melting over a wide range despite many recrystallizations (130-145°). This material showed a decided tendency to become syrupy. Eventually the syrupy material was submitted to emulsin hydrolysis by the method of Asano, Ueno and Tamaki[10]. A crystalline aglycone was obtained in about 30% yield (calculating the weight of the syrupy material as 100% tetrahydroverbenalin), several recrystallizations yielding crystals, m.p. 122-3°. Ultraviolet spectrum analysis showed a maximum at 241 Å, shifting to 275 Å in alkaline medium.

Hydrogenation of verbenalin in the presence of palladium

chloride and activated charcoal resulted in an uptake of two moles of hydrogen in 26 hours, although more catalyst had to be added during the course of the reduction. After several recrystallizations there were obtained seemingly pure crystals, m.p. 200-2°, in indifferent yield. An infrared absorption spectrum showed much fine structure, indicative of impurity.

More attention was paid to the attempted reduction of verbenalin by means of lithium aluminum hydride. Young[6] had previously determined that one mole of verbenalol consumed five moles of lithium aluminum hydride. This was apparently confirmed when the writer found that one mole of verbenalin reacted with approximately nine of the reagent. When more verbenalin was used for reduction by lithium aluminum hydride in an effort to obtain the product of reduction, no organic crystals were obtained. It was difficult to remove all traces of lithium from the oily residues, no matter what solvents were used for extraction and crystallization attempts. Acetylation of the oily residue by means of acetic anhydride and pyridine yielded nothing crystalline. Attention was then shifted to lithium aluminum hydride reduction of the aglycone, verbenalol (obtained by emulsin hydrolysis of the glycoside), in the belief the reduction mixture would be easier to work with, since no glucose or products of degradation of glucose would be present.

A brief abortive effort was made also to carry out the reductive acetylation of verbenalin by means of treatment with acetic anhydride, zinc dust and catalytic amounts of triethylamine.

PROPERTIES AND DERIVATIVES OF VERBENALOL

Verbenalol crystallizes from ethyl acetate, alcohol or acetone in colorless rods, m.p. 133° (dec.), $[\alpha]_D^{24} = -21.13°$ (in alcohol). It is quite soluble in ether and chloroform, fairly soluble in ethyl acetate, acetone, alcohol and benzene, sparingly soluble in water, insoluble in petroleum ether.

Verbenalol in solution in aqueous alkali develops an amber coloration immediately and this soon deepens to brown. The aglycone dissolves in concentrated sulfuric acid with formation of a deep brown color. It gives a positive test with Tollens' reagent (ammoniacal silver nitrate) in the cold and reduces Fehling's and Benedict's solutions on warming.

By analyses, the molecular formula of verbenalol has been determined to be $C_{11}H_{14}O_5$ as would be expected for the aglycone of a monoglucoside verbenalin, of molecular formula $C_{17}H_{24}O_{10}$.

Bourdier[1] was not able to prepare the aglycone in crystalline state but he did report certain properties and derivatives of his amorphous product. It gave a dark violet color with ferric chloride. It was readily soluble in dilute alkali, acidic to litmus, and it reduced alkaline copper solutions. With phenylhydrazine acetate, he obtained a brownish-yellow crystalline compound for which he gave no data. He noted his aglycone did not react with sodium bisulfite, hydroxylamine, or semicarbazide.

Cheymol[8] found by the Zeisel method that verbenalol contained one methoxyl group. Although an aqueous solution of verbenalol was acid to phenolphthalein, it did not effervesce with sodium bicarb-

onate solution. A study of the behavior of verbenalol towards alkali indicated to Cheymol[8] that it contained a lactone structure. He noted a deepening yellow color with alkali, and found acidification of the yellow solution caused it to become colorless. When heated with sulfuric acid, verbenalol was not decarboxylated.

Cheymol obtained negative tests for verbenalol with Schiff's Reagent and with sodium bisulfite although with phenylhydrazine, it gave a red product. It gave a positive Tollens' test and a positive iodoform reaction, as well as a positive Legal's test. He felt these observations indicated the presence of a ketone group.

Cheymol[8] found evidence for carbon-carbon unsaturation in verbenalol in the compound's decolorization of bromine in carbon tetrachloride and in acetic acid. An aqueous solution of potassium permanganate oxidized it. Verbenalol in chloroform solution did not produce any color with tetranitromethane, which he felt indicated the olefinic linkage was not adjacent to the ketone or lactone.

A determination by the Zerewitinoff method by Cheymol[8] indicated the presence in verbenalol of two active hydrogen atoms. He also obtained a violet color when he treated aqueous solution of verbenalol with ferric chloride reagent. Since the glycoside itself did not yield a positive test with this reagent, Cheymol[8] postulated that the glucose moiety was linked to verbenalol through an enolic hydroxyl. It should be noted at this point, that Karrer and Salomon[13] found verbenalin produced a weakly positive test with ferric chloride after standing in the reagent for one hour or more, and

that Young[6] found that when no heat was used to effect solution of verbenalol in water, the aqueous solution of aglycone failed to give a positive test.

Karrer and Saloman[13] found that oxidation of verbenalol by chromic acid yielded oxalic acid. Ozonolysis yielded oxalic acid and formic acid, which were identified as their benzylthiuronium salts. No crystalline products were obtained when verbenalol was treated by acetylation, benzoylation and nitrobenzoylation respectively, even at low temperatures and with exclusion of air. Results were no better for the preparation of certain carbonyl derivatives, namely the phenylhydrazone and the oxime. Contrary to the finding of Cheymol[8], Karrer and Saloman[13] found that verbenalol treated with 1N. hydrochloric acid at 60-70° was degraded, there being strong evolution of gas. No crystalline compound was isolated from this reaction.

The same workers[13] prepared a crystalline derivative of verbenalol during the course of reduction of verbenalin (see P. 18). It was desoxyverbanol, $C_{10}H_{16}O_3$, which apparently resulted from reductive cleavage of the sugar residue and the methoxyl group.

Karrer and Saloman[13] carried out the hydrogenation of verbenalol with Raney nickel at 75° and 15 atmospheres. From this, they obtained in approximately 50% yield, a product $C_{10}H_{16}O_4$, m.p. 95-6°, containing no methoxyl group, lacking reducing properties, and having 2 active hydrogen atoms. They called it norverbanol. Permanganate oxidation of this compound yielded acetic acid in the form of its

benzylthiuronium salt and a strong acid, $C_{10}H_{14}O_5$, m.p. 170°. The yield was about 10% of theory. Esterification of the acid with diazomethane yielded an ester, $C_{11}H_{16}O_5$, m.p. 82°. The acid contained two ketone groups and a hydroxyl. The other two oxygen atoms were of course part of the carboxyl group. One of the ketone groups seemingly came from the hydroxyl formed by opening of the lactone ring, since the diketo acid could not be changed back to the original lactone. This acid gave no color with ferric chloride, and did not reduce Fehling's solution, but still gave an iodoform reaction.

Karrer and Saloman[13] oxidized the compound, desoxyverbanol, m.p. 137-8° (one active hydrogen atom by the Zerewitinoff method) with potassium permanganate in alkaline media. Apparently, an acid was produced by this oxidation since the mixture was neutral within 2 hours. The rather slow oxidation yielded an acid, m.p. 157-8°. Analysis data were inconclusive for any molecular formula.

Asano, Ueno and Tamaki[10] studied the action of barium hydroxide on verbenalol. At room temperature, they found that a 0.1 N solution of the alkali degraded the aglycone to methanol and an acid which could be converted back to verbenalol by the action of diazomethane. The acid was unstable and could not be crystallized. The action of 2 moles of 0.1 N barium hydroxide on verbenalol produced, in addition to methanol, formic acid and a viscous acidic substance, which could not be crystallized, which had no crystalline derivatives and which diazomethane did not reconvert to verbenalol. This substance did not give a ferric chloride test and gave a negative response to carbonyl reagents. Asano and his co-workers likened this behavior of verben-

alol to the alkaline degradation of phenylformylacetic ester and felt that proof of a formyl group in verbenalol was furnished by the fact that hydrogenated verbenalol, treated with barium hydroxide, yielded methanol only.

By oxidizing verbenalol with hydrogen peroxide in a strongly alkaline solution, Asano, Ueno and Tamaki[10] obtained methylsuccinic acid. By oxidizing the aglycone with sodium hypoiodite, they obtained about 0.1 mole of iodoform. Since furfurylpropionic acid on similar treatment gave a like amount of iodoform, they concluded that the haloform reaction in verbenalol did not indicate a methyl ketone or methyl carbinol structure but rather a furan ring which was ruptured by oxidation. As a second product of this oxidation, they isolated α-iodocrotonic acid, $C_4H_5O_2I$, m.p. 111°. In the corresponding reaction with furfurylicpropionic acid, the second product was β-iodoacrylic acid, $C_3H_3O_2I$, m.p. 139°. From oxidation of furfurylacrylic acid with alkaline hydrogen peroxide, they prepared succinic acid. It is interesting to note that, in additional experiments, these workers found that certain furan derivatives, notably pyromucic acid (2-furoic acid), furfural and furfuryl alcohol did not yield iodoform on hypoiodite oxidation, nor were their furan rings ruptured.

Chatterjee[4] attempted to prepare carbonyl derivatives of verbenalol. She had no success with semicarbazide or hydroxylamine, but when she refluxed verbenalol with 2,4-dinitrophenylhydrazine in absolute methanol, she obtained crystals, m.p. 179°, in small yield.

Analysis for nitrogen content of these by the semimicro Kjeldahl method, indicated the possibility the crystals were those of either a di-2,4-dinitrophenylhydrazone or a 2,4-dinitrophenylosazone of verbenalol.

Chatterjee made a quantitative determination of the amount of iodine required by verbenalol in alkaline solution for the haloform reaction. Results indicated one iodoform-forming group in the molecule. Upon titrating verbenalol with bromine in chloroform, the same worker[4] was able to demonstrate the uptake of one mole of bromine per mole of aglycone.

Chatterjee[4] also attempted the preparation of hydroxyl derivatives of verbenalol. Acetylation failed to give a crystalline derivative, although a dark brown reaction mixture indicated attack by the reagent. Methylation of an hydroxyl group in verbenalol was apparently accomplished in ethereal solution by means of diazomethane, even though a crystalline derivative was not isolated, for the distillate obtained at 175-180° and 2×10^{-3} mm. pressure gave analytical data by the Zeisel method for two methoxyl groups.

From the hypoiodite degradation of verbenalol, Chatterjee[4] procured very small amounts of crystalline needles, m.p. 114-114.5°. The amounts obtained were insufficient for further study. The same product was apparently obtained when verbenalin was similarly degraded, and its properties have been noted on P. 8.

Young[6] clarified, to a considerable extent, the confusion existing concerning whether or not verbenalol gives a positive ferric

chloride test. He found that when verbenalol was dissolved in water for purposes of the ferric chloride test with the aid of heat, a positive test resulted. On the other hand, when solution of the aglycone in water was effected by shaking only, a negative test resulted. From these observations, he concluded mere boiling of an aqueous solution of verbenalol caused degradation of the molecule in such a way as to yield an enolic hydroxyl group.

Young[6], reasoned that if the second methoxyl group in Chatterjee's "methylated verbenalol" resulted from the methylation of an enolic group and if the fifth oxygen atom were a carbonyl oxygen, it should be possible to prepare a 2,4-dinitrophenylhydrazone, tried to do so by refluxing the reagent and proposed reactant in absolute methanol. However, he recovered starting reagent only. From this evidence, he concluded that if a carbonyl function were present, it was not able to condense with the reagent.

The same worker[6] undertook a lithium aluminum hydride titration of verbenalol and concluded that one mole of aglycone reacted with five moles of lithium aluminum hydride. No crystalline reduction product could be isolated.

In the present study, "methylated verbenalol" was prepared according to the method of Chatterjee[4]. The slightly yellow syrupy liquid gave a negative test with ferric chloride. Its ultraviolet absorption spectrum (to be discussed later) differed from that of verbenalol itself in that the peak did not shift in alkali.

Also in the current study, unsuccessful attempts were made to

prepare derivatives of the carbonyl and of the hydroxyl group respectively of verbenalol. The carbonyl derivatives attempted were the oxime and the 2,4-dinitrophenyl hydrazone. The hydroxyl derivatives tried were the 3,5-dinitrobenzoate and the phenylurethan.

The treatment of verbenalol with N-bromoacetamide in chloroform containing 0.75% ethanol according to the method of Buckles[20] has yielded colorless crystals, m.p. ca. 295° (sublimed).

When the Lucas test[21] was tried with verbenalol to determine the nature of the alcoholic hydroxyl, no layering resulted, but the color did deepen. Despite the fact that verbenalol's 11 carbon atoms would place it at the limit usually shown by Ceric Nitrate reagent, the test was attempted. It proved negative but it was observed that the orange color of the ceric ion was bleached after the solution had stood overnight.

DEGRADATION STUDIES ON VERBENALOL AND VERBENALIN

OTHER PRODUCTS OF EMULSIN HYDROLYSIS OF VERBENALIN

Since the best yield of aglycone obtained by emulsin hydrolysis of verbenalin has barely exceeded 60% of theory, it was decided to investigate the hydrolysis residue, i.e. the syrupy dark brown liquid remaining after crystallization of verbenalol from the ether extraction of the hydrolysis mixture. The material gave positive tests with 2,4-dinitrophenylhydrazine reagent and ammoniacal silver nitrate. When the hydrolysis residue was treated with 2,4-dinitrophenylhydrazine preparatively in sulfuric acid, water and ethanol[22], there was obtained two compounds, one light yellow and the other dark red. The original precipitate, the dark red compound, after recrystal-

lization several times from ethanol and water, had the following values, m.p. 142-3° (decomp.), $C_{13}H_{12}N_4O_6$ (calc. C= 48.75%, H= 3.78%, N= 17.49%; found C= 48.81%, H= 3.77%, N= 17.80%). This would appear to be the 2,4-dinitrophenylhydrazone of the carbonyl compound, $C_7H_8O_3$. The second compound was not crystalline. Analysis data of this bright yellow amorphous compound, m.p. 214-5° (decomp.) indicated it to be $C_{20}H_{24}N_4O_9$ (calc. C= 51.70%, H= 5.21%, N= 12.06%; found C= 51.92%, H= 5.10%, N= 11.95%). This is apparently the 2,4-dinitrophenylhydrazone of the carbonyl compound, $C_{14}H_{20}O_6$. The amounts of these two compounds obtained, have precluded further studies at this time.

ALKALINE DEGRADATION OF VERBENALIN AND VERBENALOL

Cheymol[8] found that an aqueous solution of the glycoside was neutral to litmus, but that the addition of less than one mole of sodium hydroxide produced the sodium salt of an acid apparently by cleavage of the lactone ring. Similar behavior was observed when barium hydroxide was used. These salts were found to have reducing properties with alkaline copper reagents. The color of the aqueous solution of the sodium salt of verbenalosidic acid was yellow. Verbenalosidic acid was obtained by Cheymol[9] by acidifying a solution of barium verbenalosidate with N/10 sulfuric acid, distillation in vacuo, and extraction of the residue with ethyl acetate. Its formula was $C_{17}H_{26}O_{11}$ and its physical constants, m.p. 210-2°, $[\alpha]_D^{22} = -185.99°$. It possessed reducing properties, but gave a yellow color only with ferric chloride. An aqueous solution of

verbenalosidic acid was hydrolyzable by emulsin in a sealed ampoule, closed in vacuo. Sulfuric acid hydrolysis resulted in decarboxylation. Nothing crystalline was isolated from the emulsin hydrolysis of verbenalosidic acid, although the solution was strongly acidic, effervesced with sodium bicarbonate, gave a positive ferric chloride test and reduced alkaline copper reagents. Cheymol[8] named the substance which could not be isolated verbenalic acid.

The reactions of barium hydroxide on verbenalol as studied by Asano, Ueno and Tamaki[10] have been discussed on P. 25. The attempts of Chatterjee[4] to hydrolyze verbenalin by means of alkali have been noted on P. 13.

Chatterjee[4] fused verbenalol with potassium hydroxide at 295-300° for 10 minutes. From the blackened mass after neutralization, ether extraction produced a very small quantity of an acidic product, m.p. 122-5°. Similar alkaline fusion of verbenalin yielded nothing crystalline. She carried out alkaline degradation of verbenalol also under milder conditions refluxing an aqueous solution of verbenalol with 10% aqueous sodium hydroxide for several hours. From this she obtained in small yield colorless crystals, m.p. 114-5°. Mild alkaline degradation of the glycoside was also unsuccessful. Similar mild alkaline treatment of tetrahydroverbenalol resulted in the recovery of starting material, proving that reduction of verbenalol stabilized the alkali-labile structure. Chatterjee's[4] alkaline degradation of verbenalol by means of the iodoform reaction has been discussed on P. 27.

In the current study, milder conditions still (5% aqueous

sodium hydroxide with refluxing for 2 hours) were utilized for alkaline degradation. Distillation of the alkaline mixture before neutralization gave a colorless distillate smelling of formaldehyde, and giving a chromotropic acid color test[23] for that substance although no crystalline derivative of formaldehyde could be prepared. No volatile acid was distilled off after neutralization. Ethyl acetate extraction of the non-volatile residue gave a dark brown syrup which tested positively with 2,4-dinitrophenylhydrazine reagent. The substance could not be crystallized, but there was prepared from it a 2,4-dinitrophenylhydrazone, m.p. 142-3° (decomp.), $C_{13}H_{14}N_4O_6$ (calc. C= 48.45%, H= 4.38%, N= 17.39%; found C= 49.36%, H= 4.40%, N= 17.59%). This is apparently the 2,4-dinitrophenylhydrazone of $C_7H_{10}O_3$. Extraction of another neutralized non-volatile residue of alkaline degradation by means of t-amyl alcohol, gave a brown syrup which was acid to litmus, effervesced with aqueous sodium bicarbonate, and which gave upon treatment with p-phenylphenacyl bromide a small amount of crystalline derivative, m.p. 149-151°. Attempts have been made to accomplish the alkaline degradation of verbenalol by stirring an aqueous solution of the aglycone with an alkaline ion-exchange resin, Amberlite IR-4B. There is evidence of reaction at room temperature, but the relative insolubility of verbenalol in water at room temperature, and the apparent change in verbenalol caused by heating an aqueous solution of it (positive ferric chloride test after such heating[6]) present difficulties which remain to be studied.

LITHIUM ALUMINUM HYDRIDE REDUCTION

Young[6] carried out the quantitative reduction of verbenalol with lithium aluminum hydride in tetrahydrofuran and found that one mole of the aglycone reacted with five moles of the reagent. Similar treatment of the glycoside in the present study indicated one mole of the glycoside reacted with nine moles of lithium aluminum hydride, a result in agreement with Young's observation for the aglycone. Attempts were made to utilize the reaction between verbenalin and lithium aluminum hydride to prepare crystalline reduction products (perhaps more stable than the parent compound) but nothing was achieved. Consequently attention was centered on the reduction of verbenalol by means of lithium aluminum hydride. From this reduction, carried out in tetrahydrofuran, was obtained a dark brown thick syrupy material which could not be crystallized from any of the many solvents and solvent mixtures tried. An acetyl derivative of the syrup could not be prepared but benzoylation produced a nicely crystalline colorless compound, m.p. 204.5-205.5°, apparently $C_{20}H_{14}O_4$ (calc. C= 75.46%, H= 4.43%, mol. wt. = 318.22; found, C= 75.48%, H= 4.61%, mol. wt. 290.8, 296.1). A microdetermination of the benzoyl groups yielded apparently anomalous results (consideration of the molecular formula indicates the presence of two benzoyl groups), leaving available only the conclusion that the "benzoate of reduced verbenalol" contains some other alkali-consuming structure in addition to the benzoyl groups. A mixed melting point determination with the dibenzoate of hydroquinone, $C_{20}H_{14}O_4$, m.p.

199°, proved they were not identical. In the course of lithium aluminum hydride reduction of verbenalol, methanol was cleaved from the molecule. It was identified in the distillate after oxidation by the chromotropic acid color test[23] for formaldehyde. This finding of methanol as a cleavage product would seem to indicate a methoxyl group is present as part of a carbomethoxy group, as a hemiacetal, or in a position allylic to unsaturation.

DECARBOXYLATION OF VERBENALOL AND VERBENALIN

Cheymol[8] noted that when 2.5% sulfuric acid was allowed to react with verbenalin for a prolonged time (in an effort to accomplish hydrolysis to the aglycone) one mole equivalent of carbon dioxide was given off. Cheymol heated a 1% aqueous solution of verbenalol with 2.5% sulfuric acid at 100° for 18 hours in a sealed ampoule and noted no decarboxylation. Cheymol[9] found that tetrahydroverbenalin did not undergo decarboxylation when treated in the same manner. Cheymol found that verbenalosidic acid was decarboxylated by sulfuric acid also.

Karrer and Saloman[13] reported that when they heated verbenalol with N/1 hydrochloric acid in an evacuated ampoule at 60-70°, there was reaction with strong evolution of gas. They were not able to crystallize any cleavage product. The same workers noted that when they treated verbenalol with barium hydroxide at 60-70°, barium carbonate separated and a dark brown color developed. This would seem to indicate that decarboxylation had taken place also under these conditions.

In the present work, decarboxylation of verbenalin was carried out according to the procedure of Stoll and Renz[24]. Decarboxylation was accomplished easily with N/4 sulfuric acid although nothing crystalline could be isolated from the residue. During decarboxylation, a deep yellow color developed in the aqueous solution. In another experiment in which phosphoric acid (1% concentration) was the intended decarboxylating agent in an effort to avoid the charring action of sulfuric acid, no carbon dioxide was caught in the barium hydroxide traps until the reaction was catalyzed by the addition of two drops of concentrated sulfuric acid. Another decarboxylation or decarbonylation attempt involved the use of dry sodium fluoride suspended in a solution of verbenalin in dry pyridine with refluxing for 30 minutes and for 12 hours. Verbenalin was recovered unchanged in each instance.

Decarboxylation of verbenalol was accomplished easily by refluxing its aqueous solution with 10% sulfuric acid for 8-12 hours, approximately one mole equivalent of carbon dioxide being recovered in the barium hydroxide traps. No acidic substance appeared to be developed during decarboxylation. A brown syrupy material obtained by ether extraction gave a positive test for a carbonyl function with 2,4-dinitrophenylhydrazine reagent, and with Tollens' reagent. In one case, and only after standing at refrigerator temperature for several weeks, there was obtained a crystalline sodium bisulfite addition product from which was obtained on hydrolysis, a small amount of a liquid giving positive tests with 2,4-dinitrophenyl-

hydrazine reagent and with Tollens' reagent. From the ether extract of another decarboxylation experiment was prepared an amorphous 2,4-dinitrophenylhydrazone, m.p. 110-111° (analysis data, C= 55.54, 55.36; H= 5.93, 5.94; N= 11.50, 11.50). No satisfactory molecular formula could be constructed from these data. An attempt to oxidize the liquid resulting from bisulfite complex hydrolysis by means of Tollens' reagent was abortive. The aqueous residue left after ether extraction of the carbonyl compound from the decarboxylation experiment, was submitted to distillation after it was shown to be acidic to litmus. Duclaux values obtained were 1.06, 1.32, 1.48. It was not possible to prepare a derivative of the volatile acid but qualitative tests with potassium permanganate indicated the likelihood of the unknown's being formic acid. In a refinement of previous procedure, decarboxylation with 10% sulfuric acid was carried out under reflux, after which distillation yielded an acidic alkali-consuming aqueous liquid. The distillate did not give a positive test with 2,4-dinitrophenylhydrazine reagent.

OZONIZATION AND OZONOLYSIS OF VERBENALOL

Karrer and Saloman[13] reported that on degrading verbenalol with ozone, they obtained oxalic acid and acetic acid, both in the form of their benzylthiuronium salts.

In the present study, ozonization and oxidative ozonolysis of verbenalol were undertaken in ethyl acetate solution. The rate of reaction and the time of its completion indicated the presence of but one double bond in verbenalol. The ozonolysate gave a strongly

positive test with 2,4-dinitrophenylhydrazine reagent. The solubility characteristics of the carbonyl compound were strongly polar since repeated ether extraction failed to remove much of the compound from the aqueous residue. The ozonolysate also effervesced with sodium bicarbonate indicating the presence of an acidic group stronger than carbonic acid. Small amounts of two derivatives of the carbonyl product of ozonolysis were prepared, the 2,4-dinitrophenylhydrazone, m.p. 111-2° (decomp.) and the semicarbazone, m.p. 267-8°. The ozonolysate was divided by distillation into two fractions, one volatile and the other non-volatile. The latter gave positive tests with 2,4-dinitrophenylhydrazine reagent, ceric nitrate, ferric chloride, Tollens' reagent and still effervesced with sodium bicarbonate. Since it gave positive indications of containing both carbonyl and carboxyl functions, it was protected by methylation to the methyl ester before there was prepared for analysis a 2,4-dinitrophenylhydrazone, m.p. 128°. Analysis indicated the presence of a brown non-alkaline ash as impurity and thus rendered the data (C= 46.48%, H= 4.16%, N= 18.02%, ash from CH= 4.20%) inconclusive. The volatile fraction was proved to contain acetic acid by preparation of the p-bromoanilide, m.p. 166°, and the p-toluide, m.p. 150-2°.

ULTRAVIOLET ABSORPTION STUDIES

Cheymol[8,9] reported on ultraviolet absorption spectra of verbenalin, verbenalol, verbenalosidic acid and tetrahydroverbenalin. He stated the curves of the glycoside and verbenalosidic acid were

characteristic of a ketone and that this portion of the curve was missing in the hydrogenated glycoside. He found also that the curve of the acid differed from that of the glycoside in the visible part of the spectrum only and felt that this difference was due to the opening of a lactone ring. The peak for verbenalol was the same as for the other substances but upon hydrolysis (alkaline?) suffered modification which Cheymol believed might be due to keto-enol tautomerism.

Young[6] began quantitative studies of ultraviolet absorption spectra of verbenalin and verbenalol. For the glycoside, Young reported a single peak $\lambda_{max.} = 237 m\mu$ ($\xi_{max.} = 11,900$). When treated with an equimolar quantity of alkali, there was no appreciable change in the nature of the curve. In neutral solution, verbenalol had a peak approximating that of verbenalin, $\lambda_{max.} = 239 m\mu$ ($\xi_{max.} = 4,500$). However, when treated with an equimolar quantity of alkali, the single maximum shifted, $\lambda_{max.} = 273 m\mu$ ($\xi_{max.} = 40,000$). When the alkaline solution was neutralized with an equimolar amount of hydrochloric acid, the maximum absorption band shifted back to a shorter wave length ($230 m\mu$). The curve again resembled closely the original curve for verbenalol in neutral alcoholic solution. Young noted that an alkali shift as exhibited by verbenalol was typical often of phenolic or enolic substances, and that its occurrence in verbenalol was further proof of a keto-enol system in the aglycone. He concluded that the inability of verbenalin to shift its maximum absorption band in alkali was due to the manner of linkage of the glucose moiety, possibly through the keto form in a hemiacetal type

linkage thus destroying its enolic character.

Ultraviolet absorption spectra studies were extended in the present study to derivatives of verbenalin and verbenalol. Acetylated verbenalin behaved like verbenalin in that its maximum absorption band - $\lambda_{max.}$ = 236 mμ ($\varepsilon_{max.}$ = 18,300 or 17,000 dependent on whether acetylated verbenalin is a tetra- or pentaacetyl derivative) is shifted only slightly to 242 mμ in alkaline medium immediately. Reneutralisation of the alkaline test solution did not shift the maximum absorption band back to a lesser wave length. However, on standing in alkali for 24 to 96 hours, degradation of the molecule apparently took place for a second maximum absorption peak appeared in the region, 275-285 mμ.

A qualitative ultraviolet absorption spectrum of "methylated verbenalol" (see Page 141) gave a sharp single maximum absorption band at 242 mμ, a peak which was not shifted in alkali. The "benzoate of reduced verbenalol" exhibited a maximum absorption band at 235 mμ, and it did not shift in alkali.

Tetrahydroverbenalol, even though reduction had seemingly removed a carbon-carbon double bond and a carbonyl function, behaved like verbenalol. It showed $\lambda_{max.}$ = 241 mμ ($\varepsilon_{max.}$ = 18,500), shifting to 275 mμ when treated with an equimolar concentration of alkali.

The liquid obtained once from the decarboxylation of verbenalol with 10% sulfuric acid (see P. 35) exhibited a fairly broad maximum absorption at about 232 mμ and this was not shifted by treatment with alkali.

Whatever functional group or combination of functional groups that causes the alkali shift in the case of verbenalol, was still present in the hydrogenated product. This would seem to rule out keto-enol tautomerism as the explanation for the alkali shift. It has also appeared that any group that masks the hydroxyl group of verbenalol (e.g. glucosyl, methyl) prevents the alkali shift.

Ultraviolet absorption spectra studies were made of several compounds synthesized as "type" structures. These spectra will be discussed at the same time the compounds are mentioned.

INFRARED ABSORPTION STUDIES

In the current work, infrared absorption spectra of verbenalin, verbenalol and several of their derivatives became available for study. Spectra of verbenalin, verbenalol, acetylated verbenalin and tetrahydroverbenalin were done in Nujol mulls, and of verbenalol and tetrahydroverbenalol in chloroform solutions. In addition, certain interpretive comments have been available concerning infrared absorption spectra of the "benzoate of reduced verbenalol" and the dinitrophenylhydrazone of the decarboxylation product of verbenalol (see P.153), although the spectra themselves were not made available for study.

The spectra of verbenalin and verbenalol differ markedly in the -OH stretching and bending regions, as would be expected by the presence of the glucose moiety in the glycoside. The broadening of the hydroxyl absorption band to lower frequencies in verbenalol is evidence of hydrogen bonding, and the strength of the absorption is also notable[25]. In other respects, the absorption spectra of glyco-

side and aglycone are very similar, particularly in the carbonyl stretching region. This would seem to indicate that the linkage between the hydroxyl group and glucose moiety in the glycoside is a normal, not an enolic one.

An absorption band inthe carbonyl stretching region, 1739 cm^{-1} in verbenalin, 1736 in verbenalol in Nujol mull and 1745 in chloroform solution, and obscured, if present in acetylated verbenalin by strong acetate absorption at 1745, is absent in tetrahydroverbenalol. "In the -OH stretching region, the relative intensities of the "free" and "associated" hydroxyl absorption have changed slightly and the bands themselves are closer together in tetrahydroverbenalol. In the -OH bending region there is quite a difference between verbenalol and tetrahydroverbenalol. These two changes could quite possibly indicate the appearance of a new hydroxyl group in tetrahydroverbenalol[26]." These differences between the spectra of verbenalol and tetrahydroverbenalol (in chloroform solutions) indicate the reduction of a carbonyl function to an alcohol. The high frequency of the carbonyl absorption lost on reduction could be indicative of a cyclic ketone, possibly a cyclopentanone structure[27].

A very strong absorption band at 1646 cm^{-1} in verbenalin, at 1634 in acetylated verbenalin, and at 1634 and 1640 (Nujol mull and chloroform solution respectively) in verbenalol was originally assigned[25,28] to carbon-carbon unsaturation, with the notation that the absorption in these compounds was stronger than that usually observed for olefinic linkages. One interpreter[25] suggested the strength of the band might be due to an enol-ether or to the double

bond being in a terminal position. However, although not as strong, the band has survived reduction in tetrahydroverbenalol and appears at 1632 cm^{-1}. A possible explanation might be that the band in verbenalin, verbenalol and acetylated verbenalin is due to absorption by two functional structures, a carbon-carbon double bond and a carbonyl function. The decreased strength of the absorption band at 1632 cm^{-1} in tetrahydroverbenalol would then be the result of saturation of the double bond while the carbonyl is part of a functional group not reducible by catalytic hydrogenation.

In addition to this postulated carbonyl, a band at 1692 cm^{-1} in verbenalin, at 1675 and 1710 in verbenalol (Nujol mull and chloroform solution respectively), and at 1680 in tetrahydroverbenalol is within the range of carbonyl stretching frequencies. This band is not retained in acetylated verbenalin, or if it is, it is the weaker band at 1721 cm^{-1}. That it does not disappear in tetrahydroverbenalol may be evidence that the carbonyl is neither aldehyde or ketone. That it is shifted to 1680 from 1710 would seem to indicate hydrogen bonding with the newly created alcoholic hydroxyl.

