A CHEMICAL STUDY
OF
SOME TRITERPENOID COMPOUNDS

By

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INTRODUCTION

During the past half-century the attention of many plant chemists has been focused on a number of high melting, crystalline substances of non-nitrogenous composition. A large number of these natural products has been investigated and assigned formulae containing 30 to 31 carbon atoms. These compounds were at first only vaguely characterized and each was usually designated by some name indicative of its botanical origin.

The term triterpene probably originated from the fact that the underlying hydrocarbon of these derivatives has a composition of $C_{30}H_{48}$, just three times that of the terpenes, $C_{10}H_{16}$. This nomenclature is in line with that of Wallach 1) who classified the terpenes into the various groups such as hemiterpenes, terpenes, sesquiterpenes, etc. Since he gave the terpenes the composition $C_{10}H_{16}$ and the diterpenes $C_{20}H_{32}$, it is logical to assign the name "triterpene" to the $C_{30}H_{48}$ group. In 1887, Vesterberg, 2) following the classification of Wallach, stated that "The amyrilenes (hydrocarbons corresponding to the alcohols, α- and β-amyrin) belong evidently to a class of terpenes which we can name triterpenes, because they have three times the molecular weight of the regular terpenes, $C_{10}H_{16}$."

Most of the naturally occurring triterpenoid compounds may be generally divided into the triterpene alcohols and the triterpene hydroxy-acids. They usually occur in the free state in resins and combined as glycosides in saponins. Although some hydroxy-ketones and hydroxy-aldehydes have been isolated, the great majority of the natural products fall into one of the above two groups. The presence in all of these compounds of a secondary hydroxyl group, on a hydroaromatic ring, which in most cases readily forms acetyl and benzoyl derivatives, has been responsible for their classification as sterols by many early investigators. Many of the characteristic color and chemical reactions of the sterols are given by the triterpenes. In addition, the general sources and methods of isolation are similar to those of the sterols. Because of these facts and the general structural similarity, it is not surprising that the error in the classification of the triterpenes was made.

Haworth[3] has divided these substances into two main groups. The first includes the sterols and certain sapogenins. These are characterized by the fact that they yield 3'-methyl-1,2-cyclopentanophenanthrene on dehydrogenation. The second group is made up of substances occurring in resins and many saponins which give homologs of naphthalene on dehydrogenation with selenium. The members of this latter group are the so-called "triterpene derivatives".

The determination of the molecular formulae of the triterpenes has presented numerous difficulties. Purification is often troublesome, combustion results frequently fail to distinguish between homologous formulae, cryoscopic determination of molecular weight has proved unreliable, and it is therefore not surprising that the molecular formulae of many of these compounds have been subjects for controversy. Recent investigations, embodying careful micro-combustions, determination of equivalents of acids by micro-titrations, and determination of the acetyl value of acetates and the methoxyl value of esters, have shown that most of these compounds contain thirty carbon atoms.

Work on the structural details of the triterpenes is complicated by the fact that functional groups are frequently difficult to detect. The detection of the number of double bonds is also difficult in many cases. Tetranitromethane has proved a useful reagent for this purpose, but even it will not react with α, β -unsaturated ketones. Many double bonds are also inert to catalytic hydrogenation. A useful method for the detection of double bonds in the unsaturated triterpene acids depends on the conversion by bromine to the crystalline bromolactones, from which the acid is regenerated by the action of zinc and acetic acid.[4]

From the number of double bonds and the molecular formulae, it has been shown that the triterpenes possess a pentacyclic structure of the picene type, since, with one exception, all yield 1,2,7-trimethyl naphthalene (sapotaline) on selenium dehydrogenation. Degradation of several to 1,8-dimethyl picene also lends credence to this observation. Although the pentacyclic structure has been confirmed by all workers in the field, the exact structures of the members of the triterpene series are still points of conjecture. No one structure which completely explains all the observed properties has been advanced, although several proposed structures approach this goal.