Coincident with the decrease in strength of the band found at 1640 cm^{-1} in verbenalol and at 1632 in the reduced product, there occurs the loss of absorption bands at 990, 970 and 875. These observations may possibly be explained by the presence in verbenalol (and absence due to saturation in tetrahydroverbenalol) of a group such as $CH_2=C$ or $R_1CH=CHR_2$(trans).

The only band of absorption in tetrahydroverbenalol for which there is no corresponding band in verbenalol occurs at 1340 cm^{-1}.

However, the reduced aglycone has no band corresponding to verbenalol's absorption at 1265. These two observations may be related. A possible explanation is the reduction of an unsaturated methyl ether to a saturated methyl ether. There is chemical evidence for the presence of a methoxyl group in the glycoside and aglycone.

The combinations of absorption bands at 2980 cm^{-1}, 1445, and 1388 in verbenalol and at 2970, 1445 and 1385 in tetrahydroverbenalol best fit the picture of a C-methyl group, which has been proven by chemical analysis.

The absorption bands in the hydroxyl bending region do not seem to account for a primary alcohol. A possible conclusion from this is that the hydroxyl through which glucose is linked in the glycoside is not part of a hydroxyalkyl group, but is rather probably attached to a ring, and is therefore secondary or tertiary.

That two and possibly three absorption bands must be assigned to carbonyl structures would seem to eliminate a 1,2-dicarbonyl compound from consideration since such compounds usually show a single band at a frequency higher than either of the isolated carbonyls[25].

That there is no absorption band in verbenalol between 2700 and 2900 cm^{-1} would appear to be sufficient evidence to rule out the possibility of the aglycone's containing an aldehyde group.

The loss of the absorption band at 1692 cm^{-1} in verbenalin, and at 1675 and 1710 in verbenalol upon acetylation, but its retention upon catalytic hydrogenation (tetrahydroverbenalol has a corresponding band at 1680), is an argument in favor of a lactone structure

being assigned to these bands. Its loss upon acetylation would be due to hydrolysis by the acetylating reagent, with subsequent acetylation of the new hydroxyl. It is difficult to imagine an ester structure whose carbonyl absorption would be lost due to acetylation.

The spectrum of tetrahydroverbenalin, due to lack of material was done in Nujol mull rather than in chloroform solution, and consequently is not subject to chloroform blackouts from 1250 to 1200 and from approximately 800 to 600. In these intervals, absorption peaks occur at 1250, 1220, 1200, 810 and 675.

Comments[28] on the infrared absorption spectrum of the "benzoate of reduced verbenalol" indicate absorption due to the carbonyl of the benzoate linkage at 1730 cm^{-1}, and absorption due to the aromatic ring of the benzoate at 1599 and 1506. The dinitrophenylhydrazone of the decarboxylation product of verbenalol shows a weak absorption band at 3290 cm^{-1}, presumably due to the =N-H structure, absorption at 3105, and absorption at 1619, 1591 and 1513 due to the aromatic ring of the dinitrophenylhydrazone. No band is present which can be attributed to a carbonyl group and consequently no ester or lactone is present in this compound.

A furan nucleus has been postulated for verbenalin and verbenalol by Asano, Ueno and Tamaki[10]. Consequently it is worthwhile, indeed essential, to examine the literature concerning infrared spectra of furan and its derivatives.

Thompson and Temple[29] in the infrared spectrum of furan, found eight bands due to ring deformation, six bands due to C-H bending and four due to C-H stretching. Bands due to ring deformation were

allocated at 605, 724, 744, 994, 1381, 1486 and 1579 cm^{-1}, bands due to C-H deformation at 837, 872, 1067, 1137 and 1270 cm^{-1}, bands due to C-H stretching at 3090 and 3120 cm^{-1}, and a probable fundamental unallocated at 1034 cm^{-1}.

Pickett[30] had previously reported resolution of a number of combinations and overtones found in the infrared spectrum of furan in the vapor state. Lines at 624, 725, 872, 1139, 1389, 1490 and 1580 cm^{-1} were ascribed to the furan nucleus; bands at 763, 999, 1073 and 1264 cm^{-1} were reported due to C-H bending.

Bands are found in the infrared spectra of verbenalin and verbenalol corresponding to many of those found in the spectra reported for furan. Some of the latter's bands are missing, but it does not appear possible to draw any definite conclusions concerning the presence or absence of a furan nucleus in verbenalin or its aglycone on the basis of infrared spectra alone.

COLOR TESTS FOR THE FURAN NUCLEUS

Trim and Hill[7] have carried out a series of color tests on aucubin, $C_{16}H_{22}O_9$ and asperuloside, $C_{17}H_{24}O_{11}$, both glucosides of aglycones which cannot be isolated in the free state, for the presence of the furan nucleus in these compounds. Due to the similarity of these molecular formulas with that of verbenalin, and the extreme reactivity of verbenalol, it was felt desirable in the current investigation to perform the same tests on verbenalin, verbenalol and some of their derivatives. Aucubin has been suggested by Karrer and Schmid[31] to have a substituted furan structure, to be the glucoside of α-(2,5-dihydroxycyclopentyl)-β-hydroxylfuran, the glu-

cose moiety being linked to the β-hydroxy group. Asperuloside has been shown by Trim and Hill[7] to be very similar in chemical reactivity to aucubin, and consequently has also been suggested to have a substituted furan structure.

Aucubin and asperuloside, both acid-labile, were shown to produce soluble blue products in glacial acetic acid containing a small amount of cupric ion. The presence of ketohexose (fructose) caused the positive test to assume a reddish color. Verbenalol and verbenalin yielded an amber color with the reagent in the absence of fructose, a deeper amber color in the presence of fructose. Glucose (tested because it is the sugar of verbenalin) and coumarin (tested because it is a valerolactone) yielded negative tests with the reagent, both in the absence and presence of fructose.

The antimony chloride reaction[32] with aucubin[7] developed through a series of colors (pale yellow, pale green, pale blue) to a strong blue-violet color in 30 minutes. In the same time, asperuloside gave a blue-green color[7]. Verbenalin colored the reagent pale yellow-green in 4 hours, and the solution was quite green in 20 hours. Verbenalol reacted to give the pale color in 2 hours. Tetrahydrofuran (tested because it was the only available saturated furan compound) gave a grey colored precipitate within one hour. The decarboxylation product of verbenalol (see P. 35) showed traces of a similar precipitate in 20 hours. The "benzoate of reduced verbenalol" and tetrahydroverbenalol gave negative tests with this reagent. With Shear's aniline hydrochloride reagent[33], with which aucubin did not

react in the cold[7] and with which asperuloside gave a yellow, brown and finally, an intense green color, verbenalol turned dark amber immediately, becoming red-brown within 15 minutes, and verbenalin reacted in the same manner just a bit more slowly. Glucose and coumarin yielded negative tests.

Certain phenolic substances have been shown to develop red colors with aucubin and asperuloside[7]. Phloroglucinol gave a red-brown color with asperuloside and a green color with aucubin[7]. Verbenalol and tetrahydrofuran gave yellow colors immediately deepening to amber with the reagent. Verbenalin gave a positive reaction within 5 minutes, acetylated verbenalin within 10 minutes, tetrahydroverbenalol and the decarboxylation product of verbenalol within 3 hours; the "benzoate of reduced verbenalol" was faintly positive within 20 hours. A butyrolactone, 2-phenyl,-3-acetyl,-4,5-diketo-tetrahydrofuran gave a positive test within 10 minutes while a valerolactone, coumarin, gave negative results.

Trim and Hill[7] do not suggest that positive tests with all the foregoing reagents indicate the presence of a furan ring, since some terpenes give positive reactions with the phloroglucinol, antimony trichloride and aniline hydrochloride reagents, and pyruvic acid on warming gives a positive test with the phloroglucinol reagent. Ease of reaction with antimony trichloride and aniline hydrochloride is increased by unsaturation and the presence of carbonyl groups[7].

The tests for a furan ring, as performed on verbenalin, verbenalol and some of their derivatives, do not seem to have yielded conclusive evidence for the presence of such a nucleus in these compounds.

COMPARISON OF CERTAIN PROPERTIES OF TYPE STRUCTURES AND VERBENALOL

During the course of their studies on verbenalin and verbenalol, Chatterjee[4,5] and Young[6] postulated as a possible structure for the aglycone, an α-ketobutyrolactone with a substituted cyclopentane structure in the γ-position. The latter felt the hydroxyl through which the glucose moiety was linked might well be a hemiacetal hydroxyl at the γ-position, while Chatterjee postulated an enolic hydroxyl on the carbon adjacent to the point of linkage on the lactone structure.

In the present study, 2-phenyl-3-acetyl-4,5-diketotetrahydrofuran was synthesized according to the method of Kaskin[34]. The compound, m.p. 168-168.5°, (calc. C= 66.05%, H= 4.62%; found C= 65.63%, H= 4.66%) showed maximum absorption in ultraviolet spectrum analysis at 324 mμ (ϵ=20,000), a value which shifted very little in alkali. Attempts to prepare by the same synthetic method 3-acetyl-4,5-diketotetrahydrofuran and 2-n-hexyl-3-acetyl-4,5-diketotetrahydrofuran, were unsuccessful.

In an experiment paralleling the sulfuric acid decarboxylation of verbenalol, 2-phenyl-3-acetyl-4,5-diketotetrahydrofuran did not suffer decarboxylation nor was any volatile or non-volatile acid produced. The distillate (in vacuo) had a perfume-like odor and gave a positive test with Schiff's reagent, but nothing could be prepared from it because of the lack of material.

LOGANIN AND LOGANETIN

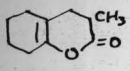
In their discussion of the possible structures of loganin, a glycoside of Strychnos nux vomica (Loganiaceae) and its aglycone,

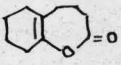
loganetin, Merz and Krebs[35] concluded that the glycoside's structure might not be too far different from that of verbenalin and aucubin. On the basis of this conclusion, it seems wise to include in a thesis about verbenalin and verbenalol, the known data and inferences made concerning the structures of loganin and loganetin.

The properties of loganin are briefly as follows. Loganin is $C_{17}H_{26}O_{10}$, m.p. 223°, $[\alpha]_D^{20} = -82.11°$ (in water); it gives no color with ferric chloride, does not decolorize bromine water, is not hydrogenated catalytically with palladium-barium sulfate or various platinum catalysts, forms pentaacetyl and pentabenzoyl derivatives, is titrated as a lactone with sodium hydroxide when heated, contains one methoxyl group, does not reduce Fehling's Solution, and with sulfuric acid produces a red color deepening to violet. The sugar of loganin was identified as glucose. In glycosidic linkage, it (i.e. glucose) contains four acetylable hydroxyl groups. The fifth such group in loganin must be extant in the aglycone. Loganetin has not been obtained in a crystalline state. It is apparently $C_{11}H_{16}O_5$, forms a di-p-nitrobenzoate (hence has two hydroxyl groups), absorbs one mole of hydrogen, decolorizes bromine water, reduces Fehling's Solution weakly, is titrated as a lactone, gives a green color with ferric chloride in neutral solution, is oxidized readily by potassium permanganate, with alkaline hydrogen peroxide yields acetic and formic acids, and with sulfuric acid gives a color test similar to that given by loganin.

Merz and Krebs[35] postulated for loganetin a structure involving a six- or seven-membered lactone fused to a cyclohexene ring, the

double bond being common to the two ring systems.





DISCUSSION OF THE STRUCTURES OF VERBENALIN AND VERBENALOL

The first point of structure worthy of discussion should be the glycosidic linkage since it is by this only that the glycoside and aglycone differ. Two facts confirm that the hydroxyl of verbenalol through which the glucose moiety is linked is alcoholic, not enolic. Young[6] has shown that pure verbenalol dissolved in water without the aid of heat gives no ferric chloride test. The infrared spectra of verbenalol and verbenalin do not differ materially in the carbonyl stretching region. One would expect a considerable difference if a potential carbonyl were linked to glucose in the glycoside.

A procedure, most useful in helping to determine structural formulas of compounds whose molecular formulas are known, and which compounds contain carbon, hydrogen, oxygen and/or nitrogen only, is the application of the following formula to determine the "degrees of unsaturation".

$$\text{Degrees of Unsaturation} = C + 1 - \frac{H - N}{2}$$

Any site of unsaturation in the molecule will be reflected in the value determined, e.g. C = Y, where Y = C, O, S, N, etc. Unsaturation of the type C = C, may be due to an olefinic linkage or a carbocyclic structure, as evidenced by the fact that C_6H_{12}(one degree of

unsaturation) may be a hexene or cyclohexane.

In verbenalin and verbenalol, there are 6 and 5 degrees of unsaturation respectively;

$$\text{Verbenalin's Degrees of Unsaturation} = 17 + 1 - \frac{24-0}{2} = 6.$$

$$\text{Verbenalol's Degrees of Unsaturation} = 11 + 1 - \frac{14-0}{2} = 5.$$

The sixth degree of unsaturation in verbenalin is, of course, the glucose ring.

At no time has there been agreement on the nature of the 5 oxygen atoms of verbenalol. One, of course must be contained in the hydroxyl through which the glucose is linked. This hydroxyl group must be concerned in the "alkali shift" of verbenalol, i.e. the shifting of the ultraviolet absorption maximum from 239 to 273 mμ, since masking it with a methyl group (methylated verbenalol) or with a glucosyl group (verbenalin) destroys this ability. If this were keto-enol tautomerism causing the shift in verbenalol, the absorption maximum for verbenalin should be the same as for the enol form of verbenalol, i.e. 273 mμ. An alkali-catalyzed reaction, the substrate of which must contain a free hydroxyl group, is difficult to explain. One possibility is that verbenalol is a hemiacetal (a partially stabilized one) and that alkali destroys this stability. Another is an α-hydroxy carbonyl structure.

A second oxygen atom is contained in a methoxyl group for which there is much chemical evidence. Asano, Ueno and Tamaki[10] claim that the methoxyl group is part of a carbomethoxy structure. How-

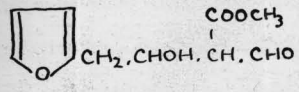
ever, their inclusion of a carbomethoxy group rather than the more generally favored lactone structure causes difficulty in assigning the five degrees of unsaturation demanded by verbenalol's molecular formula, as will be shown in subsequent reasoning. Moreover, the methoxyl group may be present in several other types of structure to account for its being sloughed in lithium aluminum hydride reduction, e.g. in hemiacetal linkage or in a position allylic to unsaturation.

A third and a fourth oxygen atom are almost surely involved in a lactone structure. The fact that glycoside and aglycone titrate as lactones, that a ferric chloride test is positive only after an aqueous solution is heated, indicate that a ring is ruptured when the compound is treated with alkali or with heat.

Since several carbonyl derivatives of acetylated verbenalin have been prepared, and since verbenalin and verbenalol possess in their infrared spectra a band which is lost by reduction (e.g. tetrahydroverbenalol), it seems reasonable to assume the fifth oxygen atom is part of a reducible carbonyl group. That carbonyl derivatives of verbenalin and verbenalol have not been prepared successfully must be due to the location of the carbonyl group in these compounds. Acetylation apparently stabilizes the carbonyl function (by unbonding?) so that its derivatives may be prepared in crystalline state. As was previously pointed out, the frequency of absorption of this reducible carbonyl, and the fact that not all aldehyde reactions are given, seem to point to a cyclic ketone, likely a cyclopentanone derivative as the basic structure for ver-

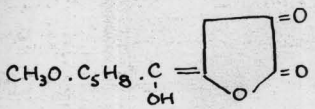
benalol.

This carbonyl group has been the subject of much controversy. Several workers, including Cheymol[8,9], Chatterjee[4,5] and Asano, Ueno and Tamaki[10] have postulated that glucose is linked to the aglycone through the enolic form of a carbonyl compound. In this event, a second carbonyl (ketone or aldehyde) is necessary to explain the carbonyl derivatives of acetylated verbenalin. Yet reduction data (hydrogen uptake, etc.) indicate but one reducible carbonyl is present. In the case of the structme advanced by Asano, Ueno and Tamaki[10] (I)



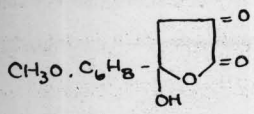
this consideration obviously makes impossible the linkage of glucose through an enolic form of the aldehyde, although it does not rule out the linkage involving the alcoholic hydroxyl of the structure (I).

In the case of Chatterjee's postulated structure (II)



there is no alcoholic hydroxyl on which to count for linkage to glucose.

Young's[6] proposed structure (III)



is not obviated by the foregoing considerations.

Other functional groups (not containing oxygen however), which have been proven to exist in verbenalol and verbenalin are a carbon-carbon double bond, and a C-methyl group.

The olefinic linkage is proven to be one only, by consideration of the hydrogenation data and the rate of ozonization. If we now consider verbenalol's five degrees of unsaturation, we find this olefinic linkage accounts for one, a lactone group for a second (the C=O structure) and a third (the ring), and a reducible carbonyl accounts for a fourth. This leaves the fifth for which we must account. If the five oxygen atoms are concerned in the various structures shown, then the only type of unsaturation left as a possibility is another carbon-carbon unsaturation, and since there is but one olefinic bond, this one must be in the form of a carbocyclic ring.

Infrared spectra observations are reasonably explained by the occurrence of the olefinic linkage in a structure such as $CH_3CH=$ attached to a ring. This structure would in addition account for the fact that verbenalin, verbenalol and acetylated verbenalin give positive iodoform tests while the reduced aglycone, tetrahydroverbenalol does not. There is no evidence for a methyl ketone structure in the infrared spectra, which group or any group oxidized to it by

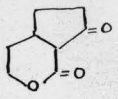
hypoiodite will give the haloform reaction.

The presence of but one double bond rules out the furan structure postulated by Asano, Ueno and Tamaki[10], or any modification of it.

Decarboxylation normally occurs in an acid medium for β-keto acids (or compounds easily converted to them such as β-keto lactones or esters) and β,γ-unsaturated acids (or compounds easily converted to them). Verbenalin and verbenalol are decarboxylated by treatment with 10% sulfuric acid. Tetrahydroverbenalol is not. This indicates that either the ketone group or the carbon-carbon double bond is in such relationship to the carbonyl of the lactone as to promote ease of decarboxylation.

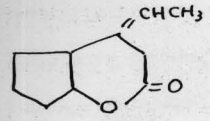
Since the reducible carbonyl group must be assigned the absorption band at 1745 cm^{-1} in verbenalol (in chloroform solution), because this band has disappeared in tetrahydroverbenalol, the band at 1710 must be due to the carbonyl of the lactone. This is in the normal ester position. γ-lactones absorb at higher frequencies while δ-lactones absorb in the same range of frequencies as esters. Conjugation and hydrogen bonding cause a lowering of this frequency. Verbenalol's absorption is in the lower range of such normal values. A comparison of the spectra of verbenalin and verbenalol in Nujol mulls shows that the glycoside is less bonded than the aglycone, evidence that the hydroxyl through which glucose is linked is sufficiently close to the carbonyl of the lactone to exert a strong influence on it.

These considerations are best answered by a β-keto-δ-lactone structure. The ketone group can be located in either the lactone ring or the cyclopentane structure. The frequency of the reducible carbonyl's absorption suggests the latter is the case. If this is so, the cyclopentanone ring can be fused to the lactone structure in just one way, at the α, β position of the lactone (IV).

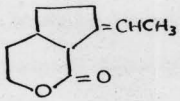


The double bond must be located in such a way as to give rise to an enol when the lactone ring is hydrolzed, since verbenalol does not give a ferric chloride test until its aqueous solution is boiled. In this latter case, the methoxyl group cannot be in hemiacetal relationship to the hydroxyl from which the lactone is formed. It can be in hemiacetal relationship to the hydroxyl through which the glucose is linked and/or in allylic relationship to unsaturation, either the double bond or the cyclopentanone carbonyl.

If decarboxylation is accounted for by a β,γ-unsaturated-δ-lactone, on the other hand, the $CH_3Ch=$ can be attached either to the lactone ring (V)



or to the carbocyclic ring (VI).



If the former be the case, the carbocyclic ring can be fused to the lactone in one position only, the γ,δ. Unless the hydroxyl through which the glucose is linked is angular and in acetal relationship with the hydroxyl from which the lactone is formed (in which case hydrolysis of verbenalin to verbenalol would cause the loss of one mole of water as well as a mole of glucose), or unless the methoxyl group is angular and in hemiacetal relationship to the hydroxyl of the lactone (in which case hydrolysis of verbenalin to verbenalol would cause the loss of one mole of methanol and one mole of glucose), a ferric chloride test would not be found in the circumstances noted with verbenalol.

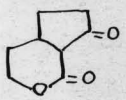
If the $CH_3CH=$ be placed in the carbocyclic ring (VI) but β to the lactone carbonyl, the carbocyclic ring can be fused to the lactone at the α,β position only. To account for the ferric chloride test given by an aqueous solution of verbenalol when heated, the methoxyl group would have to be in hemiacetal relationship to the

hydroxyl from which the lactone is formed and again we would have a situation where hydrolysis of verbenalin to verbenalol would cause the sloughing of one mole of methanol, as well as one mole of glucose.

The α-keto structures postulated by Chatterjee (II)[4,5] and by Young (III)[6] are not possible since the two adjacent carbonyls should give but one band in the infrared absorption spectrum, and there is no shortage of carbonyl bands in the spectra of verbenalin and verbenalol. In addition, 2-phenyl-3-acetyl-4,5-diketotetrahydrofuran, a type α-keto lactone synthesized in this present work, is not decarboxylated, as are the glycoside and aglycone.

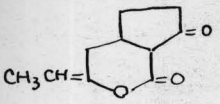
The structure postulated by Asano, Ueno and Tamaki (I)[10] is definitely disproved by two facts; the alcoholic hydroxyl through which glucose is linked, and the presence of but one carbon-carbon double bond.

As a result of all the foregoing reasoning, it would appear reasonable to postulate the basic structure (IV) for verbenalol.



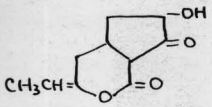
It will then be necessary to add the $CH_3CH=$, $-OH$, and $-OCH_3$ groups to the basic structure in such ways as to account for as many observations as possible.

Perhaps the most easily placed of these three groups is the $CH_3CH=$ structure, since it must account for a haloform test given by the glycoside, aglycone and acetylated glycoside (but not given by the reduced glycoside and aglycone), for the ferric chloride test given by the aglycone only after boiling (i.e. presumably after hydrolysis of the lactone ring), and for the acetic acid formed during ozonolysis. The basic structure may now be expanded to (VII).

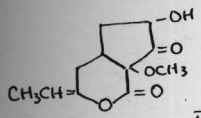


The inability of many workers to prepare either derivatives of verbenalol utilizing the hydroxyl function, or derivatives of verbenalin utilizing the carbonyl function, may well be related. Another observation whose eventual explanation may also be related, is the blockage of alkali shift in ultraviolet absorption spectra by masking the hydroxyl group of verbenalol by methylation or by glycoside formation. Presumably, an α-hydroxy carbonyl structure could be the reason for this series of observations. If an hydroxyl group is α to a carbonyl function, the latter may be either the lactone carbonyl or the ketone carbonyl. In the postulated basic structure for verbenalol, an hydroxyl α to the lactone carbonyl would, of necessity, also be α to the ketone carbonyl. Because of its angular position, resonating structures would not be possible to explain the inability to form hydroxyl and carbonyl derivatives of

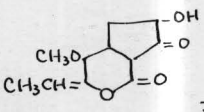
aglycone and glycoside respectively. This would leave one place only for the hydroxyl group, on the carbocyclic ring in the non-angular position, α to the ketone carbonyl. This is the alcoholic hydroxyl through which the glucose is linked in verbenalin. The basic structure may now be expanded again to (VIII).



There remains in question (assuming the foregoing to be correct) the position of the methoxyl group. It is sloughed in lithium aluminum hydride reduction. A hemiacetal relationship with the hydroxyl group involved in lactone formation is not possible due to the placement of the $CH_3CH=$ group on the δ carbon atom of the lactone ring. The postulation by Asano, Ueno and Tanaki[10] that the methoxyl group is part of a carbomethoxy structure has been ruled out previously, principally because of considerations concerning the nature of the five oxygen atoms and the number of degrees of unsaturation. This leaves as a possible explanation for the sloughing of methanol during reduction, the allylic relationship of the methoxyl group to unsaturation. Two possibilities now arise. The methoxyl group may be in the angular position, α to both the lactone and the ketone carbonyl (IX), or it may be attached to the γ carbon atom of the lactone ring, α to the carbon-carbon unsaturation (X).



IX



X

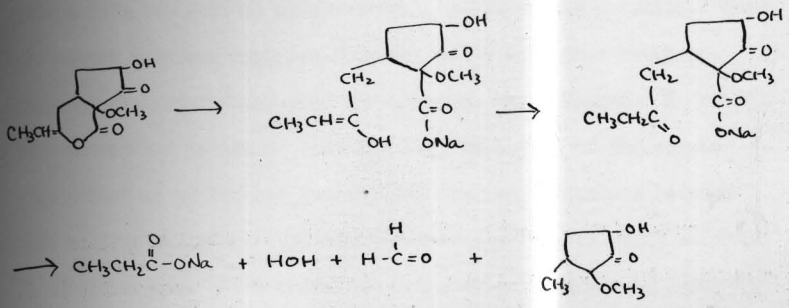
It is difficult to decide which (if either) of structures (IX) and (X) is correct, because of the paucity of well-defined and completely characterized degradation products.

It would appear to be definite that mild alkaline degradation of verbenalol does produce a 7-carbon carbonyl-containing product, since analysis of the 2,4-dinitrophenylhydrazone of the extractive of alkaline treatment gives values consistent with a molecular formula of $C_7H_{10}O_3$ for the cleavage product. Application of the formula for determination of the number of degrees of unsaturation to this molecular formula, gives the value "3".

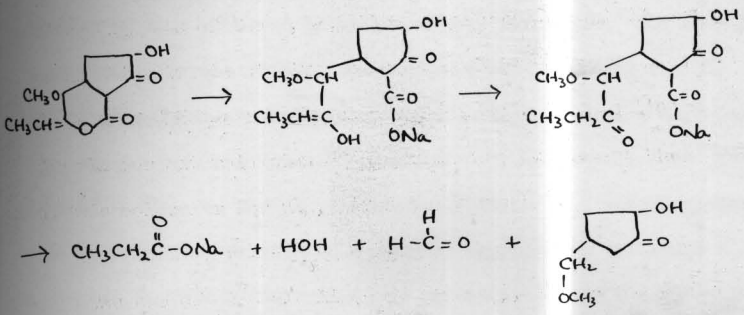
$$7 + 1 - \frac{10-0}{2} = 3.$$

The use of mild alkali (a 5% aqueous), the β-keto lactone structures postulated (IX and X) and the obtaining of a carbonyl compound would all seem to favor the view that ketonic rather than acid hydrolysis had taken place.

If the structure IX should be correct, alkaline degradation might be expected to take place as follows.



If the structure X should be correct, the following series of reactions would take place.



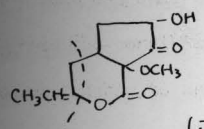
In either case, the resulting compound would have the molecular formula $C_7H_{12}O_3$. Lacking more definite information about the alkaline degradation product whose 2,4-dinitrophenylhydrazone was actually prepared, the only conclusion which may be drawn is that either of the above mechanisms could be correct (a difference of 2 hydrogen atoms only) and no facts or data yet exist to render one more likely than the other.

The obtaining of the 2,4-dinitrophenylhydrazones of two com-

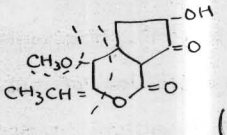
pounds from the residue of glycosidal hydrolysis is puzzling. That one parent compound contains 7 carbon atoms and other fourteen, suggests the possibility that dimerization has occurred. It would seem reasonable to assume that the further action of the enzyme preparation on verbenalin (to account for the difference between 100% and the yields of verbenalol actually obtained) might well be an oxidative one. Such oxidative degradation to give one 7-carbon fragment, might well parallel alkaline ketonic hydrolysis.

Decarboxylation of structures such as IX and X by means of 10% sulfuric acid would, of course, be complicated by a ketonic cleavage, similar to that produced by dilute alkali solutions. The absence of a molecular formula fitting the analysis data obtained for the 2,4-dinitrophenylhydrazone obtained, makes speculation here worthless.

Asano, Ueno and Tamaki[10] obtained α- iodocrotonic acid (CH_3 - CH = CI - COOH) by sodium hypoiodite oxidation and methylsuccinic acid by hydrogen peroxide oxidation of verbenalol. Although they attempted to relate these findings to the presence of a furan ring in the aglycone, they also found that pyromucic acid (2-furoic acid), furfural and furfuryl alcohol were not oxidized in a like manner. It would seem more likely then that the 4-carbon iodinated and unsaturated acid should have been derived from a side-chain or potential side-chain in the verbenalol molecule. In structures (IX) and (X) as pictured below, such possibilities exist.



(IX)



(X)

It is apparently not possible to account for the formation of methylsuccinic acid with these structures.

It is obvious from the foregoing considerations that more work is needed to elucidate completely the structure of verbenalol. Some of the research desirable may be noted here;-

(1) Ketonic hydrolysis using a dilute solution of a non-oxidizing acid would seem to be a likely possibility for useful information.

(2) If the product of alkaline (or dilute acid) ketonic hydrolysis could be isolated, a C-methyl determination would yield valuable evidence concerning the correctness of structures (IX) and (X).

(3) Periodate titration and oxidation of verbenalol could give useful information about the proposed α-hydroxy keto structure.

(4) Synthesis of a valero-lactone structure with a cyclopentanone ring fused at the α,β positions and determination of its ultraviolet and infrared absorption spectra, would seem to be desirable.

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EXPERIMENTAL

METHODS OF EXTRACTING VERBENALIN

The material used was the overground portion of Verbena stricta, collected in August, 1947, from pasture land on U.S. Highway 12 near Madison, Wisconsin. After approximately two weeks of air drying, the plant was ground to a coarse powder in a hammer mill. This air-dried ground drug was used for extraction of verbenalin.

Verbenalin was obtained in crude state by extraction of the drug for 100 hours with ether in a continuous two-phase liquid extractor[1,2,3]. The supernatant ether was poured off and used again in extracting more drug. The crude dark green deposit was then treated with Norite in alcohol, crystallized from alcohol, and re-crystallized from alcohol-ethyl acetate. Yields of glycoside represented about 0.6%.

The glycosidal extraction method of Trim and Hill[4] was applied to the preparation of verbenalin from Verbena stricta. Two kilos of powdered drug were placed in a large enamelware pot and to the drug were added 5 g. calcium carbonate and sufficient hot distilled water to render the mixture workable. It was heated at 100° for 3 hours, with constant stirring by a Lightning Mixer. The mixture was strained through a Celite bed in an International Centrifuge basket adapter. The filtrate was then stirred thoroughly with 20 g. Norite (previously boiled with distilled water, and cooled), then filtered by centrifugation and passage through a Celite bed. This filtrate was stirred with 250 g. similarly treated Norite for 20 minutes and

filtered through a Celite bed. The pad of Celite and Norite was washed with 2 l. distilled water, then eluted with 12 l. 50% alcohol. Elution was accomplished by stirring the Norite and diluted alcohol (in three lots) for 20 minute periods. The elute was concentrated to 400 ml. in a Solvent Evaporator, and further reduced in volume to 250 ml. by distillation in vacuo. The concentrated aqueous solution was filtered to remove calcium carbonate and extracted with ethyl acetate in a continuous two-phase liquid extractor twice for 72 hours each time. When the ethyl acetate extract, dried over anhydrous sodium sulfate, was concentrated by distillation in vacuo and set aside in the refrigerator, pure crystals of verbenalin, m.p. 181°, appeared. The yield was 0.5%.

PROPERTIES OF VERBENALIN

LITHIUM ALUMINUM HYDRIDE TITRATION OF VERBENALIN[3,5]

Lithium aluminum hydride reagent was prepared in the following manner. Twelve grams of lithium aluminum hydride were refluxed for 4 hours with 500 ml. tetrahydrofuran. The slurry was centrifuged for 3 hours and the supernatant titrated against reagent grade n-butanol (5%) in benzene. The standardization equivalent was; 1 ml. butanol-benzene reagent ≡ 6.808×10^{-4} equivalents of lithium aluminum hydride, (titration of 5 ml. lithium aluminum hydride reagent by 11.5 ml. butanol-benzene reagent).

A sample of 0.1386 g. verbenalin was dissolved in 20 ml. redistilled tetrahydrofuran with the aid of heat. To this solution was added 5 ml. lithium aluminum hydride reagent and the mixture was refluxed 3 hours. A control solution (no sample) was similarly

refluxed. The test and the control were titrated with butanol-benzene to determine the excess lithium aluminum hydride. Titrations were, of the sample 6.44 ml., of the control 10.90 ml.; lithium aluminum hydride reagent ≡ 4.46 ml. butanol-benzene used by the sample of verbenalol.

Standardization of butanol-benzene ≡ 6.808×10^{-4} equiv./ml.

No. equivalents of verbenalin = 0.1386/388 = 0.0003572

No. functional groups = $\frac{4.46 \times 6.808 \times 10^{-4}}{0.0003572} = 8.70$

A similar titration involved a sample of 0.1390 g. verbenalin and omitted the 3 hours refluxing period. Titration was carried out immediately after the addition of the lithium aluminum hydride to the tetrahydrofuran solution of the glycoside, and the following data was recorded.

Titrations - 7.50 ml. (test); 12.06 ml. (control)

Lithium aluminum hydride reagent used ≡ 4.56 ml. butanol-benzene

Equivalents of verbenalin = 0.1390/388 = 0.0003583

No. functional groups = $\frac{4.56 \times 6.808 \times 10^{-4}}{0.0003583} = 8.65$

A third determination yielded the following data;

Titrations - 2.29 ml. (test); 6.72 ml. (control)

Lithium aluminum hydride reagent used ≡ 4.43 ml. butanol-benzene

Weight of verbenalin = 0.1423 g.

Equivalents of verbenalin = 0.1423/388 = 0.0003667

No. functional groups = $\frac{4.43 \times 6.808 \times 10^{-4}}{0.0003667} = 8.22$

The conclusion drawn was that verbenalin contained functional groups and active hydrogens in sufficient number to combine with 9

equivalents of lithium aluminum hydride. The titration residues were worked as described on P. 96.

C-METHYL DETERMINATION OF VERBENALIN[6]

A sample of 0.0191 g. verbenalin was placed in the flask of the Roth-Kuhn apparatus. Five milliliters of cooled oxidation mixture (4 parts 5 N chromic acid and 1 part sulfuric acid, density 1.84) were added and the mixture was refluxed 1 1/2 hours.

The mixture was cooled, and the condenser, funnel and inlet tube washed with a total of 7 ml. distilled water (recently boiled and cooled). Two milliliters dilute hydrazine solution were added dropwise. After further cooling, 6 ml. 5 N sodium hydroxide were added and then the solution was acidified with 1 ml. concentrated phosphoric acid, and distilled. As the specified fractions were caught in the graduate, they were titrated with 0.0100 N sodium hydroxide with phenolphthalein as indicator, and the residue was restored to volume by the addition of water. The following titration data were recorded.

Fraction	ml.	ml. 0.0100 N NaOH
1	20	3.85
2	15	0.80
3	10	0.20
4	5	0.06
total		4.91

4.91 ml. 0.0100 N NaOH ≡ 0.7365 mg. CH_3

% CH_3- calculated for 1 CH_3- 3.866%; found 3.856%.

CLEAVAGE OF VERBENALIN

ATTEMPTED PHOSPHORIC ACID HYDROLYSIS OF VERBENALIN

A sample of verbenalin, 0.5178 g., was dissolved by shaking in

40 ml. 1% phosphoric acid, and let stand 20 days, during which time the color became pale yellow. The solution was extracted for 48 hours with ether in a continuous two-phase liquid extractor. The ether extract was dried over anhydrous sodium sulfate and concentrated by distillation in vacuo, the last trace of ether being allowed to evaporate spontaneously. Some crystalline matter was formed, m.p. 178-180°. It was verbenalin.

ATTEMPTED HYDROLYSIS OF VERBENALIN BY MEANS OF AMBERLITE IRA-50

A solution of 300 mg. verbenalin in 25 ml. distilled water was stirred with 0.5 g. acid ion-exchange resin Amberlite IRA-50 at room temperature for 18 hours. After suction filtration, the filtrate was extracted with ether for 24 hours, the extract dried over anhydrous sodium sulfate, and concentrated in vacuo. Unchanged verbenalin was obtained.

EMULSIN HYDROLYSIS OF VERBENALIN

(a) Use of Citrate Buffer (I). A solution of 10.504 g. citric acid, U.S.P. in 90 ml. carbonate-free 1.111 N sodium hydroxide was diluted to 500 ml. with distilled water. Four volumes of this citrate solution were diluted with one volume of 0.1 N hydrochloric acid to yield a buffer of pH 4.65[7]. An emulsin suspension was prepared by mixing 5 g. commercial emulsin with 100 ml. recently boiled and cooled distilled water. To a solution of 2 g. verbenalin in 40 ml. distilled water, were added 40 ml. emulsin suspension and 40 ml. citrate buffer, and the mixture was allowed to stand at 38° for 120 hours. The mixture was then filtered and the orange-yellow filtrate after saturation with sodium chloride was extracted with ether in a

continuous two-phase liquid extractor for 48 hours. A second ether extraction was made for 20 hours and the extracts were combined and concentrated *in vacuo* after being dried over anhydrous sodium sulfate overnight. A cloudy amber syrup was obtained after the last ether had evaporated spontaneously. An attempt to crystallize the material from ethyl acetate was unsuccessful.

(b) Preparation of Emulsin from Hopkins' Cosmetic Meal[8]. One hundred and twenty grams Hopkins' cosmetic meal (defatted apricot kernel meal) were shaken in approximately 350 ml. 0.1 N ammonium hydroxide for 3 hours on a mechanical shaker. The material was centrifuged at approximately 2500 r.p.m. for 30 minutes and the supernatant decanted. The marc was shaken with 350 ml. 0.01 N ammonium hydroxide for 3 hours, centrifuged, and the supernatant decanted and added to the first extract. The mixture was acidified with 70 ml. 0.5 N acetic acid and set aside in the refrigerator for 24 hours. After centrifugation, the supernatant was diluted with 3 volumes 95% alcohol and set aside in the refrigerator. The precipitate was separated by decantation and suction filtration (a tedious procedure) and dried in a vacuum desiccator. The dried material was powdered in a mortar. For use, the powdered material was triturated with succeeding small volumes of distilled water to yield a uniformly dispersed colloidal solution. The yield of crude enzyme was about 3%.

(c) Use of Citrate Buffer (II). The experimental conditions of (a) were repeated substituting the enzyme prepared in (b) for the

commercial variety. Results were the same.

(d) Hydrolysis of Salicin to test the Activity of Emulsin. To a solution of 0.5 g. salicin in 20 ml. distilled water, was added 0.5 g. emulsin (as prepared in (b)) suspended in 20 ml. distilled water and 20 ml. citrate buffer. The mixture was placed in an oil-bath at 30° for 7 days. Qualitative tests with ferric chloride solution and Fehling's solution were negative.

Two grams of salicin were dissolved in 40 ml. distilled water and to this solution were added 40 ml. acetate buffer (pH 4.7) and 40 ml. of fine suspension of 2 g. emulsin in distilled water. The mixture was placed in an oil-bath at 30° for 7 days. The mixture was filtered and after saturation with sodium chloride, the filtrate was extracted with ether for 26 hours, and with fresh solvent for another 20 hours. The ether extracts were treated separately by drying over anhydrous sodium sulfate, decantation, distillation in vacuo and spontaneous evaporation. From the first ether extract was obtained 0.7025 g. saligenin (m.p. 86°, 81% of theory); from the second, a trace only was obtained.

A similar experiment involved the use of 2 g. dried material precipitated by the addition of acetic acid in the course of the preparation of emulsin, instead of the alcohol precipitate. Apparently much enzymatic activity resided in the acetate precipitate for saligenin was obtained, in 89% yield of theory.

(e) Use of Acetate Buffer (I). Two grams verbenalin were dissolved in 40 ml. water and to this solution were added 40 ml. acetate buffer (pH 4.7). Two grams dried emulsin were suspended in distilled

water and the mixture shaken for one hour on a mechanical shaker. This latter mixture was strained through gauze into the solution and the whole set aside in an oil-bath at 30° for 7 days. After saturation with sodium chloride, the hydrolysis filtrate was extracted with ether for 32 hours and with fresh solvent for a further 45 hours. Most of the solvent was removed by distillation in vacuo. A small quantity of crystalline material was obtained from the syrup remaining after all the ether had evaporated.

(f) Use of Acetate Buffer (II). A similar experiment utilized emulsin suspension prepared by trituration rather than by shaking and filtration. Results were the same.

(g) Preparation of Emulsin from Fresh Apricot Kernels. Two hundred grams of fresh apricot kernels were scalded with boiling distilled water for 2 minutes and the skins removed mechanically. The denuded kernels were dried with a stream of compressed air, then ground coarsely in a meat grinder. The coarse material was extracted with ether to defat it. Then it was dried and ground twice more with finer cutting edges, being extracted after each grinding with several quantities of ether. It was then let stand overnight in ether, after which the ether was removed by suction filtration and the residue was spread out thinly to dry. The dry residue (weighing about 100 g.) was shaken for 3 hours with 450 ml. 0.1 N ammonium hydroxide, and the extract removed by centrifugation. The marc was shaken with 500 ml. 0.01 N ammonium hydroxide for 3 hours, and the extract again removed by centrifuging. The supernatants were combined and precipitated with 35 ml. N acetic acid. After cent-

rifuging, the supernatant was diluted with 3 volumes of 95% alcohol and set aside in the refrigerator. The precipitate was removed by centrifuging and dried in a vacuum desiccator. The weight of enzyme preparation was 6 g. This enzymatic material was tested for β-glycosidase activity against salicin as in (d). The yield of saligenin was 99% of theory.

(h) Attempt to Prepare a Specific β-Glycosidase from defatted extracted Verbena stricta. The marc of Verbena stricta, left after removal of verbenalin by prolonged ether extraction, was passed through a No. 40 sieve to yield a fairly fine powder. One hundred grams of the sieved powder were mixed with 650 ml. distilled water and shaken on the mechanical shaker for 3 hours. Approximately 100 ml. of aqueous extract were decanted, and 500 ml. water added. The mixture was shaken another 3 hours, after which time the aqueous extract was expressed from the verbena and combined with the previous extract. The supernatant (about 400 ml.) from centrifugation of the combined extracts was treated with 100 ml. 0.2 M sodium acetate and 100 ml. 0.2 M acetic acid, and allowed to stand 2 hours before it was centrifuged to remove a small amount of precipitate. The supernatant (about 600 ml.) was cooled to below 10° and 300 ml. similarly cooled 95% alcohol were added gradually with stirring. A small amount of solid material settled out and this was removed by centrifugation. The concentration of alcohol in the supernatant was increased to about 60%, and the aqueous-alcoholic solution placed in the refrigerator overnight. The small amount of solid material obtained was added to that previously removed and the mixture dried

in a vacuum desiccator. The total weight was less than 1 g.

To test the enzymatic activity of this preparation, 2 g. verbenalin were dissolved in 40 ml. acetate buffer (pH 4.7) and a suspension of 0.5 g. of the foregoing material in 10 ml. distilled water added. The mixture was placed in an oil-bath at 30° for 7 days. The supernatant, after centrifugation, was extracted with ether for 20 hours, dried over anhydrous sodium sulfate, and concentrated by distillation in vacuo. After the last traces of ether had evaporated there were obtained in good yield, crystals of what proved to be verbenalin (m.p. 180-1°).

(i) Attempted Unbuffered Hydrolysis of Verbenalin with Emulsin. Two grams of verbenalin were dissolved in 20 ml. distilled water by shaking. To this solution was added 0.5 g. emulsin (as prepared in (g)) suspended by trituration in 20 ml. distilled water. The mixture was placed for 5 days in an oil-bath kept at 30°, at the end of which time it was centrifuged and the supernatant extracted with ether for 25 hours and with fresh solvent for another 28 hours. The extracts were combined and after drying over anhydrous sodium sulfate, were concentrated under reduced pressure. Crystals were obtained as the last ether evaporated, m.p. 133-7°, weight 35 mg. The crystals were recrystallized twice from ethyl acetate, m.p. 138-9°. A solution of 3.9 mg. of these crystals in 50 ml. absolute alcohol was diluted with four volumes of absolute alcohol, and an ultraviolet absorption spectrum run. In neutral solution, maximum absorption was obtained at 239 mμ ($\varepsilon_{max.}$ = 7500). When a mole equivalent of alkali was added the maximum absorption

was shifted to 272 mμ, and back to approximately 240 mμ when the alkali was neutralized.

(j) Attempted Hydrolysis of Verbenalin in Phosphate Buffer Solution. Two grams of verbenalin were dissolved in 40 ml. phosphate buffer (pH 7.3) and the solution suspended in an oil-bath at 30° for 24 hours. The solution became yellow within 5 min. and cloudy within 15 min. However, when no further color change took place in 24 hours, 3.14 g. disodium hydrogen phosphate dodecahydrate were added to the solution to change the pH to about 8. The solution was re-suspended in the oil-bath at 30° for 11 days. The residue was then extracted in a continuous two-phase liquid extractor for 31 hours with ether, and with fresh solvent for a further 21 hours. Since there was a little white solid matter suspended in the ether extracts (combined), drying by means of anhydrous sodium sulfate was omitted, and the extract was concentrated by distillation in vacuo, the last of the solvent being allowed to evaporate spontaneously. The small amount of crystalline material which separated from the syrupy concentrate was recrystallized from ethyl acetate and dried on a porous plate, m.p. 179-81°. It was apparently verbenalin.

(k) Emulsin Hydrolysis of Verbenalin by the method of Asano, Ueno and Tamaki[9]. Three grams of verbenalin were dissolved in 100 ml. distilled water and to this solution was added a suspension (by trituration) of 1 g. emulsin (alcohol precipitate) in 50 ml. distilled water. This was saturated with carbon dioxide, generated from finely powdered dry ice in a side-arm flask, a delivery arm of rubber tubing and a burette tip delivering the gas to the solution, and a

one-hole stopper permitting the use of pressure in delivering the gas. The mixture was stoppered and stored in the refrigerator for 7 days. The suspension was shaken periodically during the 7 days and at the end of that time, it was centrifuged. The supernatant was extracted then with ether in a continuous two-phase liquid extractor for 28 1/2 hours and with fresh solvent for a further 27 1/2 hours, and again with more fresh solvent for 70 hours. The first ether extract was concentrated without drying over anhydrous sodium sulfate, thus leaving an aqueous residue when the ether was removed by distillation in vacuo. The aqueous material was concentrated by distillation at 60° and 10 mm., then set aside in the refrigerator for 48 hours. Solid matter was deposited, weight 1.01 g., m.p. 123-6° (not sharp). The solid material, which could hardly be termed crystalline, was recrystallized from ethyl acetate twice, by which means there were obtained crystals, m.p. 132-4°, weight 310 mg. Small amounts of crystalline verbenalol were used to seed the ethyl acetate residues. The total weight obtained by recrystallization and seeding was 750 mg. (30.4% of theory). The second and third ether extracts were combined and from the aqueous residue obtained by distillation in vacuo, no verbenalol was obtained even after concentration and seeding.

A second experiment, run simultaneously with the foregoing hydrolysis, involved the addition of a suspension of 0.5 g. commercial emulsin in 25 ml. distilled water, to a solution of 1.5 g. verbenalin in 50 ml. distilled water, saturation of the mixture with carbon dioxide and the setting aside of the mixture in the refrigerator for

7 days. The suspension was centrifuged and the supernatant extracted with ether in a continuous two-phase liquid extractor for 42 hours and with fresh solvent for a further 52 hours. An accident prevented further work on this experiment. A similar experiment using 3 g. verbenalin and 1 g. commercial emulsin, treated similarly and extracted for 18 and 23 hour periods respectively yielded a total of 206 mg. verbenalol, melting over a wide range. Consequently, the commercial emulsin was abandoned in favor of the enzyme prepared from Hopkins' Cosmetic Meal.

Four flasks each containing 3 g. verbenalin and 1 g. emulsin in 150 ml. distilled water saturated with carbon dioxide were centrifuged after being stored 7 days in the refrigerator, extracted with ether for 16 1/2 hours and 15 hours respectively (extracted in 2 300 ml. quantities), dried over anhydrous sodium sulfate, concentrated in vacuo, and the last ether allowed to evaporate spontaneously. From the combined extracts there was obtained a total of 6.3 g. impure verbenalol (72.2% of theory). This method was adopted for all future extractions after hydrolysis of verbenalin with emulsin.

MANNICH METHOD OF HYDROLYSIS[10]

(a) A sample of 0.3994 g. verbenalin was placed in a 250 ml. iodine flask and 20 ml. dry acetone (dried over calcium chloride) and 0.2 ml. concentrated hydrochloric acid were added. The flask was set aside at room temperature for 24 days. The mixture began to turn yellow within 24 hours but not all the verbenalin had

dissolved until the solution was brown in 72 hours. The acetone solution was reduced to a volume of 5 ml. by distillation in vacuo on the 24th day. When an equal volume of distilled water was added (the purpose was to be able to remove all acetone leaving the product(s) of reaction in aqueous solution), a dark-brown tarry material separated out. It was acetone-soluble. After the mixture was concentrated to 5 ml. to remove all the acetone, it was filtered and the yellow filtrate diluted to 25 ml., then extracted with ether for 24 hours. The ether extract was dried over anhydrous sodium sulfate and the ether removed by distillation in vacuo and by spontaneous evaporation. An amber syrupy matter was obtained, and attempts to crystallize it from chloroform and dioxan were unsuccessful.

(b) A sample of 0.4116 g. verbenalin was placed in a 250 ml. iodine flask and 20 ml. dry acetone containing 2.5% dry hydrogen chloride (dried over concentrated sulfuric acid) were added. The flask was set aside for 14 days. The solution began to turn brown on the second day and not until the fourth day had all the glycoside dissolved. At the end of 14 days, the solution was concentrated to 10 ml., 10 ml. distilled water added and the mixture heated to remove all the acetone. An odor like that of ethyl acetate was noted. The aqueous residue was then extracted with ether for 25 hours, much of the dark-brown material being soluble immediately in the solvent. The ether extract was dried over anhydrous sodium sulfate before being concentrated to a dark thick syrup. Attempts to crystallize this material from chloroform and dioxan were not successful. The syrupy material was treated with 5 ml. distilled water and the dark oily

matter which separated was filtered out. The yellow filtrate was again extracted with ether for 9 hours. The extractor broke and the aqueous material being extracted was lost but the ether extract was saved. It was dried over anhydrous sodium sulfate and concentrated in vacuo, but nothing crystalline was obtained.

METHANOLYSIS OF VERBENALIN[11,12,13,14]

Preparation of Absolute Methanol - Commercial absolute methanol was refluxed with magnesium turnings (10 g./l.) until all the metal had reacted. The methanol was then distilled, the initial and final tenths being discarded. The absolute methanol was stored in small full bottles.

Attempted Methanolysis of Verbenalin (I) - A solution of 0.3906 g. verbenalin in 10 ml. absolute methanol, containing 2.5% hydrogen chloride (dried over concentrated sulfuric acid) was placed in a glass-stoppered Erlenmeyer flask and set aside in a constant-temperature (38°) bath for 14 days. Within 24 hours, the glycoside was all dissolved and the solution developed a yellow color within 3 days. On the eighth day, 0.5 ml. absolute methanol containing 12.5% hydrogen chloride, was added. At the end of 14 days, the flask was heated briefly to remove the hydrogen chloride and set aside in the refrigerator. After 96 hours, during which nothing crystalline had appeared, the volume was reduced to 3-4 ml., 10 ml. distilled water added and the solution heated to remove all the methanol. This aqueous solution was extracted with ether in a continuous two-phase liquid extractor for 12 hours. The ether extract was dried over anhydrous sodium sulfate, concentrated by distillation in vacuo, and

the last of the ether allowed to evaporate spontaneously. An orange syrupy residue was obtained. The syrup was dissolved in hot ethyl acetate, digested with Norite, filtered and concentrated. No crystals were obtained. An attempt to crystallize the material from chloroform was also unsuccessful.

Attempted Methanolysis of Verbenalin (II) - A solution of 2 g. verbenalin in 60 ml. absolute methanol, containing 2.5% dry hydrogen chloride (dried over concentrated sulfuric acid) was set aside in a constant-temperature (38°) bath for 6 days. At the end of that time, the solution, now deep yellow in color, was divided into two portions. The first half was heated to remove hydrogen chloride and to reduce the volume to 5 ml. Thirty mils of distilled water were added and the solution heated to remove all the methanol. The yellow, cloudy aqueous solution was extracted with ethyl acetate for 26 hours, the extract dried over anhydrous sodium sulfate and most of the solvent removed by distillation in vacuo. The second half was maintained at 38° for another 8 days, then treated in the same manner as was the first half. Nothing crystalline was obtained from either portion.

Preparation of 0.5 N Barium Methoxide - Twenty grams of barium oxide were refluxed with 40 ml. absolute methanol for 3 hours. The suspension was filtered, and the precipitate washed with absolute methanol to make the volume up to 200 ml.

Attempted Methanolysis of Verbenalin using Barium Methoxide - A sample of 0.5139 g. recrystallized verbenalin was dissolved in 50 ml. absolute methanol and its optical rotation observed. To this solution was added 1 ml. 0.5 N barium methoxide and polarimetric readings

taken at the times shown in the accompanying table.

TABLE I

Polarimetric Readings in Barium Methoxide Methanolysis of Verbenalin.

TIME	READINGS	TIME	READINGS
0 mins.	1.33; 1.32	2 1/2 hrs.	1.36; 1.40
5 mins.	1.10; 1.06	3 hrs.	1.45; 1.40
10 mins.	0.56; 0.57	3 1/2 hrs.	1.47; 1.48
15 mins.	1.47; 1.49	4 hrs.	1.65; 1.72
20 mins.	1.53; 1.50	4 1/2 hrs.	1.75; 1.74
25 mins.	1.62; 1.66	5 hrs.	1.89; 1.90
30 mins.	1.67; 1.66	5 1/2 hrs.	1.90; 1.91
40 mins.	1.58; 1.56	6 hrs.	1.93; 1.92
50 mins.	1.56; 1.54	10 hrs.	2.08; 2.12
1 hour	1.57; 1.54	22 hrs.	1.93; 1.97
1 1/2 hrs.	1.79; 1.81	24 hrs.	1.94; 1.98
2 hrs.	1.50; 1.49	30 hrs.	1.90; 1.92

The barium ion was precipitated from the deep orange solution by the dropwise addition of 3N sulfuric acid. The solution was filtered and concentrated by distillation in vacuo. This concentrate was stored in the refrigerator for 11 days during which time nothing crystallized. It was then dissolved in distilled water and extracted with ether for 18 hours in a continuous two-phase liquid extractor. The ether extract was dried over anhydrous sodium sulfate, then most of the ether was removed by distillation in vacuo, and the remainder allowed to evaporate spontaneously. Attempts

were made to crystallize the brown syrupy material from ethyl acetate, ethanol, dioxan and chloroform without success.

ACETYLATED VERBENALIN

Preparation

The method of Hoffmann[15] was used in acetylating verbenalin. One gram of verbenalin was dissolved in 10 ml. anhydrous pyridine and to the solution was added 1.6 ml. acetic anhydride. The flask was stoppered with a calcium chloride tube and let stand at room temperature for 17 hours. The mixture was thoroughly cooled in an ice-bath before 75 ml. 5% sulfuric acid were added slowly with constant stirring. A brown oil formed at first but continued stirring caused it to solidify. The mixture was set aside in the refrigerator for 3 hours, after which it was possible to decant the clear yellow supernatant. The gummy brown mass was dissolved in absolute ethanol, digested with Norite, filtered, concentrated and set aside. White rosettes formed soon. The crystals were filtered off and washed on the filter with cold distilled water, then allowed to dry. Weight 975 mg., m.p. 131-2° (yield 68.0% of theory, based on a tetraacetyl derivative). In subsequent acetylations, the yield was increased to over 70%. The material was purified for analysis by treatment with Norite again and several recrystallizations from ethanol. Analysis data; 3.938 mg. sample yielded 7.788 mg. CO_2 and 2.096 mg. H_2O; C = 53.97%, H = 5.96%; calculated values for a tetraacetyl derivative, C = 53.95%, H = 5.80% ($C_{25}H_{32}O_{14}$); calculated values for a pentaacetyl derivative, C = 54.18%, H = 5.73%.

Attempted Enzymatic Hydrolysis of Acetylated Verbenalin

A sample of 500 mg. acetylated verbenalin was suspended in 70 ml. distilled water by trituration and shaking, and to this suspension was added a suspension of 0.5 g. emulsin (alcohol precipitate from fresh apricot kernels) in 5 ml. distilled water. The mixture was saturated with carbon dioxide gas and set aside in the refrigerator (with frequent shaking) for 7 days. Since no color had developed in this time, the mixture was let stand at room temperature for 3 days more. Then it was shaken out with much shaking with four 35 ml. portions of ether. The ether extract was concentrated in vacuo and then let evaporate to dryness spontaneously. White crystalline material, m.p. 87-99° was deposited. Several recrystallizations raised the melting point to 128-130° and a mixed melting point with an authentic sample of acetylated verbenalin was 124-8°. This attempted method of hydrolysis was abandoned.

Determination of the Alkali-Sensitivity of Acetylated Verbenalin

Fifty milligrams of acetylated verbenalin were dissolved by shaking in 4 ml. 5% alcoholic potassium hydroxide. The solution began to turn a deep yellow color immediately and was also cloudy within 30 minutes. It was decided not to attempt the use of alkaline hydrolysis.

Iodoform Test on Acetylated Verbenalin

One hundred milligrams of acetylated verbenalin were dissolved with the aid of a little heat in 4 ml. 2N aqueous potassium hydroxide and to this solution there was added gradually and with shaking over

a period of 20 mins. 6 ml. 1N iodine. The mixture was warmed on the steam-bath at 55-60° for 10 minutes and then refrigerated for 1 hour. The yellow precipitate was filtered off, washed with cold distilled water and dried, m.p. 116-117.5°. A mixed melting point with an authentic sample of iodoform showed no depression.

Attempted Hydrolysis of Acetylated Verbenalin by means of a Mixture of Perchloric Acid, Acetic Anhydride and Glacial Acetic Acid[16]

A transforming solution was prepared by mixing 70 ml. 95% acetic anhydride, 30 ml. 99.5% acetic acid and 1 ml. 60% perchloric acid. One gram of acetylated verbenalin was dissolved in 15 ml. of the transforming mixture at room temperature. Immediately it turned yellow and within 15 minutes it was green in color. In 24 hours, it was black. No polarimetric reading was possible. Test tube experiments were conducted to determine the action of the transforming mixture on verbenalin and verbenalol. The verbenalin solution turned green very soon while the solution of verbenalol in the transforming mixture became brown then brownish green in color. After 48 hours, the solution of acetylated verbenalin in the transforming solution was poured into a large volume of cold 5% aqueous sodium bicarbonate, a sufficient volume to effect complete neutralization. At this stage, the mixture was of a dirty orange-brown color with dark oily blobs in it. The latter were collected and with the aid of heat, dissolved in carbon tetrachloride. The aqueous residue was extracted several times with carbon tetrachloride by shaking, and the combined carbon tetrachloride solutions were dried over anhydrous sodium sulfate.

The solvent was removed to dryness (a hard brown syrupy mass) by distillation under reduced pressure. The residue was taken up in 95% ethanol, treated with Norite, filtered, concentrated and set aside. Nothing crystalline was obtained.

Attempted Hydrolysis of Acetylated Verbenalin by means of a Mixture of Sulfuric Acid, Acetic Anhydride and Glacial Acetic Acid.

A transforming mixture was prepared by mixing 70 ml. 95% acetic anhydride, 30 ml. 99.5% acetic acid and 2 ml. 95% sulfuric acid. One-half gram of acetylated verbenalin was dissolved in 7.5 ml. of this mixture at room temperature. It became yellowish-green immediately and within 3 hours was black. At the end of 48 hours, the mixture was poured slowly into 500 ml. cold aqueous 5% sodium bicarbonate. After effervescence had ceased, the dark brown solution was extracted with four 200 ml. portions of carbon tetrachloride. This extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The dry residue was taken up in ethanol. The aqueous residue of carbon tetrachloride extraction was extracted with ether in a continuous two-phase liquid extractor for 8 hours. Drying was omitted so that removal of the ether left the extract in the small amount of water carried over in the continuous extraction. This water was removed by azeotropic distillation with benzene and absolute ethanol, the extract being left in absolute ethanol. This solution was then combined with the previous ethanol solution and concentrated in volume. When nothing crystalline appeared it was concentrated again. Upon standing several days

under refrigeration, crystalline material appeared in the flask. It was filtered off, dried and its melting point determined as 102-7°. It was recrystallized twice from ethanol-water mixtures, dried, m.p. 110-110.5°. A mixed melting point with an authentic sample of glucose α-pentaacetate showed no depression. No other product was obtained from this reaction.

Phenylhydrazone of Acetylated Verbenalin.

One hundred milligrams of acetylated verbenalin and 50 mg. phenylhydrazine hydrochloride were dissolved by warming in 10 drops pyridine and let stand for 1 hour. Water was added in excess and the mixture was rubbed on the sides of the flask by means of a glass stirring-rod. An amorphous yellow precipitate formed and it was washed with cold alcohol. The filtrate was quite dark in color, and the residue not readily soluble in cold alcohol was considerably lighter in color. It was dissolved in hot alcohol, water added to the clouding point and the solution set aside in the refrigerator. Nothing crystalline was obtained.

Oxime of Acetylated Verbenalin.

One hundred milligrams of acetylated verbenalin and 20 mg. hydroxylamine hydrochloride were dissolved in 2.5 ml. pyridine and heated on a hot plate until the pyridine had been removed at which time water was added in excess and the mixture placed in the freezer compartment of the refrigerator. A small amount of crystals formed and were removed and dried on a sintered glass funnel. After two recrystallizations from ethanol and water, m.p. 170-3°. (Literature[17] m.p.

175-6°). The quantity obtained was too small for nitrogen analysis.

Dinitrophenylhydrazone of Acetylated Verbenalin.

One hundred milligrams of acetylated verbenalin and 70 mg. 2,4-dinitrophenylhydrazine were dissolved in 10 drops of pyridine containing 2 drops of concentrated hydrochloric acid by warming on a steam bath. The mixture was set aside for one hour then diluted with much water. The yellow amorphous precipitate was collected on a filter, and dissolved off the filter with hot absolute alcohol. Water was added to the clouding point and the 2,4-dinitrophenylhydrazone was obtained as lemon-yellow needles, m.p. 256-8°(decomp.). After treatment with Norite and several recrystallizations from alcohol-water, the compound was submitted for analysis, in the hope that the nitrogen value might indicate if this were the dinitrophenylhydrazone of a tetraacetyl or pentaacetyl derivative of verbenalin. The nitrogen value found was 7.15% (calculated nitrogen value for pentaacetyl compound, $C_{33}H_{38}O_{18}N_4$, 7.20%; for tetraacetyl derivative, $C_{31}H_{36}O_{17}N_4$, 7.61%). The data best fit a pentaacetyl compound.

Qualitative Tests on Verbenalin and Acetylated Verbenalin.

Fehling's Test Solution - To a solution of 50 mg. verbenalin in distilled water, 2 ml. Fehling's solution were added, and the mixture was heated to boiling. A sample of acetylated verbenalin was treated similarly. The former was immediately positive. The latter was very slowly positive.

Tollens' Silver Mirror Test - To 2 ml. freshly-prepared Tollens' reagent, was added an aqueous solution of 25 mg. verbenalin. The

test was positive almost immediately. A similar test of acetylated verbenalin was only slowly positive.