Ruzicka[5] has found that certain subgroups of the triterpenes show an unmistakable similarity in their structures and reactions and he has classified them into four subgroups: those of squalene (uncyclized), of α-amyrin, of β-amyrin, and of lupeol. Through common transformations, the relationships of most of the triterpenes to one or more of these subgroups has been demonstrated.

The triterpenes have found little use in commerce thus far. Some, such as dammarolic acid, ursolic acid, and oleanolic acid and some of the alcohols, have been used in the varnish and lacquer industry to increase gloss. The drying times of varnish or nitrocellulose lacquers in which ursolic or oleanolic acid have been dissolved have been shown to be greatly extended.6) Oleanolic and ursolic acids have also been suggested for use in cosmetic preparations. Pharmaceutically and chemically they are of interest primarily because of their close relationship to products of therapeutic value such as the sterols.

References

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4) Winterstein, A. and Stein, G., Z. physiol. Chem. 199, 46 (1931).

5) Ruzicka, L. and Marxer, R., Helv. Chim. Acta 23, 144 (1940).

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I. CHARACTERIZATION OF A TRITERPENE DIOL FROM *ARCTOSTAPHYLOS UVA-URSI* (LINNÉ) SPRENGEL

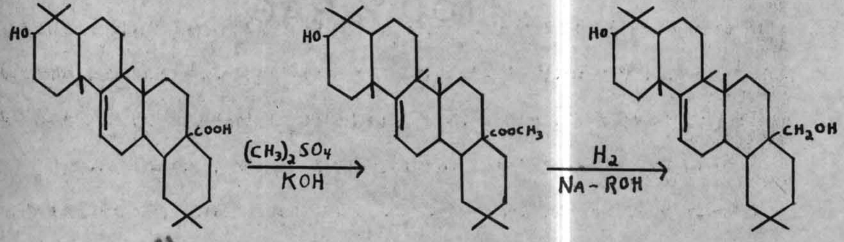
Introduction

In 1938, Parks[1] reported the isolation of a high molecular weight alcohol from Arctostaphylos Uva-Ursi (Linné) Sprengel. The crude drug had been extracted with ether and the deposit which formed in the solvent filtered off. The filtrate was concentrated, petroleum ether added, and the precipitate which formed filtered off. This filtrate was concentrated and 80 grams of dark-green material precipitated. This was extracted, first with sodium carbonate solution, and then with sodium hydroxide solution. The sodium hydroxide-insoluble portion was extracted with petroleum ether and the substance isolated, after several crystallizations, melted at 217-218°C.(uncorr.). An acetate melting at 128-129°C.(uncorr.) was prepared and saponification gave a product melting at 219-222°C.(uncorr.). An approximation of the carbon and hydrogen values indicated 79.8 per cent carbon and 11.5 per cent hydrogen.

A search of the literature in an attempt to locate the unidentified alcohol among products previously isolated was unsuccessful. Parks stated that it "very probably (was)-a new representative of a group of polycylic alcohols, commonly referred to as 'triterpene alcohols', a number of which have been isolated from natural sources." He added that "the prediction is strengthened by the fact that ursolic acid, a 'triterpene acid', exists in the plant and it is not inconceivable that upon decarboxylation ursolic acid would yield such an alcohol." It is also possible that ursolic acid might be the oxidation product of a diol contained in Uva Ursi.

The work was extended by Feiertag[2] in 1938-40, under the direction of Parks. He developed a shorter method of isolation of the alcohol from the plant and extracted a considerable quantity of the unidentified alcohol, the crude material melting at 208-210°C.(uncorr.). The acetate of this material melted at 128°C.(uncorr.) as did that prepared by Parks. However, the purified alcohol obtained by fractional crystallization melted at 238-238.5°C.(uncorr.). Two fractions of the acetate were obtained, one melting at 151-152°C.(uncorr.) and the other at 149-152°C.(uncorr.). It was concluded that the original material was a mixture and that the compound which Parks obtained was also a mixture. The identity of the contaminating material has not been investigated. A comparison of the properties of the purified material obtained by Feiertag with those of a diol prepared from ursolic aldehyde by Ruzicka[3] indicated that the Uva Ursi alcohol might be a diol, corresponding to the reduction product of ursolic acid.