Legal's (Sodium Nitroprusside) Test[18] - A few milligrams of verbenalin were dissolved in 2 ml. pyridine and to this solution were added a few drops of freshly-prepared 5% aqueous solution of sodium nitroprusside, made alkaline with sodium hydroxide. A deep red color was indicative of a positive test. A similar test of acetylated verbenalin was negative.

Determination of Acetyl Groups in Acetylated Verbenalin[6]

A sample of 0.0077 g. acetylated verbenalin was placed in the three-necked flask of a Kuhn-Roth Microacyl Apparatus and 1 ml. 1 N aqueous sodium hydroxide added as a hydrolysing agent. The mixture was refluxed 1 1/2 hours, cooled, and the condenser, funnel and inlet tube washed with a total of 7-8 ml. distilled water. Dilute solution of hydrazine (2 ml.) was added dropwise and the solution cooled again. As a neutralizing agent, 1 ml. dilute sulfuric acid was added, and the mixture was distilled. Five fractions, of 15, 15, 10, 5, and 5 ml. respectively were caught, the volume in the distilling flask being replenished periodically so that it did not become less than 3 ml. at any time. Each sample for titration was heated briefly to the boiling point after the addition of 10 mg. barium chloride, cooled and titrated with approximately 0.01 N aqueous sodium hydroxide.

Sample No.	Size of Sample	Mls. 0.009968 N Alkali
1	15 ml.	8.32 ml.
2	15 ml.	1.35 ml.
3	10 ml.	0.19 ml.
4	5 ml.	0.10 ml.
5	5 ml.	0.06 ml.
		10.02 ml.

This amount of alkali represents 0.0043 g. acetyl (CH_3CO-). In the case of a tetraacetyl derivative, the acetyl value for a sample of 0.0077 g. should be 0.0023 g., while for the pentaacetyl derivative, it should be 0.0028 g. The value obtained then is extremely high, and represents the many difficulties (see Page 16) encountered in microacetyl determinations.

REDUCTION OF VERBENALIN

HYDROGENATION OF VERBENALIN WITH PLATINUM OXIDE CATALYST

A sample of 10.0037 g. recrystallized verbenalin was dissolved in 120 ml. distilled water and to this solution were added 2 g. platinum oxide catalyst. The mixture was hydrogenated over 2 1/2 hours in the hydrogenation apparatus, the following data being recorded.

Time	Hydrogen Pressure (lbs.)
10.35 A.M.	40.0
10.41	39.0
10.49	38.0
10.53	37.0
11.00	35.0
11.10	33.0

Time	Hydrogen Pressure (lbs.)
11.30 A.M.	31.0
11.45	30.0
12.35 P.M.	28.0
1.10	27.0

Temperature - 24°C.
Sample Weight - 10.0037 g. (0.02577 mole)
Desired Hydrogen Uptake - 0.0515 mole (2 moles per mole)
Pressure drop equivalent - 12.15 lbs.
Observed pressure drop - 13.0 lbs.

The platinum oxide was removed by filtration through a fine sintered glass funnel. It was very slow. The material on the funnel was washed with hot distilled water and the washings added to the filtrate. The filtrate and washings were concentrated to about 30 ml. under reduced pressure, then set aside in the refrigerator.

The white crystalline material which formed, was filtered off and washed with cold water, then dried, m.p. 68-71°. The mother liquor deposited more crystals which were filtered off, dried, washed with ether, and dried in a vacuum desiccator for 24 hours, m.p. 83-5°. Total weight - 3.65 g. Ethyl acetate was tried as a recrystallization solvent, but the material was obtained as a crystalline mass, not as discrete crystals. The mass was filtered off by suction, dried, then washed through the filter by hot alcohol. The alcoholic solution was concentrated on a steam-bath, and then allowed to evaporate spontaneously. A syrupy residue resulted.

The syrupy material was dissolved in hot chloroform, and the solvent concentrated. A spongy solid mass was deposited. The mass was dissolved in 5 ml. hot absolute alcohol and while hot, was

diluted with 50 ml. hot chloroform. Nothing crystalline was obtained.

The hard syrupy material from chloroform-alcohol treatment was treated with boiling isopropyl ether, which treatment caused it to solidify. The mass was broken up and filtered with suction. It was rinsed with isopropyl ether and dried. In a melting point determination, a little moisture appeared at 80°, but the bulk of the material melted in the range 130-145°. The material was taken up in ethyl acetate containing 20% alcohol, concentrated and set aside to crystallize. Small discrete crystals appeared and were dried, m.p. 137-140° (not sharp). The material was again crystallized from ethyl acetate and alcohol when it appeared as large rosettes, m.p. 152-4°. In view of the difficulty experienced in the fractional crystallization of an obvious mixture, it was decided to submit the crude crystalline mass to hydrolysis by the method of Asano, Ueno and Tamaki[9], in the hope of obtaining a crystalline reduced aglycone.

EMULSIN HYDROLYSIS OF THE CRUDE PLATINUM OXIDE REDUCTION PRODUCT

The syrupy materials were combined (total weight about 3 g.) and dissolved in 100 ml. distilled water. To this solution was added in small portions a suspension of 1 g. emulsin (alcohol-precipitate) in 50 ml. distilled water. The mixture was saturated with carbon dioxide and set aside in the refrigerator for 7 days. The mixture was then centrifuged and the supernatant extracted in a separatory funnel with six 25 ml. portions of ether. The ether extracts were combined, and concentrated, the last ether being allowed to evaporate spontaneously. Crude white crystals were obtained. They were

dissolved in alcohol, treated with Norite, filtered and concentrated. After several more solvent concentrations, an oil was obtained which could not be made to crystallize by scratching. Thereupon, the solvent was removed completely, and the oil was taken up in about 30 ml. alcohol-benzene (1:3), concentrated and set aside. After several such concentrations, crystals appeared and were filtered off and dried, m.p. 115-9°, (weight 560 mg. Calculating the syrupy starting material as 100% this represented 31.8% of theory). Some material was retained for seeding, and the remainder recrystallized twice by dissolving it in the minimum amount of hot absolute alcohol, and adding benzene to the clouding point. The dried crystals thus obtained after being refrigerated 10 days melted at 122-3° (sharp). The ultraviolet and infrared absorption spectra of this sample of tetrahydroverbenalol were determined and are reported in the appropriate sections of this work.

C-METHYL DETERMINATION OF REDUCED AGLYCONE, TETRAHYDROVERBENALOL

A C-methyl determination was carried out on tetrahydroverbenalol (sample weight 0.0158 g.) as was done on verbenalin[6] (see Page 70) and the following data were recorded.

Fraction	ml.	ml. 0.01127 N NaOH
1	20	4.84
2	15	1.00
3	5	0.06
4	5	0.05
5	5	0.03
total		5.98

5.98 ml. 0.01127 N NaOH ≡ 6.74 ml. 0.01 N NaOH ≡ 1.012 mg. CH_3

% CH_3 calculated for 1 CH_3- 6.52%; found 6.405%.

HYDROGENATION OF VERBENALIN WITH PALLADIUM-CARBON CATALYST

A sample of 5 g. verbenalin was dissolved in 20 ml. distilled water and 40 ml. alcohol. To this solution was added 50 ml. 1% aqueous palladous chloride and 2 g. activated charcoal (Norite washed with nitric acid). The mixture was hydrogenated for 26 hours with the following data being recorded.

Time	Hydrogen Pressure (lbs.)
8.14 A.M.	24.9
8.16	21.5
8.20	21.0
8.55	18.9
9.30	16.0
10.09	13.1
11.00	12.5
11.01	25.2 (raised)
11.40	24.8
1.10 P.M.	24.1
2.15	23.9
3.40	23.3
4.20	23.1
4.20 - 0.25 g. Palladous chloride added.	
4.25	25.0 (raised)
4.27	23.3
4.30	22.9
6.07	13.3
6.22	12.3
6.58	10.0
7.22	8.5
8.00	6.4
8.15	5.5
10.00	0.0
10.01	25.00 (raised)
10.10	24.2
8.15 A.M.	2.5
8.16	25.0 (raised)
8.45	24.2
9.30	23.2
10.10	22.3

Sample weight - 5 g. (0.013 mole)
Desired Hydrogen Uptake ≡ 54.4 lbs. pressure drop (2 moles)
Observed pressure drop - 58.7 lbs.

The reaction mixture was centrifuged and the residue filtered off was washed with distilled water and centrifuged again. To the filtrate and washings was added a calculated amount (1.33 g.) barium hydroxide octahydrate, dissolved in 30 ml. distilled water. Then the whole mixture was concentrated in vacuo to dryness after the addition of one part of alcohol and four of benzene (to remove water). The dried material was digested with absolute ethanol, filtered, treated with Norite, filtered and set aside to evaporate spontaneously.

In 96 hours, there appeared small discrete crystals which were filtered off and dried, m.p. 168-176°. These crystals were dissolved in hot alcohol, concentrated and set aside. Within 24 hours, crystals were obtained, which when filtered off and dried, melted at 176-9° (fairly sharp). A mixed melting point with an authentic specimen of verbenalin showed almost no depression. From the mother liquor of this recrystallization, there was obtained some 10 days later, 0.8 g. of crystalline material which after drying, melted quite sharply at 200-2°. An infrared absorption spectrum of this material is reported elsewhere in this thesis.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF VERBENALIN (I)

The residues of reduction from the titrations of verbenalin by lithium aluminum hydride (see P. 69) were combined and centrifuged. The precipitate was suspended in distilled water and 5% aqueous sulfuric acid added to pH 3.9 without the precipitate dissolving completely. The suspension was then saturated with sodium chloride

and the suspension extracted with ether in the continuous two-phase liquid extractor for 42 hours. The ether extract was dried over anhydrous sodium sulfate for 24 hours, then reduced in volume to approximately 5 ml., the rest of the solvent being allowed to evaporate spontaneously. There was obtained about 3 ml. of amber colored liquid. This material was taken up in 20 ml. dioxan-benzene (1:9), then concentrated and set aside. Nothing crystalline was obtained. The remainder of the dioxan-benzene was removed by distillation in vacuo. The residue was dissolved in excess methanol, treated with Norite, filtered, concentrated and set aside. Nothing crystallized. Nothing crystallized from an acetone solution either.

The acetone solution was heated to the boiling point and isopropyl ether added dropwise. After the addition of 125 ml. solvent the solution separated into two fractions. The brown oil was freed of isopropyl ether by distillation in vacuo after the supernatant clear solvent was decanted. The syrupy oil was dissolved in 5 ml. absolute ethanol and 50 ml. chloroform added. The solution was then boiled but nothing precipitated out. The remaining solvent was removed by distillation in vacuo and taken up in ethyl acetate to which water was added to the clouding point. Nothing crystallized. The solvent was removed by distillation in vacuo and the residue was dissolved in a mixture of nine parts of benzene and one part of absolute ethanol. It was concentrated and set aside, but nothing crystallized.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF VERBENALIN (II)

A sample of lithium aluminum hydride was powdered finely and 4 g. were refluxed with 500 ml. redistilled tetrahydrofuran in a one liter ST round bottom flask for 3 hours. The mixture was then centrifuged for 1 hour and the supernatant reserved for use as lithium aluminum hydride reagent. In a three-necked one liter round bottom flask were placed 500 ml. lithium aluminum hydride reagent. The center well of the flask was reserved for a stirrer. One side well was stoppered. In the other side well was inserted a Soxhlet apparatus topped with a reflux condenser and a calcium chloride tube. In the thimble in the Soxhlet was placed a sample of 5 g. verbenalin. The flask was heated in a mantle with constant stirring. The Soxhlet was observed to drain in 7 3/4 minutes. Very little glycoside was dissolved by the refluxing tetrahydrofuran in this manner in 72 hours, so a hole was drilled in the Soxhlet beneath the thimble, after which the glycoside was all carried in solution into the reaction flask in 34 hours refluxing.

Distilled water was then added through the condenser to destroy the excess reagent. The suspension was washed into a beaker and then acidified with N/1 sulfuric acid to pH 3.8. Since much material was still undissolved, the mixture was centrifuged. The mixture separated into three parts, a white precipitate and two solvent layers. The precipitate was suspended in distilled water and acidified with N/1 sulfuric acid, until the precipitate had all dissolved at which time the pH of the solution was 1.5, and

the volume was approximately 150 ml. This aqueous solution was distilled at 10 mm. pressure and 75° to reduce the volume to 50 ml. This residue was extracted with ether in a continuous two-phase liquid extractor for 24 hours after it had been saturated with sodium chloride. The extract was then dried over anhydrous sodium sulfate. During this drying, a second extraction was made for 36 1/2 hours with fresh ether. Each extract was reduced in volume separately to about 10 ml. by distillation in vacuo, the remainder of the ether being allowed to evaporate spontaneously. In each case, an amber syrupy material was obtained. The two extracts were combined, a total of about 3 ml., and dissolved by heating in 20 ml. benzene-dioxan (9:1) and set aside. Nothing crystalline was obtained and the solvent was all removed by distillation in vacuo. Absolute methanol was added to the residue and solution effected by heating. It was concentrated and distilled water added to the clouding point. Nothing crystallized so the solvent was removed by distillation in vacuo.

ACETYLATION OF LITHIUM ALUMINUM HYDRIDE REDUCTION MIXTURE OF VERBENALIN

One-half milliliter of the syrupy residue of lithium aluminum hydride reduction of verbenalin was placed in a 50 ml. round-bottom flask and to it was added 1-1.5 ml. acetic anhydride and 3-4 drops of pyridine. The mixture was refluxed 2 hours. The addition of ether to the mixture precipitated a hard syrupy dark brown mass on the walls of the flask. When the ether was allowed to evaporate,

the mixture became homogeneous again. Distilled water was added to the mixture and a dark brown material oiled out. The aqueous material was decanted and the oily residue was taken up in methanol, concentrated to about 15 ml., water added to the clouding point and the solution set aside. Nothing crystallized, even after further concentration. The solvent was removed by distillation in vacuo and the residue taken up in ethyl acetate, concentrated and set aside. Nothing crystallized.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF VERBENALIN (III)

Using the apparatus previously described, 10 g. verbenalin were reduced by means of 8 g. lithium aluminum hydride dissolved by refluxing (and centrifuged) in 550 ml. tetrahydrofuran. All the glycoside was dissolved by the refluxing tetrahydrofuran and carried into the flask for reaction within 2 1/2 hours. The mixture was centrifuged and the precipitate was suspended in distilled water. Sufficient dilute sulfuric acid was added to bring the precipitate completely into solution. Then 4% aqueous sodium hydroxide was added to pH 7 and the precipitate of aluminum hydroxide was removed by centrifugation. The aluminum hydroxide was twice shaken with distilled water on the mechanical shaker, centrifuged and the washings added to the aqueous solution. The aqueous solution (about 1500 ml.) was concentrated in vacuo to about 75 ml., at which time it consisted of a small amount of white solid (giving a positive flame test for lithium), a dark brown oil and light brown aqueous liquid. The whole mixture was rinsed into a continuous two-phase liquid extractor and extracted with two portions of ether for 31 1/2 hours and 50 hours

respectively. From the first ether extract there separated a brown oil. Consequently drying over anhydrous sodium sulfate was omitted and the solvent was concentrated to about 15 ml. and allowed to evaporate spontaneously. Some small crystals were deposited and these proved by flame test to be inorganic. The second extract likewise deposited inorganic crystals. Since the ether extracts were not dried over anhydrous sodium sulfate, the material extracted remained in the water carried over in the extraction procedure. The aqueous material then was passed through a column of Amberlite Ion Exchange Resin IR-120 in order to remove aluminum, sodium and lithium. The effluent (pH 1.3) was then washed through a column of Amberlite Ion Exchange Resin IRA-400 and the aqueous effluent concentrated to a volume of 10 ml. by distillation in vacuo. Attempts were made to crystallize this material from ethyl acetate, chloroform, acetone and from isopropyl ether, but nothing crystalline was obtained.

REDUCTIVE ACETYLATION OF VERBENALIN

Two grams of verbenalin were suspended in 40 ml. acetic anhydride and 2 g. zinc dust and 10 drops triethylamine added. This mixture was heated slightly when no reaction was apparent at room temperature. The color turned yellow then and it was refluxed for 5 minutes. A total of 25 ml. hot acetic acid was added in three portions and the mixture filtered by gravity. The filtrate was kept at the boiling point and water added dropwise to the clouding point. It was set aside in the refrigerator. Nothing crystallized. Some

solvent was removed by distillation *in vacuo*, but nothing had crystallized after the mixture had stood for several months at ice-box temperature.

PROPERTIES OF VERBENALOL

LUCAS TEST ON VERBENALOL

The Lucas reagent was prepared [19] by dissolving 13.6 g. anhydrous zinc chloride in 10.5 g. concentrated hydrochloric acid. It was used at 26-7°. Twenty-five milligrams of verbenalol were tested with 3 ml. Lucas' reagent. Nothing separated although the color did deepen.

CERIC NITRATE TEST ON VERBENALOL

To 3 ml. ceric nitrate reagent[20], diluted 1:6, were added 3-5 drops aqueous solution of verbenalol. There was no deepening of the color, hence the test was negative. However, overnight the yellow color was bleached, probably indicating oxidation.

DERIVATIVES OF VERBENALOL

METHYLATION OF VERBENALOL

To 10 ml. dry ether were added 3 ml. 50% aqueous potassium hydroxide and the mixture was cooled below 5°. One gram of finely powdered nitrosomethylurea was added to the cooled mixture with continued cooling and shaking over a period of two or three minutes. The solution of diazomethane in ether was decanted and dried over pure pellets of potassium hydroxide for 3 hours. The dried ether solution of diazomethane was added to a solution of 50 mg. verbenalol in 5 ml. absolute methanol which was stoppered and set aside

overnight. Next day, more diazomethane was prepared from 0.5 g. nitrosomethylurea and after drying, it was added in ether solution to the methanolic solution of aglycone. Six days later, the diazomethane prepared from 1 g. nitrosomethylurea was added, the mixture stoppered and again set aside. One week later, the excess diazomethane and the ether were removed by gentle heating on the steam-bath. Attempts were made to crystallize the syrupy residue from ethyl acetate and from alcohol. Nothing crystalline was obtained. The material was then refluxed for 3 hours with absolute ethanol and the filtrate from this treatment was digested with Norite, then filtered, concentrated and set aside. In 24 hours, a brown oil had appeared. Separation of the oily material from the solvent was completed by the addition of water to the mixture. A solution of the oil in absolute alcohol was used for ultraviolet absorption spectra studies. The curves obtained in neutral and alkaline alcoholic solutions were not smooth, although they seemed to indicate peak absorption in both cases in the same region, i.e. about 240 .

Subsequently, methylation of verbenalol was accomplished using the following method. To 30 ml. 50% aqueous potassium hydroxide solution and 100 ml. ether, cooled to 5°, were added gradually and with constant shaking, 5 g. nitrosomethylurea. After reaction was complete, the diazomethane and ether were distilled into a flask (loosely attached to the end of the condenser so as not to develop pressure) containing 50 ml. dry ether, and maintained in an ice-bath.

In the ether solution of diazomethane were dissolved 250 mg. finely-powdered verbenalol with shaking. The solution of diazomethane and verbenalol in ether was set aside for 6 days. After 48 hours, the solution was tested for excess diazomethane[21] and for enolic hydroxyl by the ferric chloride test. The latter test was negative and subsequent reasoning re structure showed no reason why it should be positive. The following data were determined for the diazomethane excess.

Volume solution tested = 10 ml.

Volume 0.2 N ethereal benzoic acid used = 10 ml.

Titration with 0.1005 N sodium hydroxide = 0.75 ml.

Strength of diazomethane solution = approx. 8 mg./ml.

Excess diazomethane and ether were removed after 6 days and a solution of 1.44 mg. % of the oily material in absolute ethanol was used for ultraviolet absorption spectra studies. A spectrum was determined first in neutral solution, and subsequently the effect of the addition of 2 drops of 0.2034 N sodium hydroxide was determined, as well as subsequent overneutralization of the alkali. A quite sharp peak of absorption was observed in neutral solution at 242 mμ, with no shift in alkaline medium. (see Fig. 7)

ATTEMPTED PREPARATION OF OXIME OF VERBENALOL

Twenty-five milligrams of verbenalol and 25 mg. hydroxylamine hydrochloride were refluxed with 2 ml. absolute alcohol and 2 ml. pyridine for 4 hours. The mixture was set aside in the refrigerator overnight. Since nothing had crystallized, the mixture was concen-

trated by distillation under reduced pressure to a dark amber viscous syrup. Attempts were made to crystallize the material from ethanol and water, but nothing crystalline was obtained.

ATTEMPTED PREPARATION OF 2,4-DINITROPHENYLHYDRAZONE OF VERBENALOL

Four hundred and fifty milligrams of 2,4-dinitrophenylhydrazine were dissolved in 50 ml. absolute methanol by refluxing. When the solution was cool, 0.25 g. finely-powdered verbenalol was added and the mixture refluxed for 30 minutes. It was cooled to room temperature, then set aside in the refrigerator. In five days, there was what appeared to be a heterogeneous crystalline mass adhering to the sides of the flask. When the mother liquor (after filtration) was concentrated, more material was deposited.

The two precipitates were combined and refluxed with methanol. After concentration, a dark-brown amorphous material was deposited. This was filtered off and dried, m.p. 142-9° (decomp.), designated A1. A second deposit, orange this time, designated A2, was obtained from the mother liquor of A1. It was filtered off and dried, m.p. 128-133°. It was not crystalline. The fraction A1 was dissolved in hot chloroform, concentrated and set aside. Nothing crystalline resulted. A further crystalline fraction, B, was obtained after 2 weeks from the original mother liquor. It was dark red-brown and after filtration and drying, melted at 132-5°. However, when a small amount of B was mixed with a small amount of A2, there was no appreciable depression of melting point. Consequently the two fractions were mixed and dissolved in hot ethyl acetate, filtered, concentra-

ted, and set aside. Nothing crystalline resulted. The solvent was removed and the material dissolved by refluxing in a mixture of equal parts of toluene and nitrobenzene. An oil resulted and the attempt to prepare a crystalline dinitrophenylhydrazone of verbenalol was abandoned.

ATTEMPTED PREPARATION OF 3,5-DINITROBENZOATE OF VERBENALOL

A sample of 75 mg. verbenalol was dissolved in 2 ml. anhydrous pyridine and to this solution were added 100 mg. 3,5-dinitrobenzoyl chloride. The mixture was heated gently over a low flame, then poured into 7 ml. distilled water with stirring. Nothing crystalline was precipitated although the color of the solution deepened to orange. It was set aside in the refrigerator, but nothing crystalline was obtained. The material was concentrated by distillation under reduced pressure. It turned dark brown but nothing crystalline was obtained.

ATTEMPTED PREPARATION OF PHENYLURETHAN OF VERBENALOL

One hundred milligrams of purified, dried verbenalol were placed in an air-oven dried test tube. To it were added 2 ml. phenyl isocyanate. There was no spontaneous reaction. The test tube was stoppered with a cotton plug and suspended in a steam-bath for 10 minutes to effect solution or reaction. The tube was then placed in a beaker of ice and the sides of the tube scratched. A solid white material was precipitated. It was filtered off, dried, m.p. 122-6°. A mixed melting point with authentic verbenalol showed no depression.

REACTION OF N-BROMOACETAMIDE AND VERBENALOL[22]

One gram of verbenalol was dissolved in 20 ml. chloroform containing 0.75% V/V ethanol with the aid of heat. When the solution was cool, 1.25 g. N-bromoacetamide were dissolved in the solution by shaking. The mixture was set aside in the refrigerator for 48 hours with the light excluded. Most of the chloroform was removed by distillation *in vacuo*. Three days later, white crystals appeared. The crystals were filtered off and dried; weight 50 mg., m.p. over a broad range above 140°. The crystals were recrystallized from ethanol and water, filtered off, dried, m.p. 295° (sublimed), weight 25 mg.

DEGRADATION STUDIES ON VERBENALIN AND VERBENALOL

DECARBOXYLATION OF VERBENALIN BY MEANS OF SULFURIC ACID[23]

To 40 ml. N/4 sulfuric acid in a 200 ml. 3-neck flask, was added 1.0072 g. recrystallized verbenalin and the mixture was heated to 95° for 7 hours. During the heating, a slow stream of nitrogen, freed from water and carbon dioxide by passage through a soda-lime tube, was passed through the mixture, the effluent gases being led into two traps in series, each containing 75 ml. 0.1 N barium hydroxide. After 1 1/2 hours, the solution began to turn yellow and some charring was also noted. After 2 1/2 hours, a further 50 ml. of the barium hydroxide solution were added to the second trap. The loss of volume in the heating flask was made up by periodic addition of distilled water which had been boiled, cooled and had a stream of nitrogen passed through it. The approximately 0.1 N barium hydroxide was standardized as 0.0982 N by titration of 25 ml. pipetted samples

with 4.05 ml. 0.6061 N hydrochloric acid. After the 7 hours' heating, the barium hydroxide solution in the traps was titrated with 0.6061 N hydrochloric acid. The following data were recorded.

Mls. 0.0982 N barium hydroxide in traps = 225.00

Mls. 0.6061 N hydrochloric acid = 35.49

Carbon dioxide equivalent per mole of verbenalin =

$$\frac{388}{1.0072} \times \frac{((225 \times 0.982)-(35.49 \times 6.061))}{10 \times 1000} \times 44.01 = 10.24 \text{ g.}$$

Fifty milliliters of 0.0982 N barium hydroxide were placed in each of the washed and flushed (with nitrogen) traps, and heating continued at 95° for another 32 hours. Again the barium hydroxide solutions were pooled and titrated with 0.6061 N hydrochloric acid, with the following data being recorded.

Mls. 0.0982 N barium hydroxide in traps = 100.00

Mls. 0.6061 N hydrochloric acid = 14.79

Carbon dioxide equivalent per mole of verbenalin =

$$\frac{388}{1.0072} \times \frac{((100 \times 0.982)-(14.79 \times 6.061))}{10 \times 1000} \times 44.01 = 14.52 \text{ g.}$$

Twenty-five milliliters of 0.0982 N barium hydroxide were placed in each trap, and heating resumed, but no carbon dioxide was obtained. The total carbon dioxide released from verbenalin in the experiment was 24.76 g. (0.56 mole) per mole of verbenalin.

The aqueous residue and charred material were placed in a continuous two-phase liquid extractor and extracted for 60 hours with ether. The ether extract was dried over anhydrous sodium sulfate, then concentrated *in vacuo* and set aside. Nothing crystalline was

obtained. The brown syrupy matter was taken up in chloroform and concentrated to a volume of 3 ml. and set aside. Nothing crystalline was obtained.

DECARBOXYLATION OF VERBENALIN BY MEANS OF PHOSPHORIC ACID

A sample of 1.0030 g. verbenalin was placed with 40 ml. 1% aqueous phosphoric acid in a Claisen flask. Nitrogen gas, passed through a soda-lime tube and aqueous barium hydroxide solution to remove water and carbon dioxide, was led through the flask at a pressure of 10 p.s.i. The effluent gases, as previously, were led into two traps, containing a total of 50 ml. 0.1176 N barium hydroxide solution. The glycoside was dissolved in the aqueous phosphoric acid under the influence of gaseous agitation within a few minutes. The solution was maintained at room temperature for 3 1/2 hours. Since no carbon dioxide was given off, the temperature was raised to 50°. Still no carbon dioxide was given off and consequently, the temperature was raised to 70° and later to 90°. Even at this temperature, the barium hydroxide solutions remained clear. Two drops of concentrated sulfuric acid were added to the Claisen flask with the temperature at 95° and carbon dioxide began to be given off immediately. Heating was continued for 23 hours (with the volume being replenished periodically) at the end of which time the solution was yellow and contained a small amount of charred matter. The titration resulted in the following data.

Mls. 0.1176 N barium hydroxide in traps = 50.00

Mls. 0.6061 N hydrochloric acid = 4.42

Carbon dioxide equivalent per mole of verbenalin =

$$\frac{388}{1.0030} \times \frac{((50 \times 1.176)-(4.42 \times 6.061))}{10 \times 1000} \times 44.01 = 54.49 \text{ g.}$$

54.49 g. carbon dioxide ≡ 1.239 mole.

ATTEMPTED DECARBOXYLATION OF VERBENALIN WITH SODIUM FLUORIDE AND PYRIDINE.

One gram of verbenalin and 0.5 g. dry sodium fluoride were suspended in 20 ml. pyridine and refluxed for 30 minutes, during which time the mixture became dark brown. The undissolved sodium fluoride was separated by filtration. The pyridine filtrate was concentrated by distillation *in vacuo* and to the concentrate was added an excess of ether. The ether-pyridine mixture was poured off and the solvent evaporated, at which white crystals appeared. These were recrystallized from methanol and water, and dried, m.p. 178-80°. A mixed melting point with verbenalin was 179.5-182°.

DECARBOXYLATION OF VERBENALOL. I. QUANTITATIVE[24].

In the same apparatus used for the decarboxylation of verbenalin (see P. 107), were placed 1.0361 g. verbenalol and 100 ml. 10% aqueous sulfuric acid. The mixture was refluxed 8 hours. In each of the two traps were placed 100 ml. 0.0881 N barium hydroxide. A stream of nitrogen was passed through the flask to carry any gaseous reaction products into the traps. Carbon dioxide was observed to be given off almost immediately. The following data were recorded.

Mls. 0.0881 N barium hydroxide in traps = 200.00

Mls. 0.2220 N hydrochloric acid = 57.05

Carbon dioxide equivalent per mole of verbenalol =

$$\frac{226}{1.0361} \times \frac{((200 \times 0.881)-(57.05 \times 2.220))}{10 \times 1000} \times 44.01 = 47.37 \text{ g.} \quad (1.076 \text{ mole})$$

In a second determination, 75 ml. 1.934 N sulfuric acid were pipetted into the reaction flask and hydrolysis carried out until no further carbon dioxide was given off. The contents of the reaction flask were then titrated with standardized solution of sodium hydroxide. The following data were recorded.

Mls. 1.934 N sulfuric acid = 75.00 ≡ 145.05 ml. 1 N sulfuric acid.

Mls. 2.120 N sodium hydroxide = 67.73 = 143.58 ml. 1 N sodium hydroxide.

The conclusion was reached that little or no non-volatile acid was generated during the decarboxylation.

After titration with alkali, this residue was concentrated under reduced pressure to dryness, at which time it consisted of a salt (sodium sulfate) and a brown oil. This mixture was extracted with ether by refluxing three times, the ether extracts were combined, concentrated and set aside, "A". The residue, "B", was dissolved in water with the aid of heat to keep the volume small, then acidified to pH 4.0 with 10% aqueous sulfuric acid, and extracted with ether in a continuous two-phase liquid extractor for 20 hours. The extract was dried over anhydrous sodium sulfate, concentrated and allowed to evaporate spontaneously. The concentrate, still smelling of ether, was tested with Schiff's Fuchsin-Aldehyde reagent, yielding negative results.

A few specks of solid matter appeared in "A", but a flame test showed that they were inorganic. After concentration, apart from the few specks, "A" was an orange syrupy material. It was dissolved in absolute alcohol, filtered (to remove the inorganic matter), treated with Norite, filtered, concentrated and set aside. Nothing crystallized. It was shaken out with ether in a separatory funnel and concentrated. The concentrate was distilled azeotropically with alcohol and benzene so as to leave the material in absolute alcohol. This solution, "G", was tested for the presence of a carbonyl (aldehyde or ketone) function and yielded a positive test with 2,4-dinitrophenylhydrazine reagent. The remainder of "G" was treated with hydroalcoholic solution of sodium bisulfite but no addition product was obtained.

DECARBOXYLATION OF VERBENALOL. II. PREPARATIVE.

Time of Hydrolysis

A sample of verbenalol (0.5659 g.) was heated at 95° with 50 ml. 10% sulfuric acid in the decarboxylation apparatus for 5 hours. The residue was extracted by shaking with ether in a separatory funnel. When the ether was removed by distillation in vacuo, a brown syrupy material resulted. The addition of absolute alcohol at room temperature caused the formation of a grayish precipitate, which was collected by filtration and dried, m.p. 95-100°. The crystals (a very small sample) were dissolved in alcohol, treated with Norite, filtered, concentrated in vacuo and set aside. In 5 days, crystals appeared. They were filtered off and dried, m.p. 128-131°. They

proved to be verbenalol. The conclusion was drawn that decarboxylation was incomplete in 5 hours.

Characterization of Products of Decarboxylation

Decarboxylation was carried out as in the previous experiment, and after 48 hours' heating, the residue in the flask consisted of three parts - a yellow liquid "A", suspended dark brown particles "B", and a brown gummy deposit "C" adhering to the sides of the flask. The aqueous yellow liquid, "A" was extracted with ether for 18 hours in a continuous two-phase liquid extractor. The ether extract, "A1", was dried over anhydrous sodium sulfate, then concentrated under reduced pressure, and allowed to evaporate spontaneously. The extract gave a strongly positive test with Schiff's fuchsin-aldehyde reagent. To the extract, "A1", were added 50 ml. hydro-alcoholic solution of sodium bisulfite and the mixture was set aside under refrigeration for 3 weeks, at the end of which time, there were colorless crystals in the bottom of the flask. The crystals were filtered off, dried, dissolved in 20 ml. water, acidulated with dilute hydrochloric acid and extracted with ether in the continuous two-phase liquid extractor for 18 hours. The ether extract, "A2", was dried over anhydrous sodium sulfate, concentrated under reduced pressure, and allowed to evaporate spontaneously. When no odor of ether could be detected, there remained almost 2 ml. pale yellow liquid, "A3".

One-half milliliter of "A3" was placed in a small erlenmeyer flask and to this was added 1 ml. Tollens' reagent. Nothing crystal-

line was obtained from the attempted oxidation.

One-half milliliter of "A3" was used in alcoholic solution for a qualitative determination of the ultraviolet absorption spectra in neutral and alkaline solutions. Absorption peaks were obtained at 232 mμ and 229 mμ respectively in neutral and alkaline solutions, as shown in Fig. 10.

One milliliter of "A3" was dissolved in 10 ml. alcohol and to this was added a solution of 400 mg. 2,4-dinitrophenylhydrazine in sulfuric acid, water and alcohol (total volume 10 ml.). A precipitate began to form immediately. The orange precipitate was filtered off after the mixture had stood overnight. It was dried, m.p. 107-110°. The material was redissolved in a small quantity of alcohol and water was added to the clouding point. After standing several days, an orange precipitate was filtered off and dried, m.p. 110-111°. The material was purified once more and submitted for analysis. No satisfactory molecular formula could be constructed to account for the analysis date (C = 55.54, 55.36%; H = 5.93, 5.94%; N = 11.50, 11.50%).

The dark brown particles, "B", and the gummy brown deposit, "C", were combined and dissolved in hot alcohol. The solution yielded a positive test with 2,4-dinitrophenylhydrazine reagent. The alcoholic solution was treated with Norite and filtered. The filtrate did not give as strongly positive a test with the reagent as had the solution before the Norite treatment. Consequently the Norite pad was washed thoroughly three times with hot alcohol and the washings added to the

alcoholic filtrate. The last washing tested negatively with 2,4-dinitrophenylhydrazine reagent. The solution was concentrated and set aside. Nothing crystalline was obtained.

In another decarboxylation experiment, 1 g. verbenalol was powdered finely and placed in a 250 ml. round-bottom flask. To it were added 100 ml. 10% sulfuric acid. A reflux condenser was connected and the mixture was refluxed 14 hours. The mixture was then distilled to remove any volatile acids or carbonyl compounds, 20 ml. being collected. The distillate gave a negative test with 2,4-dinitrophenylhydrazine reagent. Titration of the distillate with approximately 0.1 N sodium hydroxide solution consumed 1.28 ml. with phenolphthalein as indicator. The solution of sodium salt was reduced to dryness. The dried residue was then dissolved in 5 ml. distilled water, one drop concentrated hydrochloric acid added and 550 mg. p-phenylphenacyl bromide and 10 ml. alcohol added. The mixture was refluxed 1 hour, then allowed to stand at room temperature. Crystals soon formed as long needles. They were filtered off and dried, m.p. 125-6° (p-phenylphenacyl bromide as shown by mixed melting point). The filtrate was set aside and within 4 hours, more crystals appeared. These were filtered off and dried, m.p. 125-7° (again p-phenylphenacyl bromide).

Sixty-five milliliters of aqueous acidic residue from a previous decarboxylation were diluted to 150 ml. and a 10 ml. aliquot titrated with approximately 0.1 N sodium hydroxide. The amount of alkali consumed was 50.12 ml. One hundred milliliters of the dilution were

distilled in a Claisen flask, three 10 ml. portions being collected and titrated with the same approximately 0.1 N alkali. The titrations consumed 1.06, 1.32 and 1.48 ml. respectively. The titrations were combined and evaporated to dryness, after which the sodium salt was dissolved in a minimum amount of water, and acidified to phenolphthalein with dilute hydrochloric acid. A drop of acidic permanganate solution was added and it was decolorized immediately. This reaction and the Duclaux values (modified) would seem to indicate the presence of formic acid.

An attempt was made to detect an acidic substance in the syrupy ether extract (acid to litmus) of another decarboxylation. One-quarter gram of the ether extract was heated with 5 ml. water and 5 ml. alcohol to effect solution. Then 0.25 g. p-phenylphenacyl bromide was added and sufficient alcohol (10 ml.) to dissolve the reagent. The mixture was refluxed 3 hours and set aside. Crystalline material was filtered off after 24 hours, and dried, m.p. 114-7° (it was starting reagent). A second crop of p-phenylphenacyl bromide was also removed.

ACTION OF BARIUM HYDROXIDE ON VERBENALOL

Reaction of Verbenalol with 1 mole equivalent of Barium Hydroxide

Asano, Ueno and Tamaki[9] reported that, at room temperature, a molar equivalent of 0.1 N barium hydroxide solution degraded verbenalol to methanol and an acid which they reconverted to verbenalol through the action of diazomethane. They further reported that the action of two moles equivalent of 0.1 N barium hydroxide on verben-

alol was to produce besides methanol, formic acid and another acid which could not be reconverted to verbenalol by means of diazomethane. (see P. 25). Attempts were made in the present study to reproduce these results.

A sample of 0.45 g. finely powdered verbenalol was dissolved with shaking in 20 ml. 0.1 N barium hydroxide. The barium hydroxide solution was prepared with recently boiled and cooled distilled water and was filtered with suction through a fine sintered glass funnel. The solution, which began to turn yellow immediately, was allowed to stand overnight at room temperature. At the end of this time, the dark brown solution contained a small amount of very fine sediment. When carbon dioxide was bubbled in, more precipitate was formed, contrary to the observations of Asano et al[9]. The mixture was centrifuged and the supernatant neutralized with dilute hydrochloric acid, then extracted by shaking seven times with ether. The ether extract was concentrated, the last solvent being allowed to evaporate spontaneously. A yellow syrupy liquid resulted. One portion of this material was treated with diazomethane generated from 1 g. nitrosomethylurea and set aside. A dark amber material oiled out of the ether solution. This was taken up in hot alcohol, treated with Norite, concentrated after filtration and set aside. Nothing crystalline, but rather a brown oil, was obtained. All the alcohol was removed by distillation <u>in vacuo</u>, and the oily residue was dissolved in ethyl acetate with the aid of heat, then concentrated. Nothing crystalline was obtained.

A second portion of the yellow syrup obtained by ether extraction, was submitted to flame test for the presence of barium. A green color imparted to the flame indicated the presence of barium.

The aqueous residue remaining after ether extraction (volume 36 ml.) was submitted to oxidation by refluxing with 0.25 g. sodium dichromate and 1 ml. concentrated sulfuric acid for 10 minutes. Then the mixture was distilled under reduced pressure and the distillate (10 ml.) diluted with 10 ml. alcohol. In this solution was dissolved 0.25 g. dimedon. The mixture was warmed briefly and set aside. In two weeks, a white crystalline material, m.p. 117-21°, appeared. One recrystallization from ethanol and water raised the melting point to 148-150°. A mixed melting point with dimedon showed no depression.

Preparation of Dimedon of Formaldehyde

In 35 ml. 50% alcohol, there were dissolved 3.45 g. dimedon (5,5-dimethyl-1,3-diketocyclohexane). This solution was added along with 1 ml. ethanol to 1 ml. 37% formaldehyde solution. The mixture was warmed briefly on the steam-bath and set aside. In a short time, a large amount of crystalline material precipitated. This was filtered off, rinsed with 50% ethanol (cold) and dried, m.p. 181-4°. It was recrystallized from ethanol and water, and dried, m.p. 187-187.5°.

Reaction of Verbenalol with 2 moles equivalent of Barium Hydroxide

Three hundred fifty milligrams of verbenalol were dissolved in 20 ml. 0.1 N barium hydroxide and set aside for 24 hours, after

which time it was brown in color. The mixture was distilled and 10 ml. distillate caught. Reduced pressure was used and since there was much foaming, a 1000 ml. round bottom flask was used for the distillation. The distillate was refluxed with 1 ml. concentrated sulfuric acid and 0.25 g. sodium dichromate, after which 8 ml. distillate were collected under reduced pressure. The Schiff fuchsin-aldehyde test was applied to 2 ml. of this distillate and was faintly positive, indicative of an aldehyde. The remainder of the distillate was utilized for the preparation of a dimedon derivative (in 50% alcohol with a drop of piperidine). Impure crystalline dimedon was recovered.

The residue after distillation, was acidified to pH 5 with 20 ml. 0.1 N hydrochloric acid. It was then extracted five times with 150 ml. portions of ether in a separatory funnel. The ether extract was concentrated under reduced pressure and let evaporate spontaneously. A syrup only (in small amount) was obtained and it could not be crystallized from ethanol and water.

INVESTIGATION OF RESIDUE OF GLYCOSIDAL HYDROLYSIS

General

The material investigated was a dark brown syrupy mass, left after crystallization of verbenalol from the ether extract of glycosidal hydrolysis. The material was dissolved in ethanol and this solution was tested with 2,4-dinitrophenylhydrazine reagent and with Tollens' reagent. Both tests were decidedly positive. No sodium bisulfite addition product was obtained when a sample of the solution

was treated with aqueous-alcoholic solution of sodium bisulfite.

Treatment with 2,4-Dinitrophenylhydrazine

A second hydrolysis residue was treated with a solution of 200 mg. 2,4-dinitrophenylhydrazine in sulfuric acid, water and ethanol. Almost immediately, an orange precipitate appeared. It was designated A. It was filtered off and dried. The filtrate soon deposited a second precipitate, yellow in color. It was designated B. It appeared to be relatively homogeneous whereas A was obviously heterogeneous, consisting largely of an orange precipitate contaminated with traces of B. The weight of the latter was 155 mg. (from hydrolysis residue of 12 g. verbenalin) and the melting range of the undried material was 132° and up (decomp.). The weight of A was 35 mg., and its melting range was 93-96° (decomp.).

The material A was precipitated from ethanol and water, filtered off, dried, m.p. 142-3° (decomp.). It was precipitated again from ethanol with water added to the clouding point, filtered off, dried, m.p. 142-3° (decomp.). The dark red material was dried 18 hours at 100° and 1 mm. pressure, then submitted for analysis. Analysis data, calculated for $C_{13}H_{12}N_4O_6$, C = 48.75%, H = 3.78%, N = 17.49%; found C = 48.81%, H = 3.77%, N = 17.80%. The compound A was therefore the 2,4-dinitrophenylhydrazone of the carbonyl compound $C_7H_8O_3$.

The second material B was purified by treatment with alcohol and water twice. Before drying in a pistol, its melting point was 203° (decomp.) and after drying for 18 hours at 100° and 1 mm.

pressure, its melting point was 214-5° (decomp.). Analysis data for $C_{20}H_{24}N_4O_9$ were (calc. C = 51.70%, H = 5.21%, N = 12.06%; found C = 51.92%, H = 5.10%, N = 11.95%). The compound was therefore the 2,4-dinitrophenylhydrazone of a carbonyl compound, $C_{14}H_{20}O_6$.

ALKALINE DEGRADATION OF VERBENALOL. I.

One-half gram of verbenalol was powdered finely and added to 25 ml. 5% aqueous sodium hydroxide and refluxed 2 hours. It turned yellow, then brown, almost immediately. The solution was distilled (with the addition of several small portions of water) and the distillate of about 20 ml., and smelling of formaldehyde, was labelled "A". The residue was acidified to litmus with 10% phosphoric acid and distilled, with the addition periodically of small amounts of water, 35 ml. being collected. This distillate "B" was neutral to litmus. The residue "C" was extracted for 24 hours with ether in a continuous two-phase liquid extractor. The ether extract "D" was dried over anhydrous sodium sulfate for 24 hours, then concentrated to about 20 ml. and the remaining ether allowed to evaporate spontaneously. The ether-insoluble and non-volatile residue "E" was then extracted with t-amyl alcohol for 24 hours to give extract "F" and residue "G".

The distillate "A" yielded a negative test for a carbonyl compound with 2,4-dinitrophenylhydrazine reagent. Two hundred milligrams of dimedon were dissolved in a mixture of 10 ml. distillate "A" and 10 ml. absolute ethanol, one drop of piperidine was added and the mixture was refluxed gently 5 minutes. Sufficient alcohol

was removed by distillation to leave the mixture cloudy. When no crystalline material appeared, the mixture was concentrated still more and set aside in the refrigerator. In two weeks, crystals were obtained, which proved to be dimedon, m.p. 146-8°.

The ether extract "D" after removal of all solvent, was a dark brown syrup. It was dissolved in absolute ethanol, refluxed with Norite for 15 minutes, filtered, concentrated and set aside. Nothing crystalline was obtained. The material was diluted to 5 ml. with ethanol and to this solution was added a solution of 200 mg. 2,4-dinitrophenylhydrazine in sulfuric acid, water and alcohol, and the mixture was set aside at room temperature. A small amount of non-crystalline derivative was obtained. After drying on porous plate, its melting point was 127-8° (decomp.). There was insufficient material for purification for analysis.

The t-amyl alcohol extract "F" was concentrated by distillation in vacuo. A brown syrup resulted. It was dissolved in absolute ethanol and treated with Norite (refluxed 15 minutes), filtered, concentrated, water added to the clouding point, and set aside. Nothing crystalline was obtained. The material was dissolved in 5 ml. water, which solution was quite acidic to litmus. The solution was neutralized with five drops 5 N sodium hydroxide, and a drop of dilute hydrochloric acid added. To this solution were added 5 ml. alcohol and 500 mg. p-phenylphenacyl bromide. The mixture was refluxed 4 hours, then set aside. In 24 hours, a heterogeneous precipitate was apparent. From this mixture there were picked out

two products since one adhered to the sides of the flask. The first was white, crystalline and after drying, had m.p. 110-4°. A mixed melting point with authentic p-phenylphenacyl bromide was 108-115°. The second product, brown and non-crystalline was washed on a funnel several times with absolute ethanol to remove traces of p-phenylphenacyl bromide. After drying, it had m.p. 149-151°. The amount of material was very scant, too little for further study.

ALKALINE DEGRADATION OF VERBENALOL. II.

Two grams of verbenalol were refluxed in 50 ml. 5% aqueous sodium hydroxide for 2 hours. After 1 hour, a 1 ml. sample was withdrawn and layered carefully over 5 ml. chromotropic acid reagent[25], before being heated for 15 minutes in a steam-bath. A second 1 ml. sample was tested after 2 hours. Both were considered doubtful positives, because of the dark brown color of the degradation mixture. At the end of the reflux period, the condenser was reversed and 10 ml. were distilled off, "A". Sufficient 10% aqueous phosphoric acid was added to neutralize the alkali of the residue and 10 ml. more, "B", were distilled. The residue was extracted with ether by shaking with six 25 ml. portions of ether in a separatory funnel and then for 24 hours in a continuous two-phase liquid extractor. The ether extracts were combined and dried for 24 hours over anhydrous sodium sulfate, and then concentrated, "C". The aqueous residue of ether extraction was then extracted for 48 hours with t-amyl alcohol in the same extractor. The t-amyl alcohol extract, "D", was dried over anhydrous sodium sulfate for 24 hours,

then concentrated by distillation in vacuo. The aqueous residue, "E", was then extracted by refluxing with ethyl acetate for 36 hours. The ethyl acetate extract was concentrated to an oil, "F", by distillation in vacuo. The distillates "A" and "B" failed to give positive tests for carbonyl compounds (with 2,4-dinitrophenylhydrazine reagent) and the ethyl acetate extractive "F" gave a more strongly positive test than did the ether or t-amyl alcohol extractives, "C" and "D" respectively, with 2,4-dinitrophenylhydrazine reagent. Consequently, "F" was used for the preparation of a carbonyl derivative.

The extractive "F" was dissolved in 10 ml. absolute ethanol. To this solution was added a solution of 500 mg. 2,4-dinitrophenylhydrazine in sulfuric acid, water and ethanol. The mixture was set aside at room temperature. One hour later, the red precipitate (non-crystalline) was filtered off and dried, m.p. 136-8° (decomp.). It was purified for analysis by several treatments with alcohol and water. It was dried for 18 hours at 100° and 1 mm. pressure, after which it had m.p. 142-3° (decomp.). Analysis data for $C_{13}H_{14}N_4O_6$ are (calc. C = 48.45%, H = 4.38%, N = 17.39%; found C = 49.36%, H = 4.40%, N = 17.59%). This is the 2,4-dinitrophenylhydrazone of the carbonyl compound, $C_7H_{10}O_3$.

ATTEMPTED DEGRADATION OF VERBENALOL WITH AMBERLITE RESIN IR-4B.

A sample of 250 mg. finely-powdered verbenalol and 2 g. Amberlite ion-exchange resin IR-4B (previously washed several times with 5% aqueous sodium hydroxide and rinsed thoroughly twice with distilled water) were suspended in 50 ml. water and stirred at room

temperature with a magnetic stirrer for 24 hours.

The mixture was centrifuged and the yellow supernatant poured off. The precipitate (apparently mostly resin, but with some undissolved verbenalol still present) was stirred with 25 ml. distilled water for 30 minutes, then centrifuged. The supernatant from this centrifugation was added to the previous one and the mixture extracted with ether in a continuous two-phase liquid extractor for 18 hours. The ether extract was dried over anhydrous sodium sulfate for 24 hours, then concentrated under reduced pressure. The last ether was allowed to evaporate spontaneously. Three days later, crystals appeared in the ether-free extract, m.p. 130-2°. A mixed melting point with authentic verbenalol showed no depression, m.p. 130-3°.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF VERBENALOL

A 250 ml. 3-neck ST flask was fitted with a mercury-seal stirrer in the center well, with a ST condenser in one side well, and with a 60 ml. dropping funnel in the other. Fifty milligrams of finely-powdered lithium aluminum hydride were placed in the flask, and 40 ml. tetrahydrofuran (redistilled) added. The mixture was heated to effect complete solution. In the dropping funnel was placed a solution of 100 mg. verbenalol (dissolved with the aid of heat) in 25 ml. tetrahydrofuran. This solution was gradually added with constant stirring to the reagent in the flask. The mixture was refluxed 2 hours after all the solution had been added.

After the solution had been allowed to cool to room temperature,

distilled water was added dropwise to destroy the excess lithium aluminum hydride. The mixture was then washed with water into a large beaker. Sufficient 10% aqueous sulfuric acid was added to put all undissolved matter into solution. The pH was 1.5. Sufficient 4% aqueous sodium hydroxide was then added to the solution to raise the pH to 4, and this mixture was distilled under reduced pressure to remove the tetrahydrofuran. This distillate, "A", was reserved for further treatment.

The residue "B" was extracted with two portions of ether in a continuous two-phase liquid extractor for 16 and 18 1/2 hours respectively. The extracts were combined and dried over anhydrous sodium sulfate. Then the combined ether extracts were distilled under reduced pressure, the last ether being allowed to evaporate at room temperature. A small amount of dark brown syrup "C" resulted.

To the aqueous residue, "D", of ether extraction, measuring 5 ml., were added 25 ml. absolute methanol and 50 ml. benzene. The azeotropic mixture, with ethanol in excess, was distilled under reduced pressure to remove all the water and benzene. Then the balance of the alcohol was removed by distillation <u>in vacuo</u>, leaving a dark brown hard syrupy material. This material was rinsed with cold absolute ethanol then filtered. A small amount of granular material which did not dissolve in the solvent yielded a positive flame test for lithium. The alcoholic solution was concentrated and set aside, but nothing crystallized. Consequently, the sol-

vent was removed completely and the residue "E" was reserved for further treatment.

Attempted Acetylation of Lithium Aluminum Hydride Reduction Product

One gram of "E" was dissolved in 10 ml. anhydrous pyridine, and to the solution were added 4 ml. acetic anhydride. The flask was stoppered with a calcium chloride tube and let stand at room temperature for 17 hours. At the end of that time, the flask was cooled in an ice-bath and there were gradually added 150 ml. 5% aqueous sulfuric acid with constant stirring. The mixture was refrigerated for 3 hours, and at the end of that time, a dark brown gummy material had settled out on the sides of the flask. The supernatant was decanted. The gummy material was dissolved in absolute ethanol, treated with Norite (refluxed 15 minutes), concentrated after filtration, and water added to the clouding point. Nothing crystalline was obtained; however, a brown syrupy material oiled out. This material was dissolved in hot ethyl acetate, and the solution concentrated and set aside. Nothing crystalline was obtained.

Benzoylation of Lithium Aluminum Hydride Reduction Product

Ten milliliters of pyridine were added to 1 g. "E" to effect solution and to the solution was added 1 ml. benzoyl chloride. The mixture was refluxed 5 hours. The reaction mixture was rinsed into a separatory funnel with dry ether and 5% aqueous sodium bicarbonate was added to the mixture slowly until effervescence ceased, when the bottom aqueous layer was drained off. The ether extract was washed in turn with 5% aqueous hydrochloric acid, 5% aqueous sodium bicarbonate

and distilled water, before it was concentrated on a steam-bath. Shortly after the concentrated solution was set aside, crystalline material began to appear.

There appeared to be two distinct crystalline shapes, one needles, the other more granular. A small sample of each type was picked out with a dissection needle and separate melting point determinations made on a Fisher-Johns melting point apparatus. The needles were observed to melt at 195-202°, the granular material at 194-200°. They apparently represented the same material, total weight 45 mg. An alcoholic solution of 25 mg. crystals was treated with Norite (reflux), filtered, concentrated, and set aside. Crystals were obtained which were crystallized twice more for analysis. They were dried 24 hours at 100° and 1 mm. pressure, and submitted for analysis, m.p. 204.5-205.5°. The following data were recorded for $C_{20}H_{14}O_4$ (calc. C = 75.46%, H = 4.43%; found C = 75.48%, H = 4.61%). The molecular formula $C_{20}H_{14}O_4$ probably represents the dibenzoate of $C_6H_6O_2$.

Determination of Molecular Weight of "Benzoate of Reduced Verbenalol"[26]

A mixture of 0.0198 g. of the benzoylated product of lithium aluminum hydride reduction of verbenalol, and 0.2435 g. resublimed camphor (m.p. 174°) was melted in a small test tube, and the material allowed to cool when it was powdered finely on a watch-glass. Two melting point determinations of the mixture gave the values 63° and 65°.

The molecular weight was then determined as:

$$\frac{39.7 \times 0.0198 \times 1000}{(174-63) \times 0.2435} = 290.8$$

$$\frac{39.7 \times 0.0198 \times 1000}{(174-65) \times 0.2435} = 296.1$$

The calculated value for $C_{20}H_{14}O_4$ is 318.22.

Preparation of Hydroquinone Dibenzoate

Fifty milligrams of hydroquinone were dissolved in 5 ml. anhydrous pyridine, 1 ml. benzoyl chloride was added and the flask was stoppered and set aside. Crystals formed very easily. They were filtered off and washed thoroughly with cold absolute alcohol, dried, m.p. 199°. A mixed melting point with the product obtained by benzoylating the syrupy product of lithium aluminum hydride reduction of verbenalol showed the compounds were not identical, m.p. 123° up (gradually).

Determination of Benzoyl Groups in "Benzoate of Reduced Verbenalol"[11]

A sample of 9.0 mg. of the crystalline product obtained by benzoylating the syrupy product of lithium aluminum hydride reduction of verbenalol was placed in the three-necked flask of a Kuhn-Roth microacyl apparatus and 1 ml. 1 N aqueous sodium hydroxide added as a hydrolyzing agent. The mixture was refluxed 1 1/2 hours, cooled, and the condenser, funnel and inlet tube washed with a total of 7-8 ml. distilled water. Dilute solution of hydrazine (2 ml.) was added dropwise and the solution cooled again. Dilute sulfuric acid (1 ml.) was added as a neutralizing agent, the condenser reversed, and the mixture was distilled. Five fractions, of 20, 20, 10, 10, and 5 ml.

respectively were caught, the volume in the flask being replenished periodically so that it never became less than 3 ml. at any time. Each sample for titration was heated briefly to the boiling point after the addition of 10 mg. barium chloride, cooled and titrated with approximately 0.01 N aqueous sodium hydroxide.

Sample No.	Size of Sample	Mls. 0.009968 N alkali
1	20 ml.	6.59 ml.
2	20 ml.	0.77 ml.
3	10 ml.	0.15 ml.
4	10 ml.	0.10 ml.
5	5 ml.	0.05 ml.
		7.66 ml.

This amount of alkali represents 0.0080 g. benzoyl (C_6H_5CO-). In the case of a dibenzoate of molecular formula $C_{20}H_{14}O_4$, the amount of benzoyl in a sample of 0.0090 g. should be 0.0059 g. The value obtained then is very high and is indicative of the many difficulties (see Page 16) encountered in microbenzoyl determinations.

Examination of Tetrahydrofuran Distillate for Methanol and other Products

Chromotropic acid reagent was made[25] by dissolving 500 mg. of the sodium salt of chromotropic acid in 100 ml. 75% sulfuric acid. The latter was prepared by adding 90 ml. concentrated sulfuric acid cautiously to 40 ml. distilled water.

Fifty milliliters of tetrahydrofuran distillate, "A", were refluxed with 0.5 g. sodium dichromate and 1 ml. concentrated sulfuric

acid for 30 minutes, then distilled. This distillate was tested for the presence of formaldehyde.

Five milliliters of reagent were placed in a test-tube and 1 ml. of the distillate suspected of containing formaldehyde was added. A control was set up containing a small amount of formaldehyde, and another was set up containing a small amount of tetrahydrofuran, and still another containing a small amount of distillate "A" before oxidative treatment. The tubes were placed in a boiling water-bath for 15 minutes. The test with the formaldehyde-containing control was strongly positive and the test of the oxidized tetrahydrofuran distillate was also positive. A positive test was evidenced by a deep purple ring between the layers of reagent and test solution. The controls of tetrahydrofuran and distillate "A" were negative.

Carbonyl Compounds

Five milliliters of the tetrahydrofuran distillate, "A", were diluted with 15 ml. alcohol and 0.5 g. p-nitrophenylhydrazine added. The mixture was brought to the boiling point, a few drops of glacial acetic acid added, and the mixture refluxed for 20 minutes. Upon cooling to room temperature and standing for some time, there was no precipitate. Consequently, the mixture was heated again and while warm, it was diluted to the clouding point with distilled water. Nothing crystalline was obtained.

Attempt to Prepare Crystalline Derivatives Relating to Methanol

A quantity of tetrahydrofuran distillate, "A", (15 ml.) was diluted with an equal volume of water, 0.5 g. sodium dichromate and 1 ml. concentrated sulfuric acid added, and the mixture refluxed for

20 minutes. The mixture was then distilled under reduced pressure, 20 ml. being collected. To the distillate was added 0.5 g. p-nitrophenylhydrazine and a few drops of glacial acetic acid. This mixture was refluxed for 20 minutes and then allowed to cool slowly to room temperature, at which temperature it was maintained for 24 hours. No precipitate appeared.

The solution was concentrated under reduced pressure and on cooling, crystalline matter precipitated out. The crystals were filtered off and a close examination revealed two kinds of crystals, one red (probably unreacted p-nitrophenylhydrazine), the other yellow. A micro melting point determination was carried out on the yellow material, m.p. 170-185°. The yellow material (a very small amount) was recrystallized from alcohol and water, m.p. 202-5°. This melting point was too high for the p-nitrophenylhydrazone of formaldehyde, and the amount of material was too little for further study.

OZONIZATION AND OZONOLYSIS OF VERBENALOL

Five hundred milligrams of dried and powdered verbenalol were dissolved in 125 ml. redistilled ethyl acetate and ozonized. The reaction was complete in 45-50 minutes as evidenced by the blue color of the ethyl acetate at the temperature of the dry ice and acetone bath. The time of ozonization and the rate of generation of ozone by the ozonizer showed the presence of but one carbon-carbon double bond. The solvent was removed by distillation *in vacuo*, leaving the ozonide as an oil. Ten milliliters of distilled water were added and the mixture heated briefly on the steam-bath

to effect ozonolysis and solution. The solution gave a positive test with 2,4-dinitrophenylhydrazine reagent, and it effervesced with sodium bicarbonate solution. The solution was distilled in vacuo, after the system had been flushed out with nitrogen. The distillate was reserved as "K". The dark yellow oil, "A", still effervesced with sodium bicarbonate solution, was decidedly acidic to litmus, gave a positive ceric nitrate test, a strongly positive ferric chloride test (purple brown), and positive tests with 2,4-dinitrophenylhydrazine reagent and with Tollens' reagent.

The oil, "A", was neutralized to litmus with aqueous sodium bicarbonate solution and extracted with five 15 ml. portions of ether by shaking in a separatory funnel. The ether extract, "B", was pale yellow in color. When the ether extract was washed with aqueous sodium bicarbonate solution, most of the color left the ether layer and appeared in the aqueous phase. This aqueous material, "C", (bicarbonate and aqueous washings) gave a positive test with 2,4-dinitrophenylhydrazine reagent.

The ether extract, "D", after washing with bicarbonate solution and with water, was dried over anhydrous sodium sulfate and concentrated. There remained after evaporation of the last ether but a trace of yellow oil.

The aqueous residue, "E", remaining after ether extraction, "B", of the neutralized "A", was stirred for 4 hours with Amberlite Ion-Exchange Resin IR-120 (previously stirred with dilute hydrochloric acid and washed with distilled water until it no longer gave a precipitate with aqueous silver nitrate solution). The solution,

"F", which was separated from the resin by filtration, was strongly acidic to litmus and gave a positive test with 2,4-dinitrophenylhydrazine reagent. Since both carbonyl and carboxyl functions appeared to exist in the same molecule, it was deemed wise to methylate the carboxyl group. This was accomplished by drying the ether extract, "H", of solution "F" (shaking with fresh portions of ether repeatedly in a separatory funnel), then placing it with an ethereal solution of diazomethane, generated from nitrosomethylurea and dried over potassium hydroxide pellets after distillation. Nitrogen was evolved for 1 1/2 hours, after which there was no further evidence of reaction. The mixture was allowed to stand 36 hours at room temperature before the excess diazomethane and ether were removed. An orange oil, "I", remained when the last of the ether had evaporated.

Dinitrophenylhydrazone of Methyl Ester of Ozonolysate

The orange oil, "I", was dissolved in 10 ml. alcohol, and to this was added a solution of 50 mg. 2,4-dinitrophenylhydrazine in sulfuric acid, water, and alcohol (total volume 10 ml.). A precipitate began to appear immediately. After several hours, it was filtered off, dried, found to be amorphous, m.p. 121-5°. It was redissolved in alcohol, water added to clouding and set aside to stand. Again an amorphous precipitate was obtained which was dried, m.p. 128°. Analytical data indicated the presence of brown non-alkaline ash as impurity. The data obtained were as follows: C = 46.48%, H = 4.16%, N = 18.02%, ash from CH = 4.20%. Based on the assumption

that the total nitrogen content of this compound would be involved in the four nitrogen atoms of a 2,4-dinitrophenylhydrazine and on the assumption that there must be three oxygen atoms in the parent carbonyl compound to account for a ketone and a carboxyl group, a partial molecular formula of $C_{12}H_{12-14}N_4O_6$ can be worked out for the 2,4-dinitrophenylhydrazine of a carbonyl parent compound, $C_6H_{8-10}O_3$. Since this is the methyl ester of the carbonyl product of ozonolysis, the latter must be $C_5H_{6-8}O_3$. A small amount of the 2,4-dinitrophenylhydrazone of this carbonyl compound was obtained, m.p. 111-2° (decomp.), insufficient in amount for analysis and further study. A very small amount of semicarbazone of ozonolysate, m.p. 267-8° was also prepared.

Characterization of Distillate "K"

The distillate, "K", was found to be acidic to litmus and it was neutralized with 8.50 ml. 0.1 N sodium hydroxide, then reduced to dryness by careful heating. One-half the residue of sodium salt was mixed with 0.2 g. p-toluidine and 0.1 ml. concentrated hydrochloric acid in a small test-tube, and heated in an oil-bath at 155° for 1 hour. The mixture was allowed to cool, upon which it solidified to a crude mass. The mass was boiled with 1 ml. 95% alcohol and the solution was decanted into 5 ml. distilled water, after which the solution was concentrated to about 2 ml. and cooled in the refrigerator. A small amount of crystalline material (needles) appeared. They were dried, m.p. 150-2°. A mixed melting point with authentic acet-p-toluide (m.p. 152-3°) showed no depression.

The other half of the residue of sodium salt of distillate "K" was mixed with 0.2 g. p-bromoaniline and 0.1 ml. concentrated hydrochloric acid in a small test-tube, and heated at 155-160° in an oil-bath for 1 hour. After the mixture had cooled to a solid mass, it was boiled with 1 ml. 95% alcohol and the solution was decanted into 5 ml. distilled water. The hydroalcoholic solution was concentrated to 1-2 ml. and cooled in the refrigerator. After several days, a very small amount of crystalline matter was obtained which, after filtration and drying, had m.p. 166°. A mixed melting point with authentic acet-p-bromanilide (m.p. 166-7°) showed no depression. It was concluded that the volatile acid was acetic acid.

COLOR TESTS FOR THE FURAN NUCLEUS

ANTIMONY TRICHLORIDE REAGENT[27]

To a few mg. of solid material to be tested for the presence of a furan ring were added 0.1 ml. acetic anhydride and 0.4 ml. saturated solution of antimony trichloride in pure chloroform containing 1% V/V alcohol. The appearance of color was regarded as positive. The following observations were recorded.

TABLE II

Results of Antimony Trichloride Color Test with Verbenalin, Verbenalol and some Related Compounds

	0 hrs.	1 hr.	2 hrs.	4 hrs.	20 hrs.
Control (Reagent only)	-	-	-	-	-
Verbenalin Control	-	-	-	-	-
Verbenalol Control	-	-	-	-	-
Verbenalin	-	-	-	±	+

Table II continued.

	0 hrs.	1 hr.	2 hrs.	4 hrs.	20 hrs.
Verbenalol	-	-	±	+	+
Glucose	-	-	-	-	-
"Benzoate of Reduced Verbenalol"	-	-			-
Tetrahydroverbenalol	-	-			-
Tetrahydrofuran	-	gray ppte.			gray ppte.
Liquid Decarboxylation Product	-	-			gray ppte.

Verbenalin and verbenalol gave yellow green colors (no precipitates) which gradually darkened to green. The gray precipitates obtained from the tests with tetrahydrofuran and the liquid decarboxylation product (see P. 35) did not, in the least, resemble the tests with glycoside and aglycone.

Trim and Hill's Reagent "A"[4]

Trim and Hill's Reagent "A" was prepared by mixing 10 volumes glacial acetic acid, 1 volume of 0.2% aqueous solution of copper sulfate and 1/2 volume of concentrated hydrochloric acid. Tests were then carried out on verbenalin, verbenalol and related compounds by adding to 11 ml. Reagent "A", 1 ml. aqueous solution containing a few mg. test material and 20 mg. fructose. Results were recorded after 18 hours. The reagent alone, the reagent with fructose, with glucose, with fructose and glucose, with coumarin, and with fructose and coumarin, did not change color. However, the tests involving reagent and verbenalin, and reagent and verbenalol became light amber

in color, while the same two compounds gave deeper colors in the presence of fructose.

Shear's Reagent[28]

Shear's reagent was prepared by mixing 1 volume concentrated hydrochloric acid and 14 volumes of aniline. For the tests, a few mg. solid material under test were mixed with 1 ml. reagent. The following observations were recorded.

TABLE III

Results of Aniline Hydrochloride Color Test (Shear's Reagent) with Verbenalin, Verbenalol, Glucose and Coumarin.

	0 min.	5 min.	15 min.	30 min.	24 hrs.
Control	-	-	-	-	-
Glucose	-	-	-	-	-
Verbenalin	-	+	+	+	+
Verbenalol	-	+	+	+	+
Coumarin	-	-	-	-	-

A positive test consisted of an amber color, becoming red and later dark red-brown. However, these colors are not those obtained by Trim and Hill[4] when aucubin and asperuloside were treated with Shear's reagent.

Phloroglucinol-Hydrochloric Acid Reaction[4]

The reagent was prepared by dissolving 2 mg. phloroglucinol in a mixture of 2 ml. concentrated hydrochloric acid, 1 ml. water and 1 ml. alcohol. To the reagent, a few mg. solid material to be tested were added. Trim and Hill[4] obtained blue and blue-green colors with the glycosides, asperuloside and aucubin. The colors obtained

with verbenalin and verbenalol and related compounds varied from yellow to orange to cherry-red. The positive tests in the table of recorded observations were, in no instance, blue.

TABLE IV

Results of Phloroglucinol-Hydrochloric Acid Color Test with Verbenalin, Verbenalol and some Related Compounds

	0	1/12	1/6	1/2	3/4	1	2	3	8	20 hrs.
Control	-	-		-		-	-			
Verbenalin	-	+		+		+	+			
Verbenalol	+	+		+		+	+			
Tetrahydro-verbenalol	-	-		-		-	-	±		+
Liquid Decarboxylation Product	-	-		-		-	-	±		+
Tetrahydrofuran	+									
"Benzoate of Reduced Verbenalol"	-	-		-		-	-	-		+
2-phenyl-3-acetyl-4,5-diketotetrahydrofuran	-		±		+					
Acetylated Verbenalin	-		±		+					
Coumarin	-	-		-		-	-			-

ULTRAVIOLET ABSORPTION SPECTRA ANALYSES

The graph shown in Fig. 1 represents the ultraviolet absorption spectrum of a solution of 1.00 mg. of acetopyruvic acid per 50 ml. solution in absolute ethanol. In neutral solution, the acid shows

maximum absorption at a wave length of 287mμ, which, upon addition of an equimolar portion of alkali, is shifted to a longer wave length 310mμ. When the alkaline solution was neutralized with an equivalent amount of hydrochloric acid, the maximum absorption band shifted back to the shorter wave length (287mμ) corresponding almost completely with the original curve for the acid in neutral solution.

Fig. 2 represents the ultraviolet absorption spectrum of 1 mg. of the sodium enolate of ethyl acetopyruvate per 50 ml. of solution in absolute ethanol. Maximum absorption for the sodium enolate occurred at a wave length of 316mμ, but upon the addition of one equivalent of hydrochloric acid (to neutralize the enolate only), the maximum band was shifted to the shorter wave length 286mμ. Addition of excess hydrochloric acid did not shift the peak further.

Fig. 3 depicts the ultraviolet absorption spectrum of a solution in 50 ml. absolute ethanol of 0.05 mg. 2-phenyl-3-acetyl-4,5-diketotetrahydrofuran. In neutral solution, maximum absorption appeared at a wave length of 324mμ, and this peak was shifted very little (321mμ) by the addition of one drop of 1 N sodium hydroxide solution. The curve which represents the absorption in alkaline solution refers to a concentration of 0.025 mg. per 50 ml. solvent since the addition of alkali caused a great increase in the molar extinction coefficient.

The absorption curve shown in Fig. 4 is that of the product of reaction of formaldehyde and methyl acetopyruvate in methanol with diethylamine as catalyst. The graph is qualitative only and shows

end absorption at 220mμ, but no maximum absorption band within the scope of the instrument.

Fig. 5 shows the ultraviolet absorption spectra of verbenalol (1.02 mg. per 50 ml. solution in absolute ethanol) in both neutral and alkaline medium. Maximum absorption will be noted in neutral solution at 239mμ, and in alkaline solution at 272mμ, both curves corresponding very closely to those obtained by Young[3].

The ultraviolet absorption spectrum in Fig. 6 is that of tetrahydroverbenalol, in a concentration of 0.44 mg. per 50 ml. of solution in absolute ethanol. In neutral solution, the maximum absorption band is seen at a wave length of 242mμ, while the addition of alkali shifts the peak to 275mμ. Neutralization of the alkali with hydrochloric acid returns the peak to approximately that observed in neutral solution.

Fig. 7 represents the ultraviolet absorption spectrum of the brown syrup obtained when methylation of verbenalol was attempted by means of diazomethane. The concentration of non-crystalline product is 0.72 mg. per 50 ml. absolute ethanol. Maximum absorption occurs in neutral solution at 242mμ, and this peak is not shifted by the addition of alkali, nor by the latter's neutralization with hydrochloric acid.

The ultraviolet absorption spectra shown in Fig. 8 are those of a solution of 1.18 mg. acetylated verbenalin in 50 ml. absolute ethanol in neutral, alkaline, and reneutralized media. The maximum absorption band in neutral solution occurs at 236mμ, and this peak

shifts but slightly to 242 mμ in alkaline medium. However, as the compound stood in alkali for 24 to 96 hours, there was evidence of degradation as shown by the appearance of a second peak in the region, 275-285 mμ.

Fig. 9 depicts the ultraviolet absorption curves of the "benzoate of reduced verbenalol" in neutral and alkaline solution. The curves are qualitative only, due to the lack of essential information on the compound. The maximum absorption band is obtained at 242 mμ in neutral, alkaline and reneutralized media.

A qualitative ultraviolet absorption spectrum was determined for the liquid degradation product obtained once by decarboxylation of verbenalol. This is shown in Fig. 10. The maximum absorption band (quite sharp) appears at 232 mμ in neutral medium, and this is shifted slightly to 229 mμ in alkaline medium.

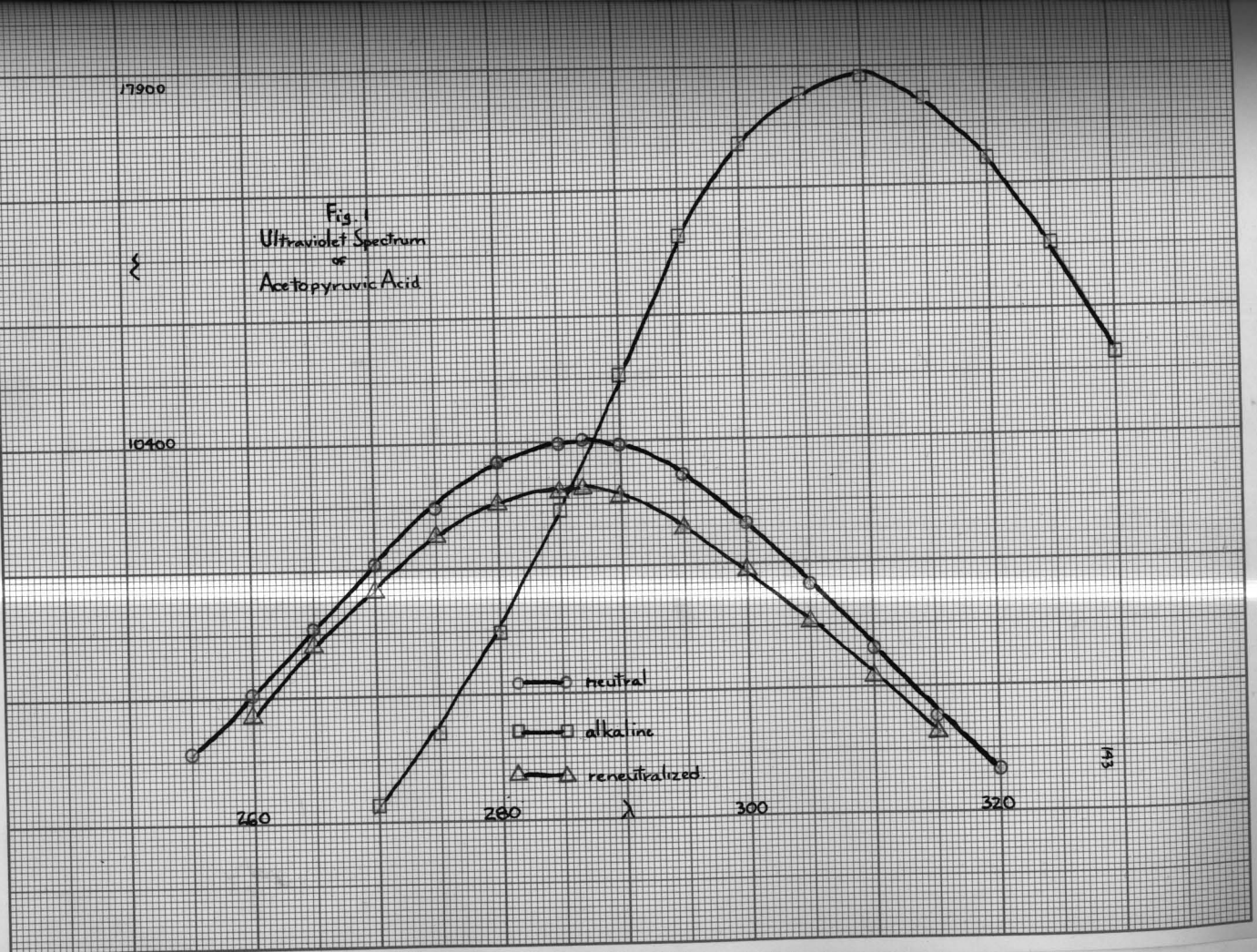
INFRARED ABSORPTION SPECTRA ANALYSES

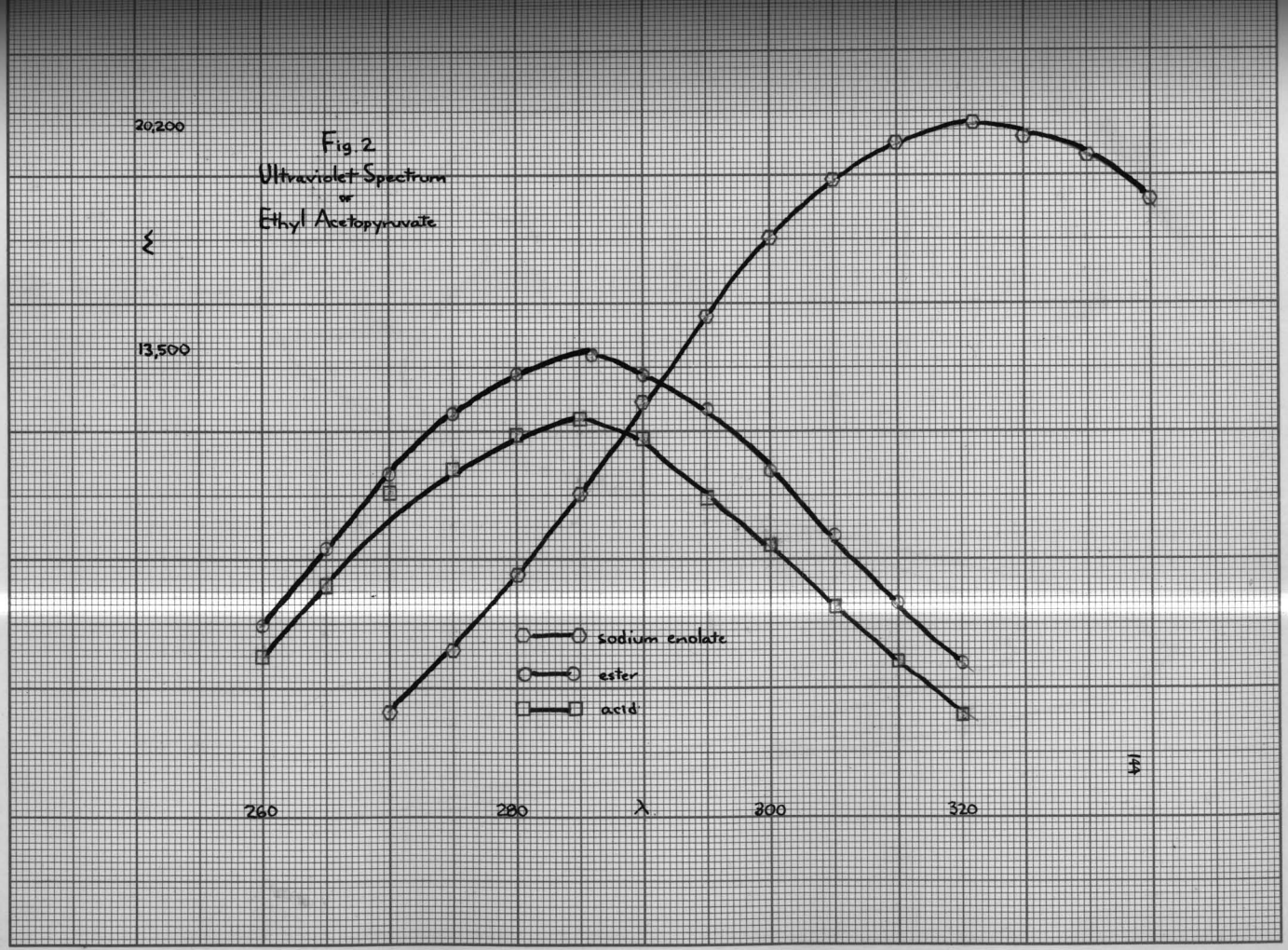
Figs. 11-16 are reproductions of infrared absorption spectra of verbenalin in Nujol mull, verbenalol in Nujol mull, acetylated verbenalin in Nujol mull, tetrahydroverbenalin in Nujol mull, verbenalol in chloroform solution, and tetrahydroverbenalol in chloroform solution respectively.

The spectra represented in Figs. 11, 12, and 13 were determined by the Upjohn Company, Kalamazoo, Mich. The interpretative remarks which were appended to the spectra by V.V.E.[29] are here reproduced.

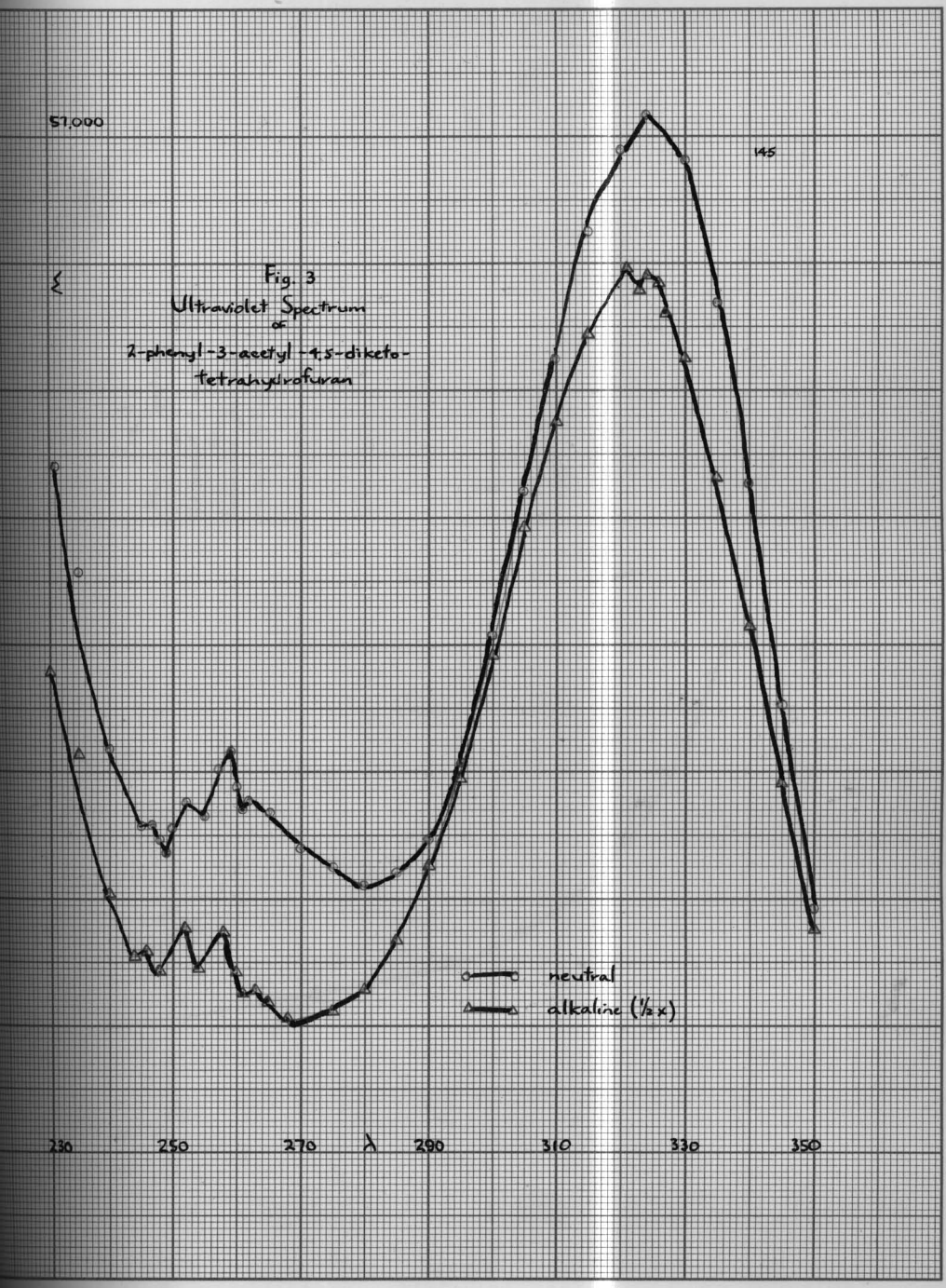
" Verbenalol

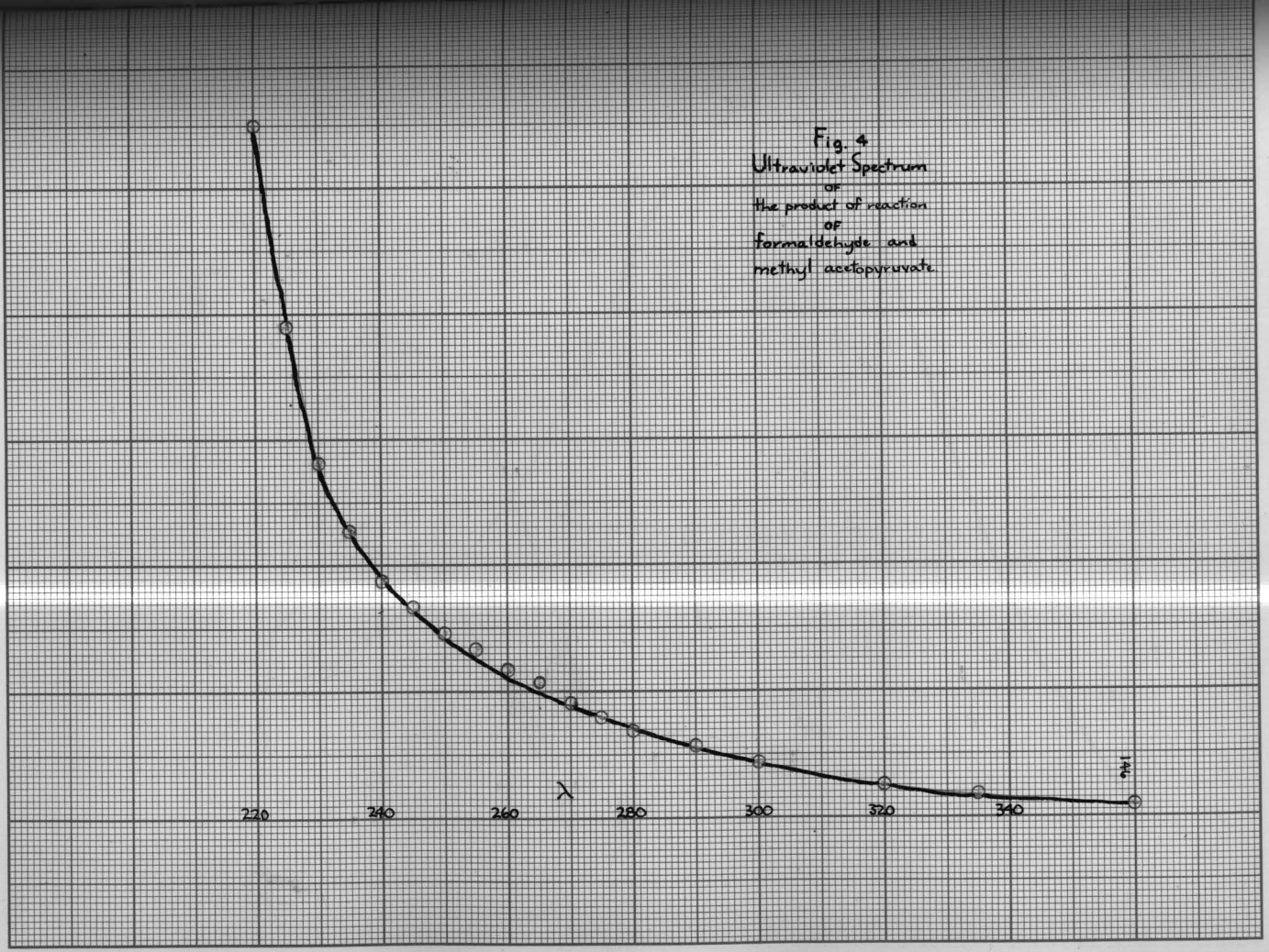
Absorption		Possible Groups and Comments.
3400 cm-1	OH	The strength of the absorption suggests the

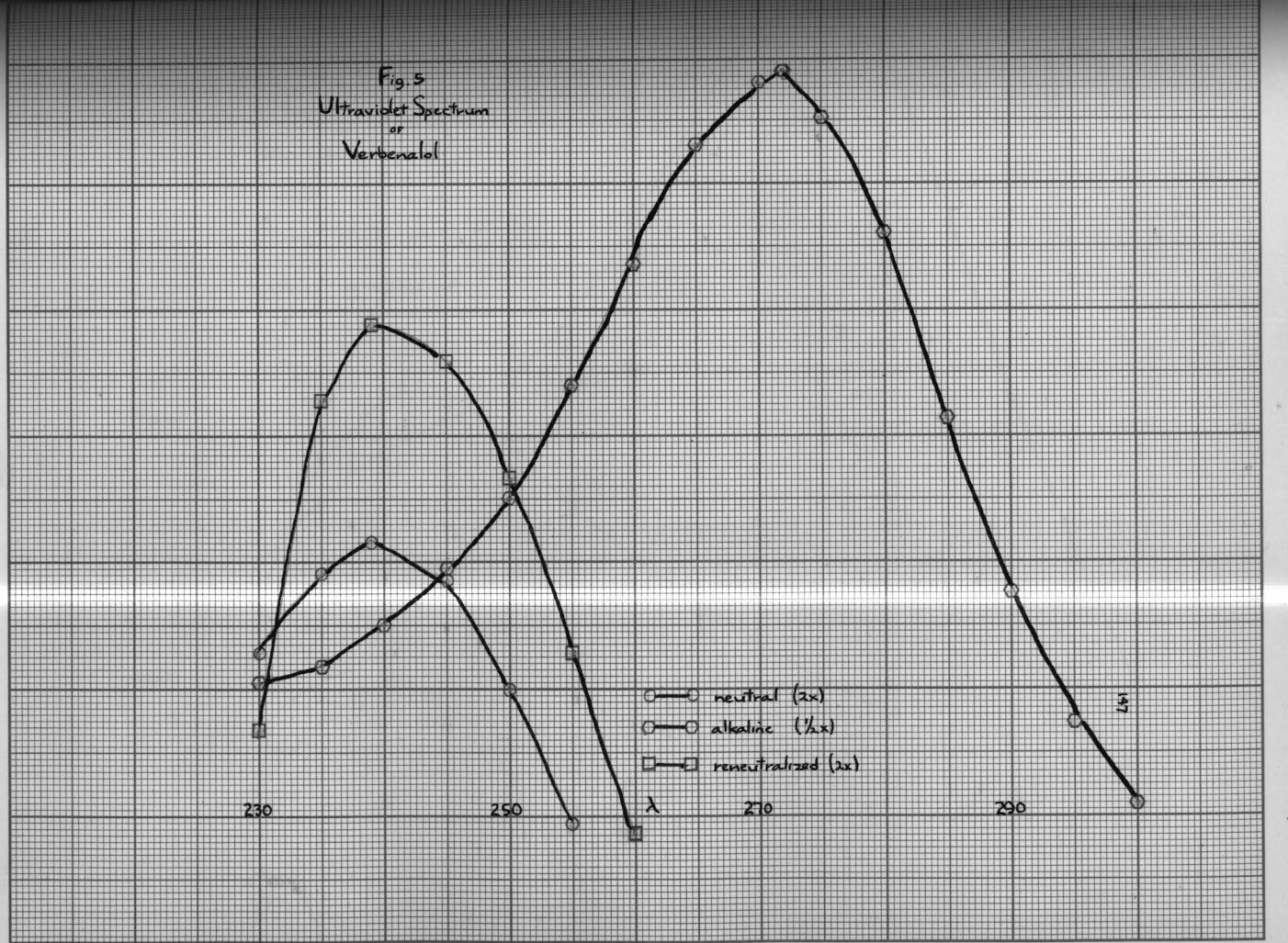

17900
10400
ε
Fig. 1
Ultraviolet Spectrum
of
Acetopyruvic Acid
neutral
alkaline
reneutralized
260
280
λ
300
320
143



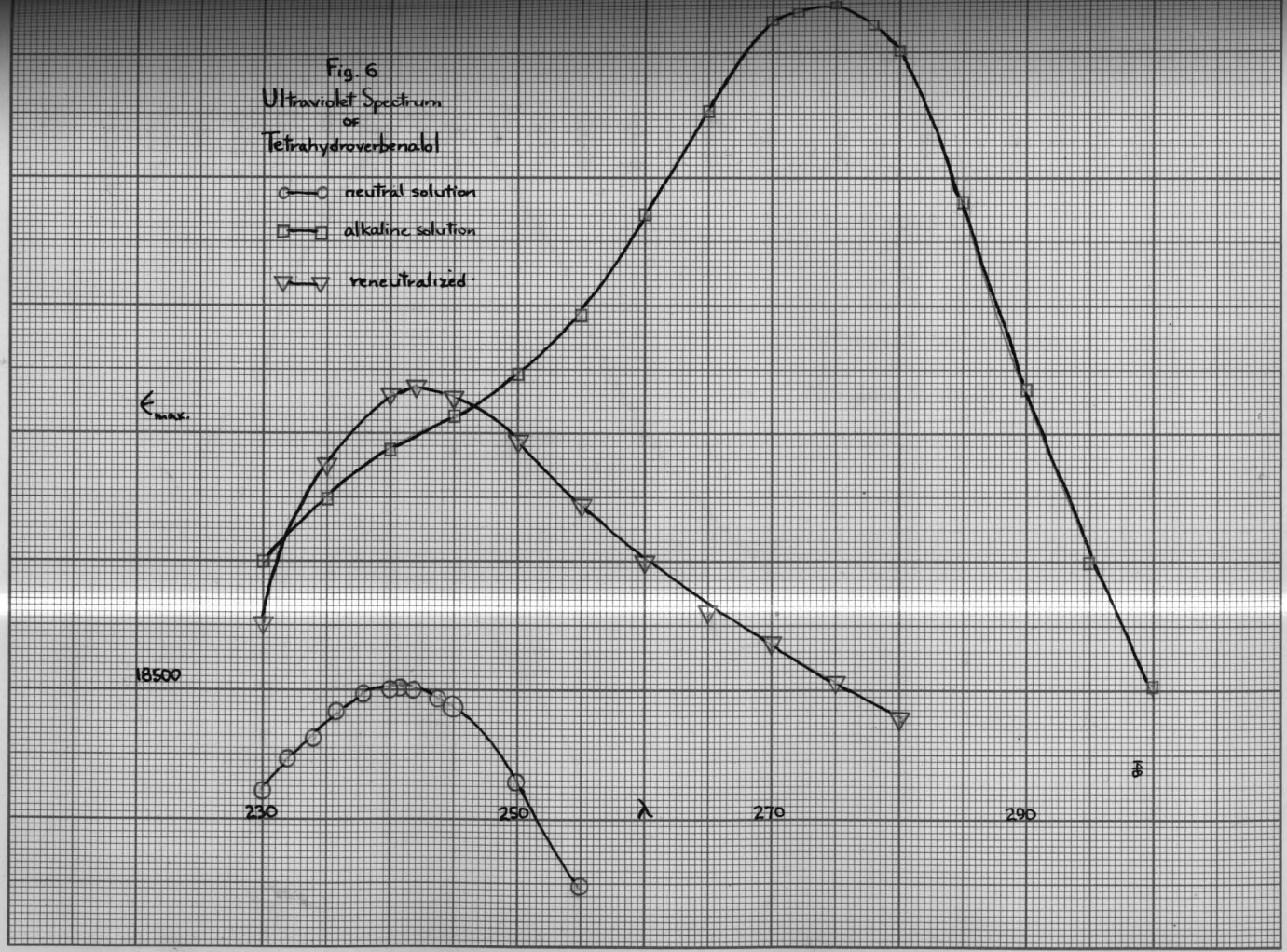
HUGHES OWENS 315 E 20x20


57,000
145
ε
Fig. 3
Ultraviolet Spectrum
of
2-phenyl-3-acetyl-4,5-diketo-
tetrahydrofuran
neutral
alkaline (1/2 x)
230
250
270
λ
290
310
330
350


Fig. 4
Ultraviolet Spectrum
of
the product of reaction
of
formaldehyde and
methyl acetopyruvate
220
240
260
280
300
320
340
λ
1.4c



HUGHES OWENS 2155 20x20



HUGHES OWENS 315 F 20x20

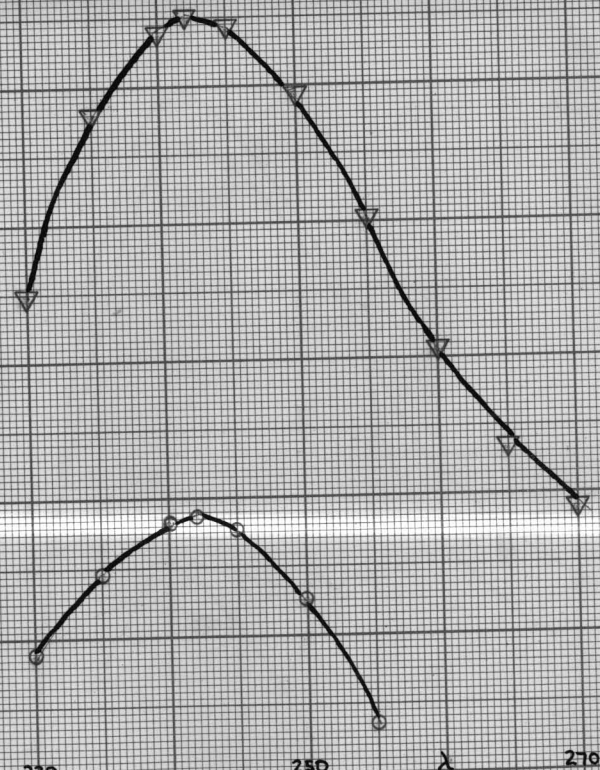
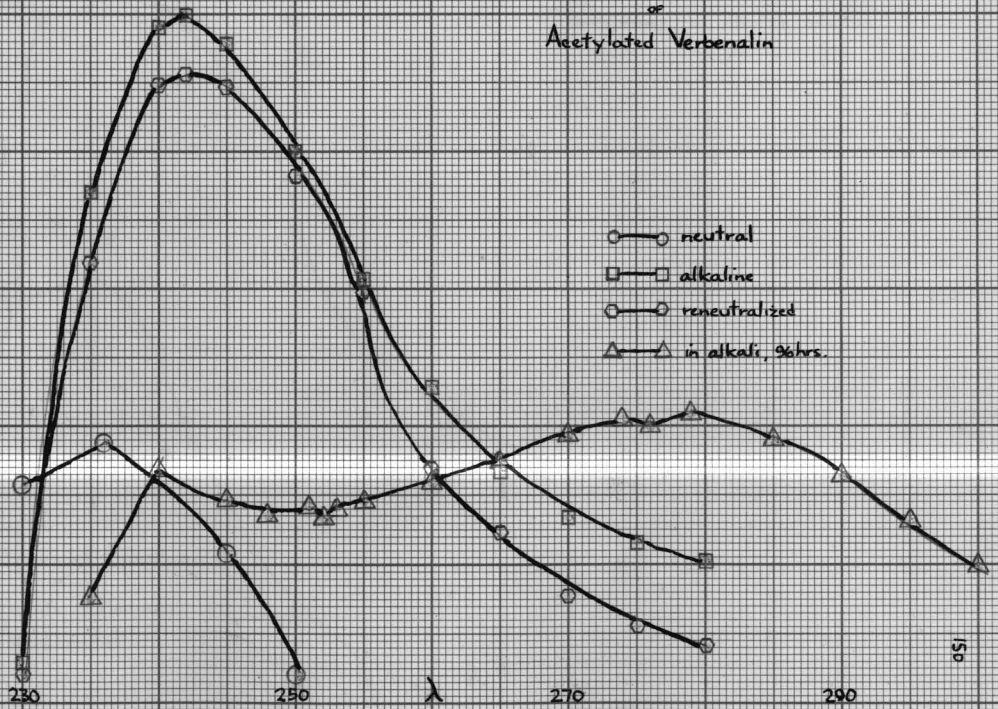


Fig. 7
Ultraviolet Spectrum
of
Methylated Verbenatol

Fig. 8
Ultraviolet Spectrum
of
Acetylated Verbenalin



HUGHES OWENS 315 E 20x20

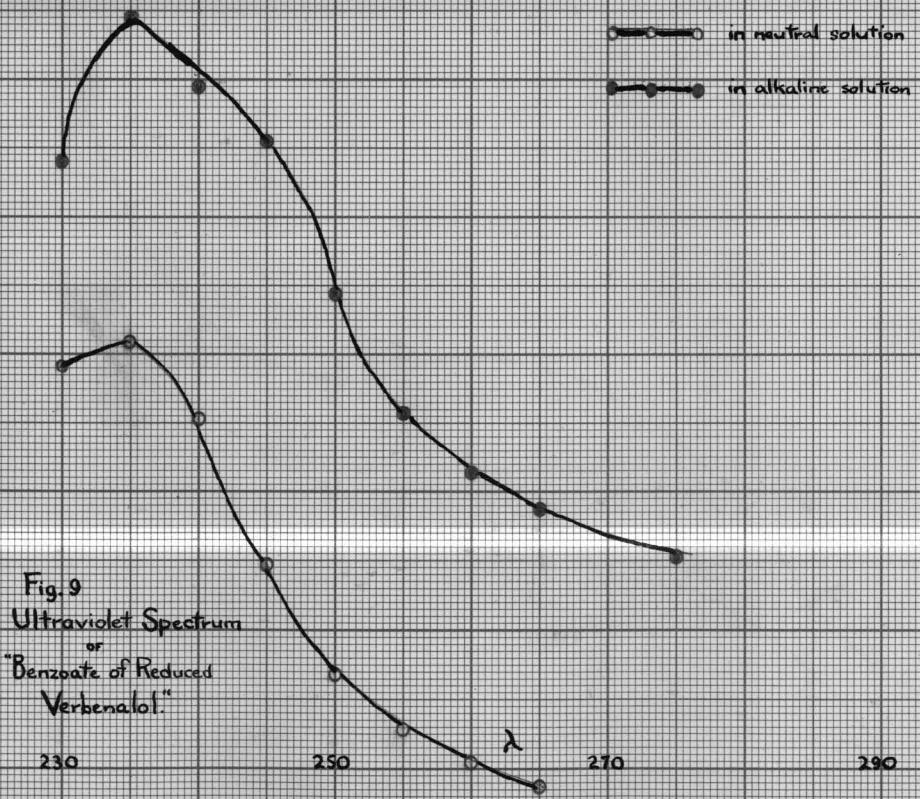
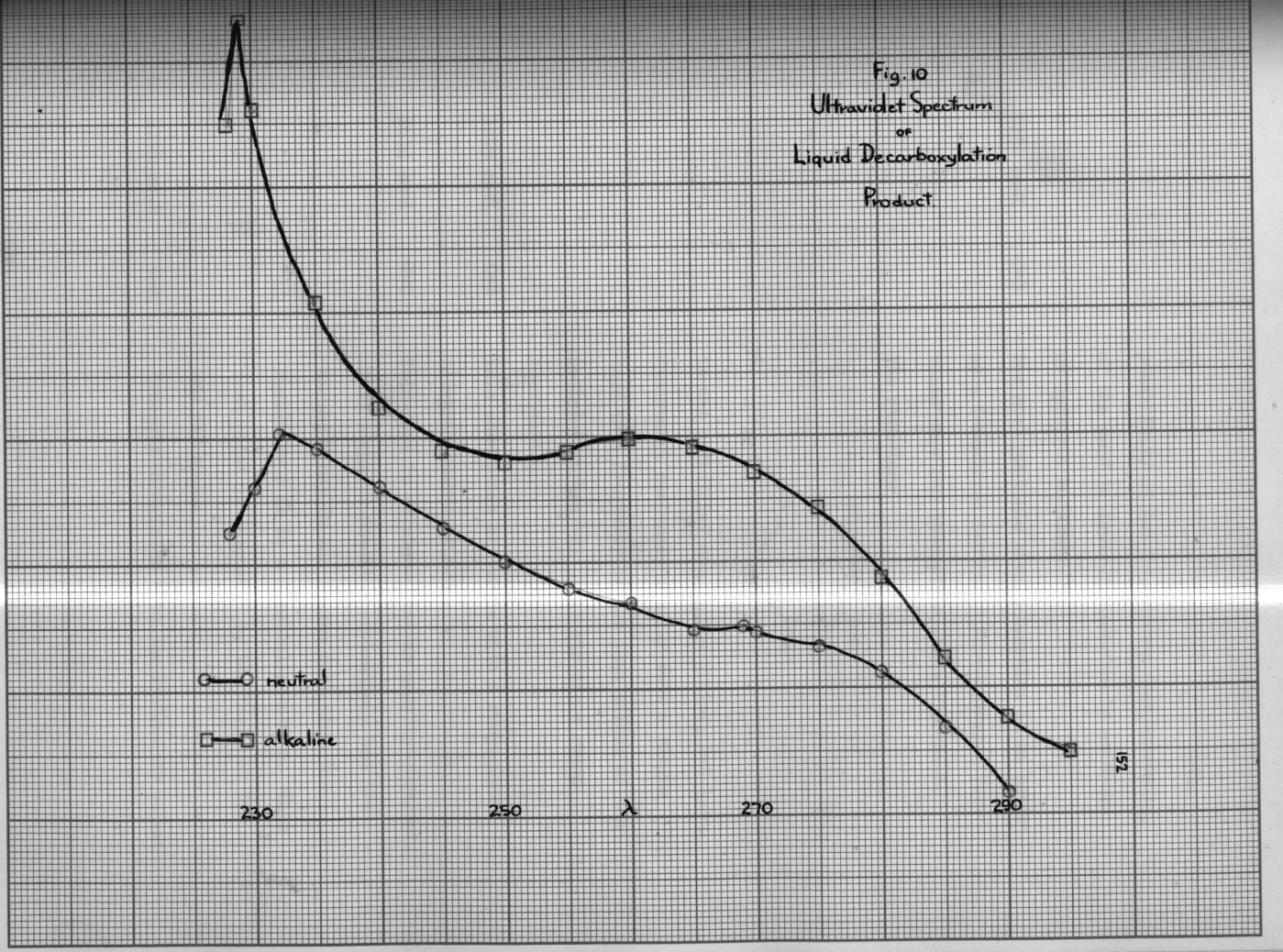


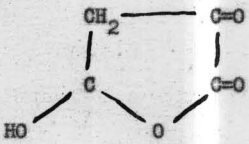
Fig. 9
Ultraviolet Spectrum
of
"Benzoate of Reduced
Verbenalol."

HUGHES OWENS 315.F 20x20


Fig. 10
Ultraviolet Spectrum
of
Liquid Decarboxylation
Product
neutral
alkaline
230
250
λ
270
290
751

possibility of two functions. The broadening of the band to lower frequencies is evidence of hydrogen bonding.

1736 cm-1 C=O The most probable simple function is an ester or a 6-membered lactone. Five-membered lactones show C=O absorption above 1750 cm-1. The structure



is not a familiar one to the writer, but 1,2-dicarbonyl compounds usually show a single band at a frequency higher than either of the isolated carbonyls. This structure seems improbable.

1675 cm-1 C=O This absorption may arise with a conjugated aldehyde ketone or acid.

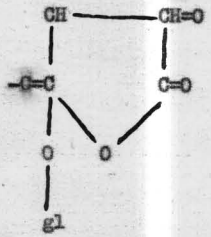
1634 cm-1 C=C This band is stronger than is usually observed. This suggests possible enol-ether, but the strength may be due to the fact that the double bond is in a terminal position. This last point is supported by the bands at 996 and 913 cm-1.

1171 cm-1 C-O Probably associated with the lactone or ester.

1131, 1111, & 1081 cm-1	C-O	Indicative of alcoholic OH groups and/or with C-O-C of an ether. Probably none of the alcoholic groups is primary.
996, 913 cm-1		This pair of bands is suggestive of a terminal vinyl group. An internal CH = CH function should show a strong band near 970 cm-1.

Verbenalin

3495 cm-1	OH	unbonded
3300 cm-1	OH	strongly H-bonded
1739 cm-1	C=C	cf. verbenalol. Also, in the structure



the lactone is a vinyl ester; this would tend to raise the frequency even above that of the simpler five-membered lactone. This structure can be excluded with reasonable certainty.

1692 cm-1	C=O	Aldehyde, ketone or acid, which may be conjugated or non-conjugated. In the verbenalol spectrum conjugation was definitely indicated.
1646 cm-1	C=C	cf. verbenalol

1132 cm-1	C-O	Probably associated with ester or lactone.
1098, 1081-1056 cm-1	C=O	Ether and/or alcoholic OH groups. Those of the sugar residue are not distinguished from others.
1029 cm-1	C-O	Probably primary alcohol.
991, 912 cm-1	C=C	cf. verbenalol

Acetylated Verbenalin

3490 cm-1	?	Probably not an OH band because it is weak. It is probably an overtone of the strong band at 1745 cm-1. This indicates the penta-acetate.
1745, 1721 cm-1	C=O	The strong 1745 cm-1 band is due to the acetates. The weaker 1721 cm-1 band is probably due to the verbenalol moiety. A band near 1736 cm-1 would be obscured. It is odd that the band observed below 1700 cm-1 in verbenalin and verbenalol is not retained. The band at 1721 cm-1 could be due to non-conjugated aldehyde or ketone.
1634 cm-1	C=C	cf. verbenalol
1231, 1215 cm-1	C-O	Due to acetoxy groups.
1071-1031 cm-1	C-O	Alcoholic OH groups.
998, 916 cm-1	C=C	cf. verbenalol

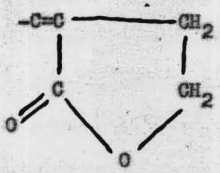
These comments may be helpful in further considering these spectra

since the above interpretations are of necessity the simpler ones.

Aldehydes and Ketones: C=O in the range 1650-1720 cm-1 with conjugated or H-bonded groups occurring below 1700 cm-1, and non-conjugated groups occurring above 1690 cm-1. Note the region of overlapping.

Esters and 6-membered lactones: Near 1736 cm-1. Shifted about 20 cm-1 to lower frequencies by conjugation, and upward about 20 cm-1 if the function involves a phenolic or enolic hydroxyl. These effects may be additive.

5-membered lactones: A double bond in the ring would increase the strain and raise the frequency of the absorption. Conjugation as in the structure:



would lower the frequency of the absorption by approximately 25 cm-1.

General: The writer is not familiar with the absorption of furan rings, so is unable to point to evidence for or against the presence of this structure in verbenalin. Spectra of the two tetrahydro derivatives would probably yield helpful information."

The original spectra represented in Figs. 14, 15, and 16 were obtained through the courtesy of the Science Service Laboratory, Department of Agriculture, London, Canada. The following represents the substance of a personal communication from R. W. White[30] containing a comparative interpretation of the spectra of verbenalol and

tetrahydroverbenalol. It should be noted that the two spectra are quite comparable since each spectra was run on a chloroform solution containing 13 mg. per ml. of verbenalol and tetrahydroverbenalol respectively.

(1) "In the OH stretching region (3650-3100) the relative intensities of the "free" and "associated" hydroxyl absorption have changed slightly and the bands themselves are closer together in tetrahydroverbenalol.

In the OH bending region (1100-1000) there seems to be quite a difference between verbenalol and tetrahydroverbenalol. These two changes could quite possibly indicate the appearance of a new hydroxyl group in tetrahydroverbenalol.

(2) "One of the carbonyl absorption peaks has disappeared in tetrahydroverbenalol and the other has been shifted to a lower frequency (i.e. 1710 shifted to 1680). This could indicate the reduction of a carbonyl to an alcohol and the association of this new hydroxyl with the other carbonyl (1710) causing a shift to 1680.

(3) "The peak at 1640 which ... was assigned to a carbon-carbon double bond is still very much in evidence. However, the peak at 990 in verbenalol (previously accepted as proof of an ethylenic linkage) has disappeared in tetrahydroverbenalol. This creates some doubt as to the peak at 1640 in verbenalol being due to a carbon-carbon double bond. If it were not, then the peak at 1640 would have to be assigned to a carbonyl band strongly associated with the hydroxyl group."

It should be noted that the carbonyl band found at 1710 in

verbenalol, is missing from the spectrum of tetrahydroverbenalin (Fig. 14).

An infrared absorption spectrum of the crystals obtained by palladium-carbon catalytic reduction of verbenalin (see P. 21) was also made available[30]. It contains much fine structure indicative of impurity, but easily seen and worthy of note is the fact that unlike the product of reduction with platinum oxide catalyst (Fig. 14) there appears an absorption peak at 1735. Also present are peaks at 1710, 1695 and 1645. In connection with the same compound, Papineau-Couture[31] reports the complex OH absorption is almost identical with that of verbenalin, "which would suggest that the glycosidic linkage is intact".

Papineau-Couture[31] also comments on several other compounds whose spectra were not made available.

"Benzoate of Reduced Verbenalol"

" 1730 - carbonyl of the benzoate linkage.

1599 and 1506 - aromatic ring of the benzoate.

No OH present.

The possibility that this might be a relatively simple by-product is not excluded.

"Dinitrophenylhydrazone of Decarboxylation Product of Verbenalol"

3290 (weak) - N-H

3105 - C-H in aromatic ring

1619, 1591, 1513 - C=C in aromatic ring

There is no band that can be attributed to a C=O group. Consequently

there is no ester group. Any olefin present would have its characteristic bands obscured by the presence of the aromatic bands."

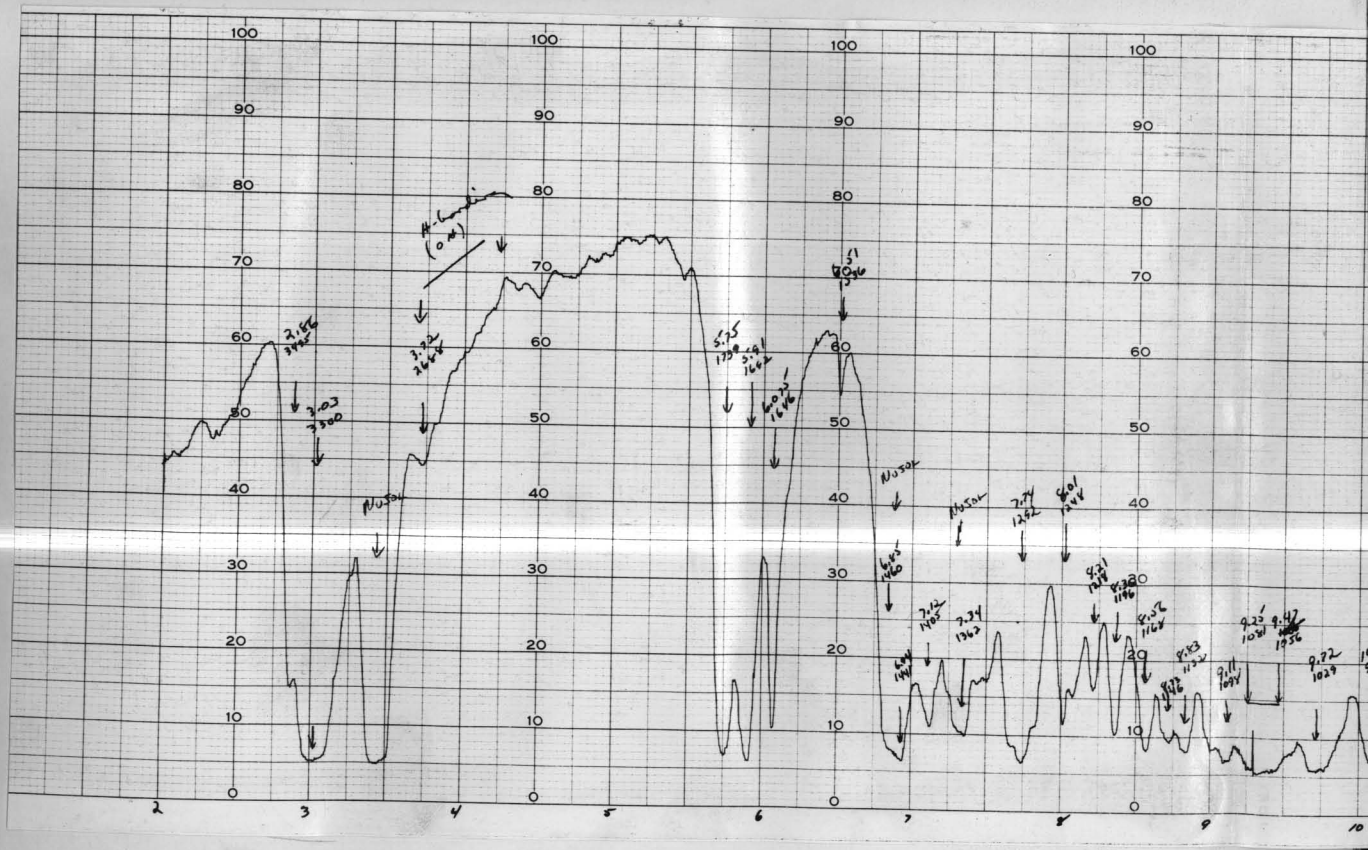
SYNTHESIS OF TYPE STRUCTURES

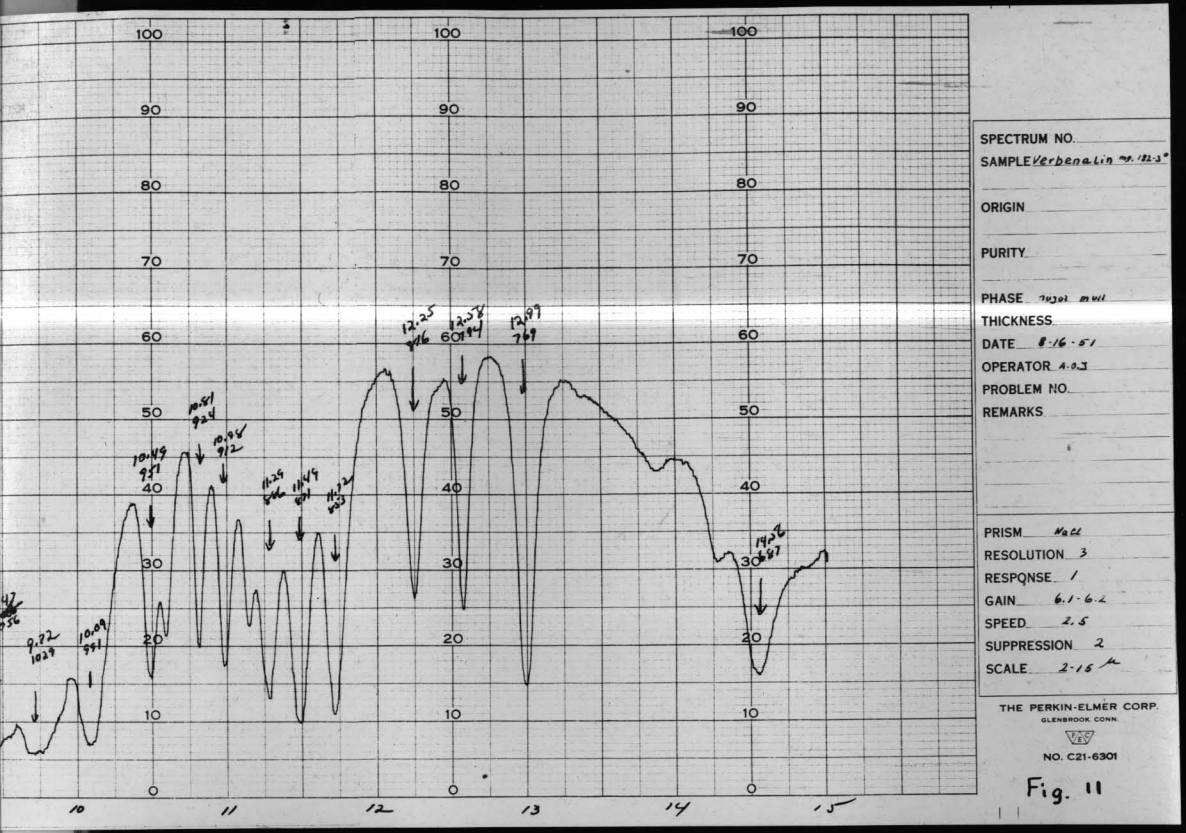
PREPARATION OF SODIUM ETHYL ACETOPYRUVATE[32,33]

Two hundred eighty milliliters absolute ethanol were placed in a one liter round bottom flask, fitted with a reflux condenser, a stirrer and a dropping funnel. Through the condenser, over a period of 2 hours, were added 12.5 g. sodium metal. The mixture was cooled to room temperature, the stirrer started and a mixture of 67.3 ml. (73.0 g.) dried ethyl oxalate (over calcium chloride) and 36.6 ml. (29.0 g.) acetone (dried one week over calcium chloride, then distilled) was added during the next 90 minutes. Stirring was continued for another hour. The mixture was centrifuged and the supernatant discarded. The yellow sodium salt of ethyl acetopyruvate was dried in a vacuum desiccator.

PREPARATION OF ACETOPYRUVIC ACID

Ten grams of powdered sodium ethyl acetopyruvate were dissolved in 150 ml. distilled water and 13.9 ml. 4 N sodium hydroxide were added with constant stirring. Exactly 2 minutes later, the solution was made acid with 3 N sulfuric acid, and the acidic solution was extracted with ether in a continuous two-phase liquid extractor for 36 hours. The ether extract was dried over anhydrous sodium sulfate for 24 hours, and then the ether was removed by distillation at 30° and 10 mm. pressure. The brown oily liquid was set aside in a


(K-Limit)
(0.04)
2.156
3.03
3300
3.32
5.75
1739
5.91
1662
6.023
1646
6.45
1546
7.12
1405
7.34
1362
7.74
1292
8.01
1248
8.31
1203
8.38
1196
8.52
1168
8.63
1152
9.11
1098
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4
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6
7
8
9
10

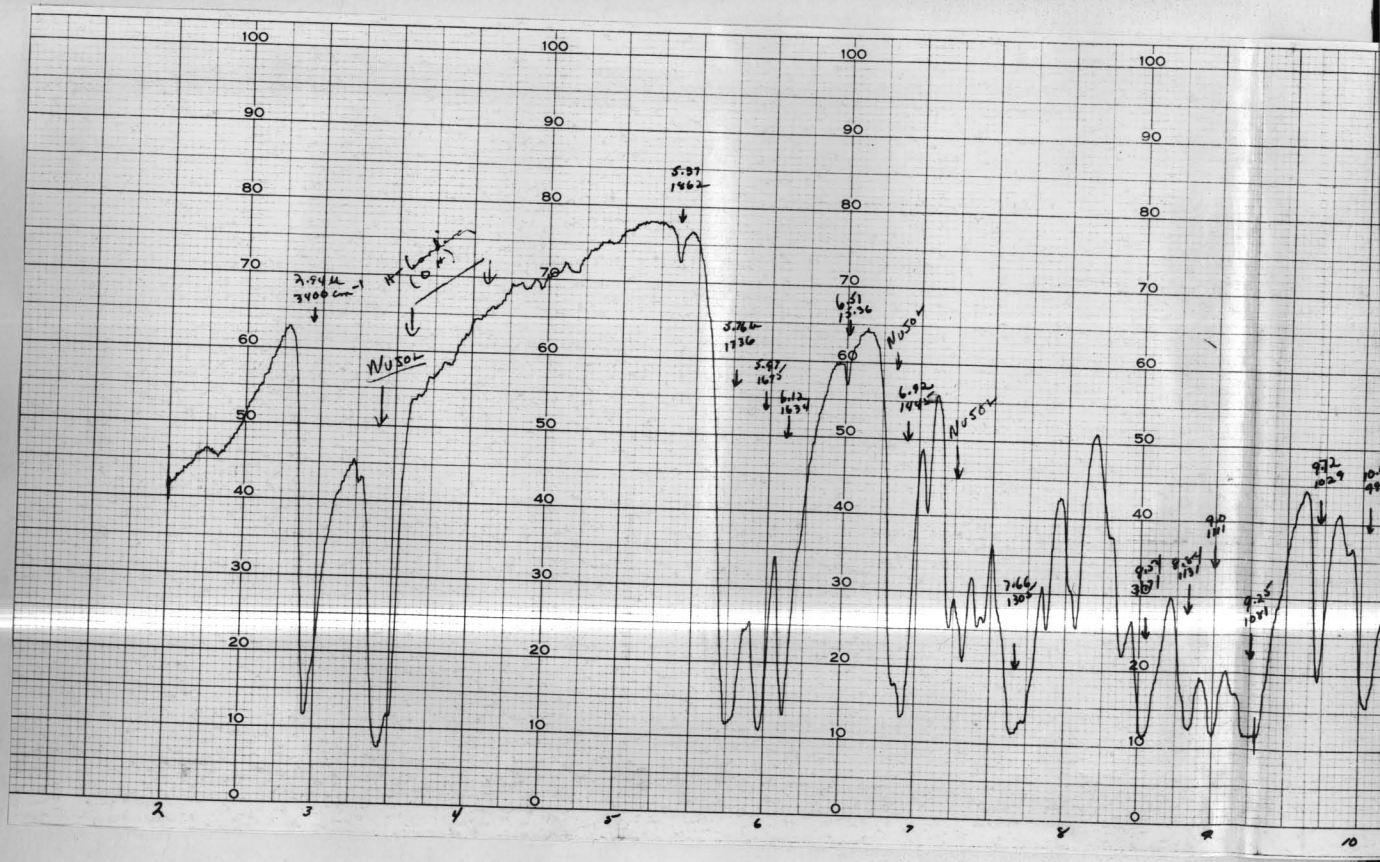


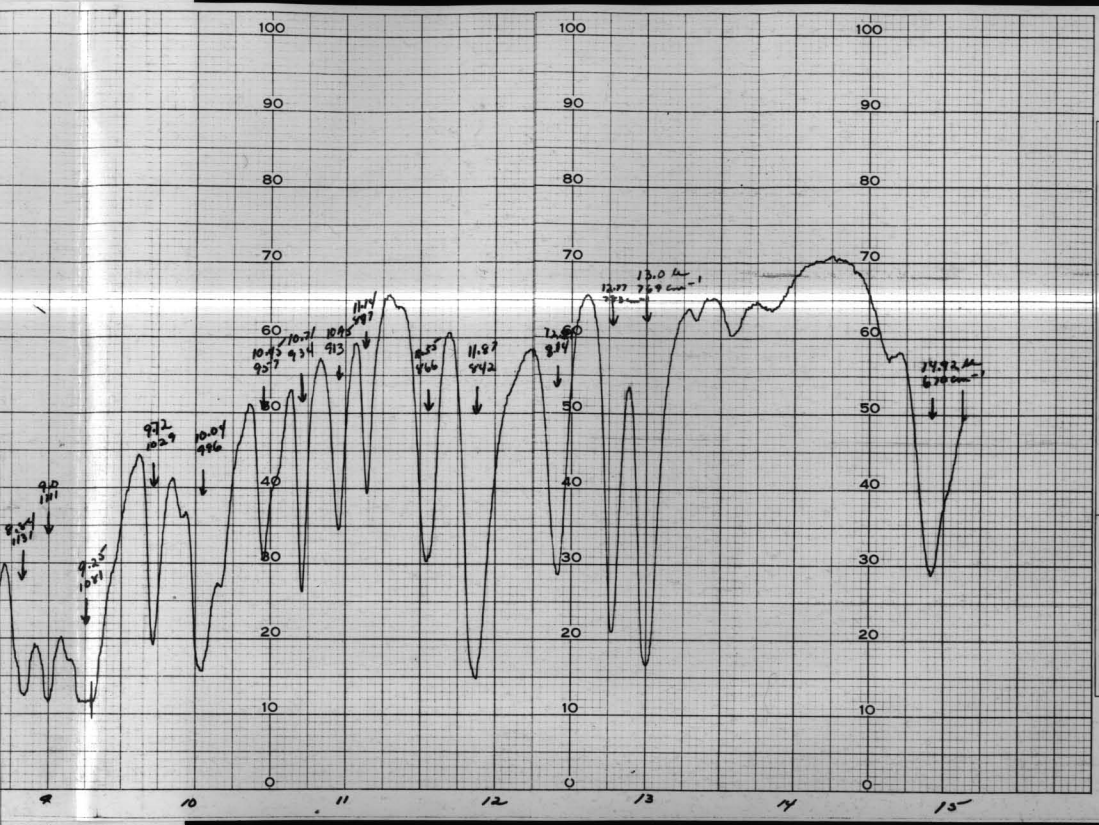
SPECTRUM NO.
SAMPLE Verbenalin mp. 182-3°
ORIGIN
PURITY
PHASE Nujol mull
THICKNESS
DATE 8-16-51
OPERATOR A.O.J
PROBLEM NO.
REMARKS

PRISM Nacl
RESOLUTION 3
RESPONSE 1
GAIN 6.1-6.2
SPEED 2.5
SUPPRESSION 2
SCALE 2-15 μ

THE PERKIN-ELMER CORP.
GLENBROOK, CONN.
NO. C21-6301

Fig. 11


2.94μ
3400 cm⁻¹
NUJOL
5.87
1662
5.76
1736
5.97
1675
6.12
1634
6.51
1536
NUJOL
6.92
1445
NUJOL
7.46
1305
8.54
1171
9.12
1081
9.72
1029
9.35
1041
2
3
4
5
6
7
8
9
10

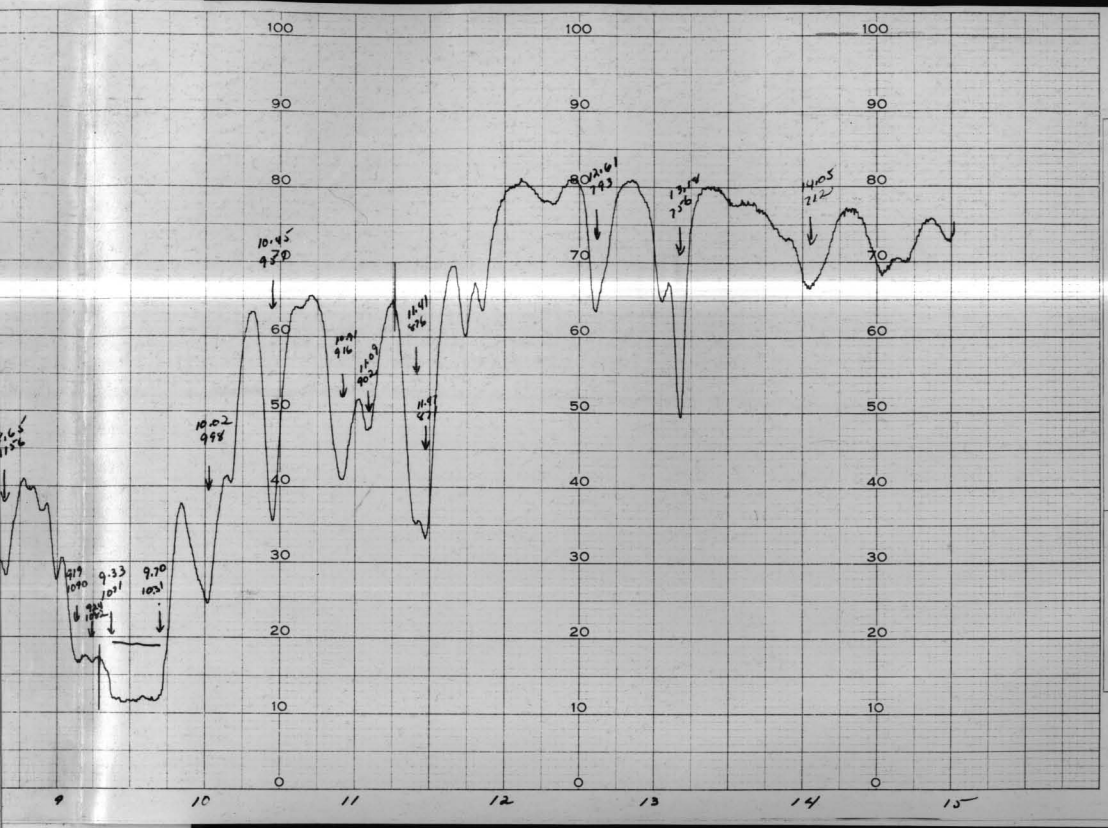


SPECTRUM NO.
SAMPLE Verbenalol
m.p. 131.5 - 132.5°
ORIGIN
PURITY
PHASE Nujol mull
THICKNESS
DATE 8-16-57
OPERATOR A.D.J.
PROBLEM NO.
REMARKS

PRISM Salt
RESOLUTION 3
RESPONSE 1
GAIN 6.1 - 6.2
SPEED 2.5
SUPPRESSION 2
SCALE 2 - 15 μ

THE PERKIN-ELMER CORP.
GLENBROOK, CONN.
NO. C21-6301

Fig. 12



SPECTRUM NO.

SAMPLE *Acetylated Verbenalin*
mp. 132.5 - 133.5°

ORIGIN

PURITY

PHASE *wet mull*

THICKNESS

DATE *8-16-51*

OPERATOR *A.O.J.*

PROBLEM NO.

REMARKS

PRISM *NaCl*

RESOLUTION *3*

RESPONSE *1*

GAIN *6.1 - 6.2*

SPEED *2.5*

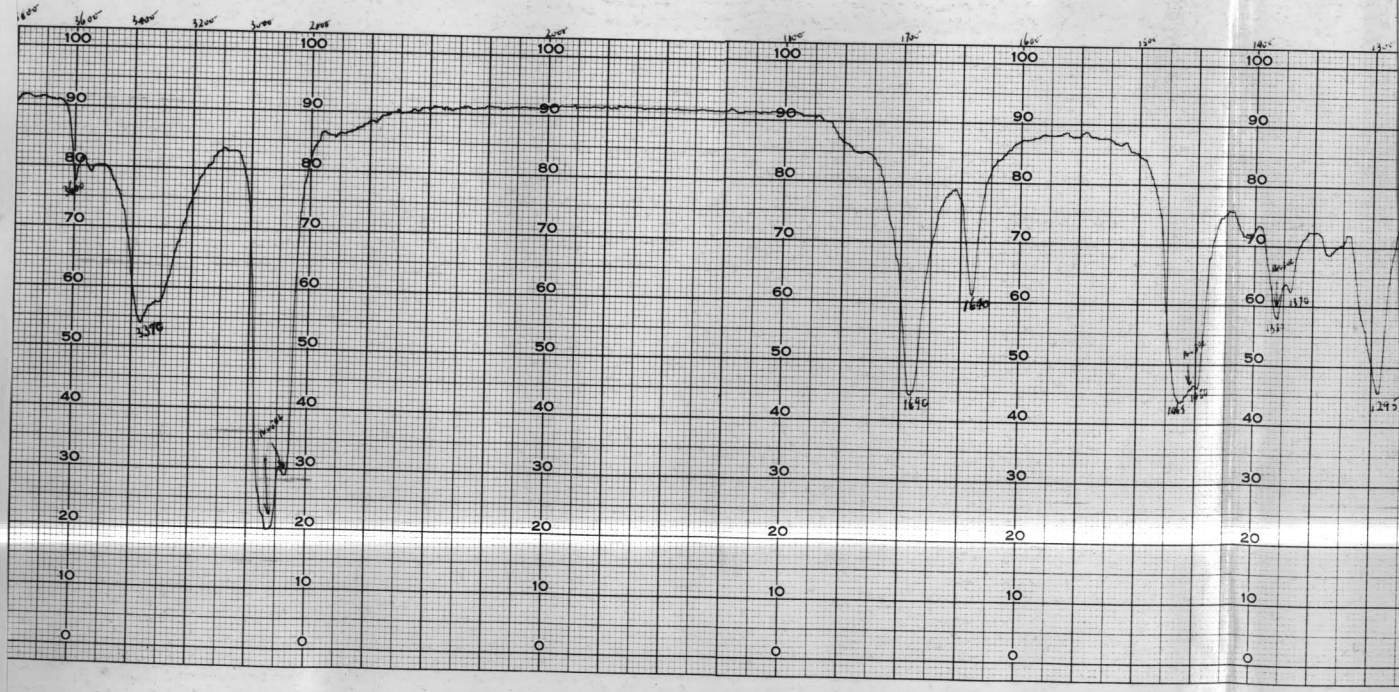
SUPPRESSION *2*

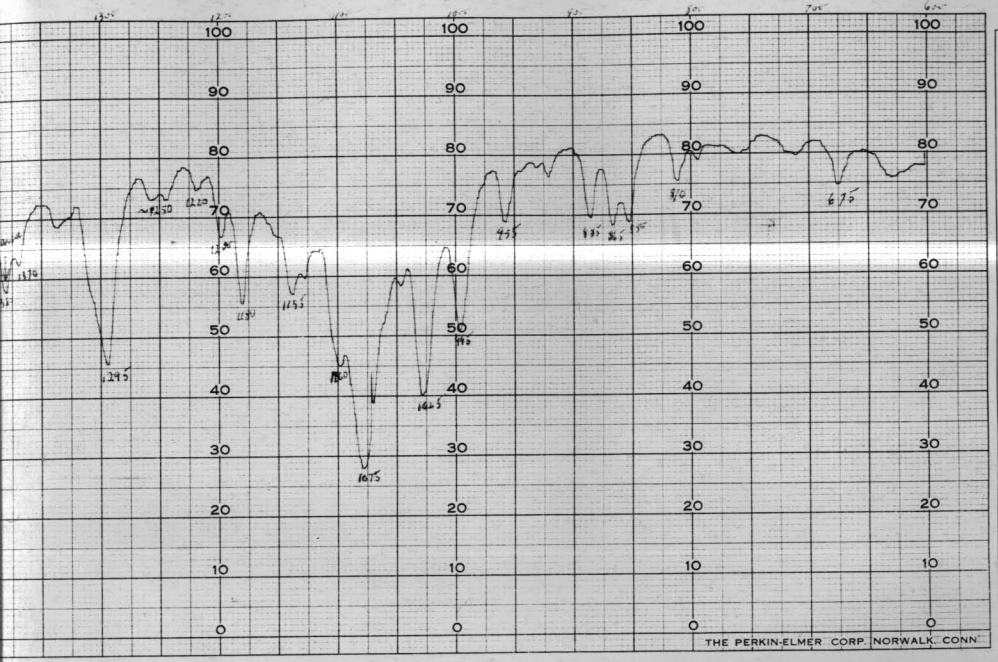
SCALE *2 - 15 μ*

THE PERKIN-ELMER CORP.
GLENBROOK, CONN.

NO. C21-6301

Fig. 13


100
90
80
70
60
50
40
30
20
10
0

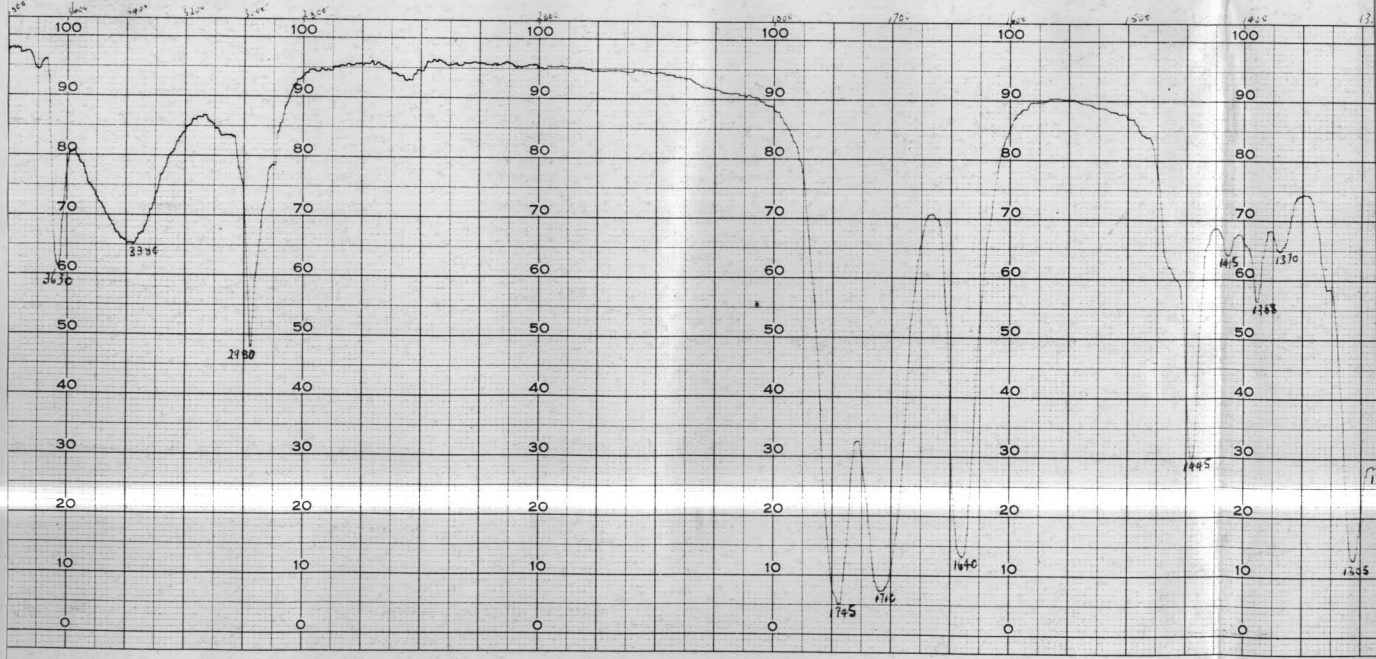


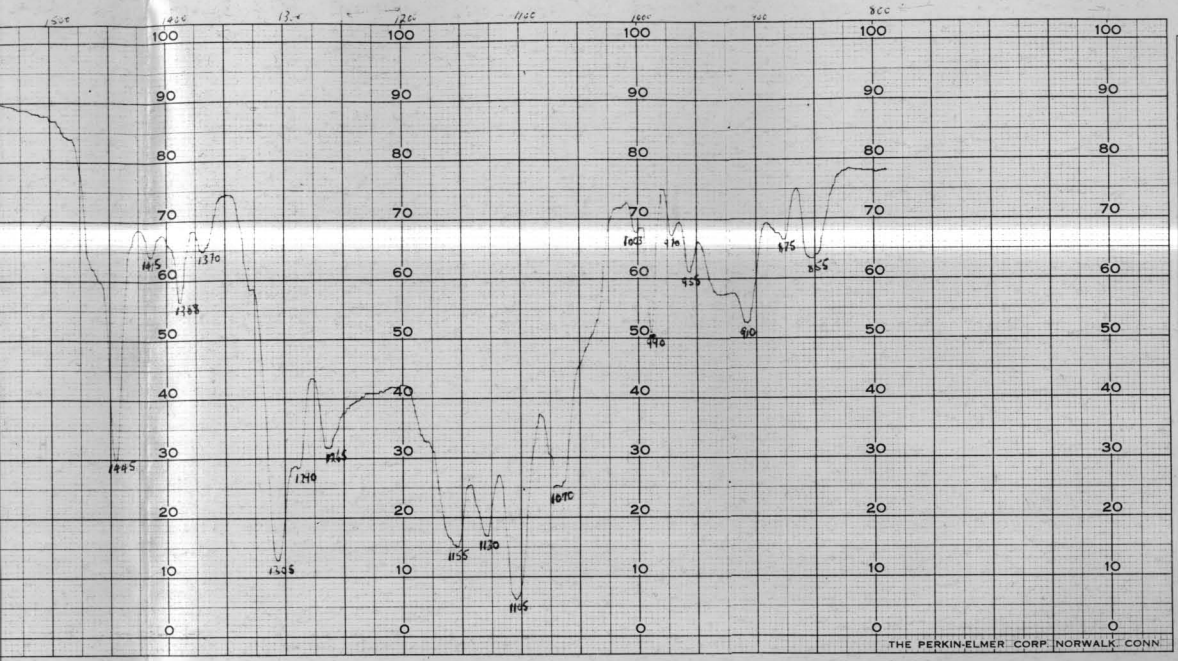
SPECTRUM NO.
SAMPLE Tetrahydrofuran-perchloroethylene
ORIGIN
PURITY
PHASE Neat Film
THICKNESS
1.
2.
3.
DATE 23 Nov 53
OPERATOR
REMARKS

PRISM
RESOLUTION
RESPONSE
GAIN
SPEED
SUPPRESSION
SCALE

Fig. 14

NO. C21-6308


100
90
80
70
60
50
40
30
20
10
0
3636
3396
2980
1745
1640
1445
1370
1303



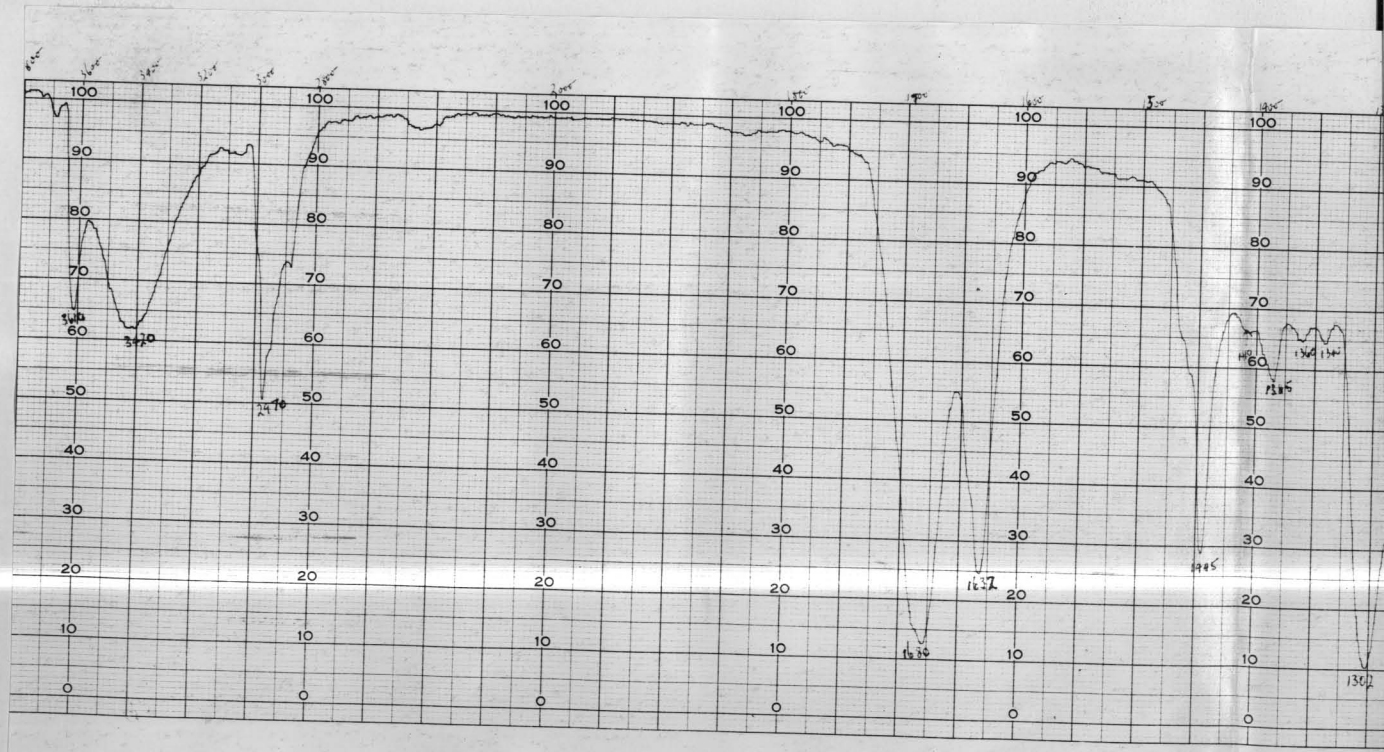
SPECTRUM NO.
SAMPLE
Lanthanol
ORIGIN
PURITY
PHASE $CHCl_3$ soln ~13 mg/ml
THICKNESS 0.5 mm
1.
2.
3.
DATE 25 Nov '53
OPERATOR K. L. W.
REMARKS

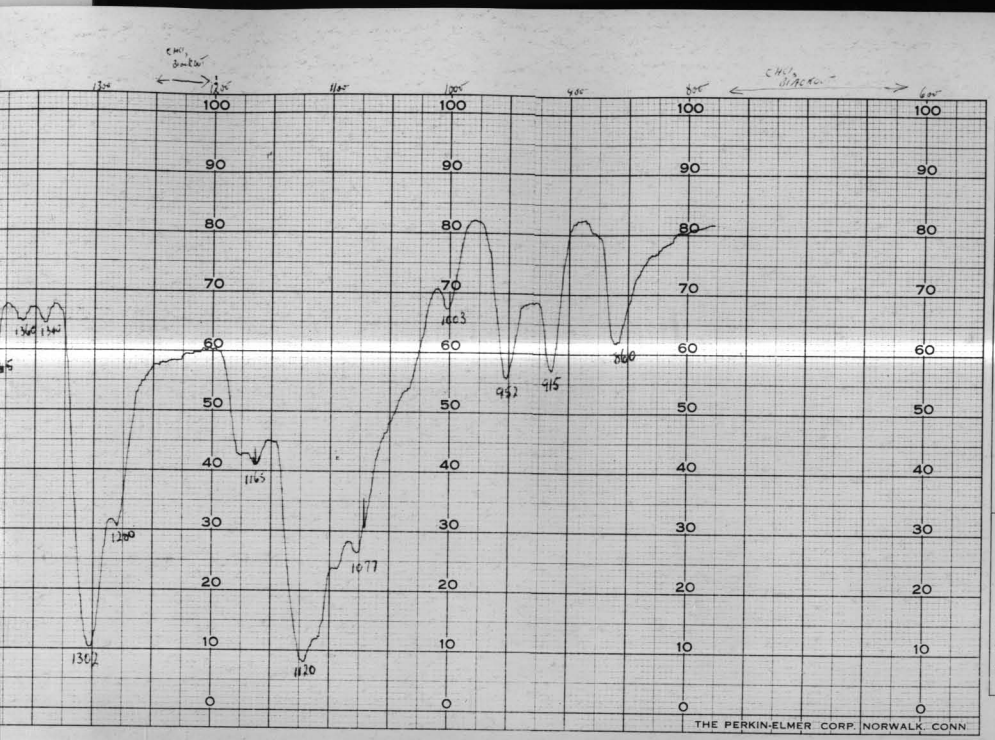
PRISM NaCl
RESOLUTION 927
RESPONSE 1:1
GAIN 5
SPEED 2
SUPPRESSION 2
SCALE 3800 – 2600 @ 100 cm/4 cm
2600 – 800 @ 100 cm/4 cm

THE PERKIN-ELMER CORP. NORWALK, CONN.

Fig. 15

NO. C21-6308


100
90
80
70
60
50
40
30
20
10
0



SPECTRUM NO.
SAMPLE Tetrahydrofuran-carbonyl-d6
ORIGIN
PURITY
PHASE $CHCl_3$ soln ~ 13 mg/ml
THICKNESS 0.5 mm
1.
2.
3.
DATE 17 Nov. 53
OPERATOR R.N.H.
REMARKS

PRISM NaCl
RESOLUTION
RESPONSE
GAIN
SPEED
SUPPRESSION
SCALE

Fig. 16

NO. C21-6308

vacuum desiccator to crystallize, which it did in 72 hours. The crude crystals were sublimed *in vacuo* immediately prior to their use in alcoholic solution for ultraviolet absorption spectra studies.

PREPARATION OF METHYL ACETOPYRUVATE[34,35]

Freshly-cut sodium metal (2.30 g.) was added over the period of 1 hour to 60 ml. absolute methanol. To this solution was added a mixture of 7.3 ml. dry acetone and 13.5 ml. dry ethyl oxalate over a period of one hour with constant sitrring. The mixture was stirred 4 hours longer, then let stand overnight.

The mixture was cooled to 0° in a salt-ice bath and a cooled solution of 3 ml. concentrated sulfuric acid in 20 ml. distilled water was added quickly. The mixture was stirred for 15 minutes and then poured into 100 ml. distilled water. The yellow solution was extracted by shaking in a separatory funnel with three 60 ml. portions of benzene. The benzene extracts were combined, washed twice with water, and dried for 24 hours over anhydrous sodium sulfate. Most of the benzene was then removed by distillation at atmospheric pressure. Crystals were obtained which were pale yellow in color and which after drying, had m.p. 61.5-62.5°, weight 3.6 g.

PREPARATION OF 2-PHENYL-3-ACETYL-4,5-DIKETOTETRAHYDROFURAN[36]

A mixture of 1.7 g. methyl acetopyruvate, 1 ml. benzaldehyde and 3 drops diethylamine was dissolved in a small quantity of absolute methanol and set aside for a few days at refrigerator temperature. Most of the solvent was removed by distillation *in vacuo*, upon which long pale yellow needles appeared. The crystals were filtered off,

washed with cold absolute alcohol, dried, m.p. 62° (apparently starting material).

A mixture of 1.7 g. methyl acetopyruvate, 1 ml. benzaldehyde and 3 drops of diethylamine was placed in a small beaker and heated slightly to effect homogeneity. After several hours, the mixture set to a yellow crystalline mass, impure in appearance. The mass was dissolved in alcohol, concentrated and set aside. In three hours, a mass of white needles was filtered off, washed with cold absolute alcohol and dried in a vacuum desiccator, m.p. 173-4°. Kaskin's[36] product melted at 165-6°, Ruhemann's[37] at 170-1°. The product was recrystallized twice from alcohol and submitted for analysis, m.p. 168-168.5° (calc. C = 66.05%, H = 4.62; found C = 65.63%, H = 4.66%). The product gave a deep red color with ferric chloride solution as reported by Ruhemann[37] and also dissolved in cold sulfuric acid to form a yellow solution from which it was precipitated by the addition of water.

ATTEMPTED PREPARATION OF 3-ACETYL-4,5-DIKETOTETRAHYDROFURAN

In a small beaker were placed 0.85 g. methyl acetopyruvate, 5 ml. absolute methanol and 2 drops diethylamine. Into the solution was bubbled 0.15 g. formaldehyde (generated by heating trioxymethylene) and the mixture was set aside. Twenty-four hours later, the solution was heated to drive off the methanol and the sticky orange residue was dissolved in dioxan, concentrated and set aside. A very few small crystals appeared several hours later. A melting point determination on a micro scale showed slow melting and decomposition

above 185°. There was insufficient material obtained for analysis. However, ultraviolet absorption spectrum studies were undertaken on this product.

ATTEMPTED PREPARATION OF 2(N-HEXYL)-3-ACETYL-4,5-DIKETOTETRAHYDRO-FURAN

One milliliter n-heptaldehyde and 3 drops diethylamine were placed together in a small beaker and 0.85 g. methyl acetopyruvate was dissolved in the mixture with the aid of a little heat. The mixture was set aside at refrigerator temperature. After two weeks, the orange thick syrup was dissolved in alcohol, concentrated and set aside. Nothing crystalline was obtained. The remaining solvent was removed by distillation *in vacuo* and the syrupy residue was dissolved in hot ethyl acetate, then concentrated and set aside. Nothing crystalline was obtained.

ATTEMPTED DECARBOXYLATION OF 2-PHENYL-3-ACETYL-4,5-DIKETOTETRAHYD-ROFURAN

In the decarboxylation apparatus, previously described for verbenalin and verbenalol, 0.5025 g. 2-phenyl-3-acetyl-4,5-diketo-tetrahydrofuran was refluxed with 75 ml. 1.934 N sulfuric acid for 7 1/2 hours, two traps, each containing 100 ml. 0.0881 N barium hydroxide, being set up to catch any carbon dioxide entrained in the nitrogen passed continuously through the refluxing mixture. Just a trace of precipitate appeared in the traps, indicating that the compound was not subject to decarboxylation under the same conditions as verbenalin and verbenalol. After cooling to room temperature, crystalline material settled out. This was filtered off and dried,

m.p. 165-7° (weight 250 mg.). A mixed melting point with the starting material showed no depression. The filtrate and washings of the unreacted material were titrated with 63.93 ml. 2.120 N sodium hydroxide ≡ 135.53 ml. 1 N sodium hydroxide. The 75 ml. 1.934 N sulfuric acid in the apparata during refluxing is equivalent to 145.05 ml. 1 N sulfuric acid, showing that no acid was generated during the reflux period. The neutralized mixture (after titration) was distilled under reduced pressure, and there was obtained a clear distillate with an aromatic (perfume-like) odor which gave a positive test with fuchsin-aldehyde reagent.

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CONCLUSIONS

1. The partial structures proposed by Chatterjee and by Young (i.e. α-keto lactones) have been disproved by the behavior of 2-phenyl-3-acetyl-4,5-diketotetrahydrofuran, and by consideration of infrared absorption spectra.
2. (a) The glycosidic linkage (through the enol of an aldehyde) postulated by Asano, Ueno and Tamaki has been disproved by consideration of infrared absorption spectra.

   (b) The furan ring in the structure postulated by Asano, Ueno and Tamaki, has been placed in serious doubt by consideration of the functions which must be attributed to the five oxygen atoms of verbenalol, and by the rate of ozonization.

   (c) The carbomethoxy structure postulated by Asano, Ueno, and Tamaki has been discredited by considerations of the functions in which verbenalol's five oxygen atoms are involved, and by the number and distribution of degrees of unsaturation in the molecule.
3. Ozonization and ozonolysis of verbenalol have been demonstrated to produce acetic acid.
4. Ketonic hydrolysis by means of 5% aqueous alkali has been shown to produce a 7-carbon fragment whose 2,4-dinitrophenylhydrazone has been prepared.
5. Lithium aluminum hydride reduction of verbenalol has been shown to produce a 6-carbon dihydroxy compound, whose dibenzoate has been prepared. In addition, it has been established that methanol is cleaved from verbenalol during this reduction.

6. From the residue of glycosidal hydrolysis (after the removal of crystalline verbenalol) have been prepared 2,4-dinitrophenyl-hydrazones of two carbonyl-containing compounds, one having 7 carbons, the other 14.

7. The method of hydrolyzing verbenalin developed by Asano, Ueno and Tamaki, has been demonstrated to give the best yields of aglycone.

8. Two alternate structures have been advanced for verbenalol.

9. More work is required to elucidate the structure of verbenalol.

SUMMARY

1. Degradation studies have been continued on verbenalin, the glycoside of Verbena stricta, and on verbenalol, its aglycone.

2. Attempts were made to find methods of hydrolyzing verbenalin which would give better yields of aglycone.

3. A compound, similar in type to the partial structures proposed for verbenalol by Chatterjee and by Young, was prepared and its comparative behavior was studied.

4. Infrared absorption spectra of verbenalin and verbenalol and of some related compounds have been prepared and reported.