The work which is reported in the following was begun with the object in mind of showing the relationship of this unidentified alcohol from Uva Ursi to ursolic acid, and to characterize the compound as a diol by partial synthesis from ursolic acid, the constitution of which has been fairly well established. If the carboxyl group of ursolic acid were reduced to a primary alcohol group, a diol would be obtained which might very conceivably be identical with the natural product described above. It was decided that reduction of the methyl ester would probably be the most fruitful approach. The reaction may be graphically represented by the following equations:



Ursolic Acid4)   Methyl Ursolate   Uva Ursi Diol?

While this work was in progress, it was learned that the Japanese workers, Husii (Fujii) and Ōsumi,5) had reported in 1939 the isolation of a diol, which they named "uvaol", from Uva Ursi. It had a melting point of 232-233°C. and its diacetate derivative melted at 158-159°C. This diol was also isolated, along with ursolic acid, from the leaves of *Leucothoe Keiskei* Miq. by Simada6) in 1939. An identical diol was prepared by Husii and Ōsumi7) by reduction of the phenyl ester of acetyl ursolic acid with sodium and amyl alcohol. It is very probable that this diol is the same as the one described in this report although the melting points reported for uvaol are higher. If such is the case, the work herein described confirms that of the Japanese workers.

The experimental work which follows is a description of the partial synthesis of the Uva Ursi diol from ursolic acid, the purification of some of the natural diol isolated by Feiertag, and a comparison of the properties of the two and their acetate derivatives. All melting points herein reported are corrected by calibration of the thermometer used with a Bureau of Standards approved set of Anschütz thermometers.

Experimental

Purification of Ursolic Acid

A fairly large quantity of crude ursolic acid obtained by Dr. Parks from Uva Ursi was crystallised from methyl alcohol. A considerable amount of the material did not dissolve in the hot solvent and was filtered off. The filtrate was chilled in an ice bath and the white crystals which formed were found to melt at 282-283°C. Recrystallization from ethyl alcohol raised the melting point to 283.5-284.5°C. Another crystallization from the same solvent raised the melting point to 285-287°C.

The methyl alcohol-insoluble portion from the first crystallization was recrystallised from ethyl alcohol and was again not entirely soluble. The mixture was filtered while hot and the residue dried. It melted at 281-283°C. The filtrate was cooled in an ice bath and the crystals obtained were likewise found to melt at 281-283°C. These two fractions were combined and recrystallised from ethyl alcohol, the melting point rising to 285-286.5°C. All of the mother liquors from the above were combined and concentrated. The crystals obtained melted at 282-284°C. The extreme difficulty encountered in trying to separate ursolic acid from minute amounts of impurities accounts for the variation in melting points. The tenacity with which the compound apparently holds impurities is evidenced by the great variation of melting points reported in the literature for it.

Preparation of Methyl Ursolate

To 3 grams (0.0066 mole) of ursolic acid dissolved in 90 ml. of absolute methyl alcohol was added 3.7 ml. of a methyl alcoholic solution of potassium hydroxide, containing 0.06 gram per ml. To this was added 1.04 gram (0.0083 mole) of dimethyl sulfate and the mixture was refluxed for 4.5 hours on a steam bath. At the end of this period, the reaction mixture was poured into water and a heavy, white precipitate formed. This was filtered off, dried, and dissolved in ether. The ether solution was washed with three 100 ml. portions of 3 per cent aqueous sodium hydroxide solution and once with water. During the first washing a quantity of flocculent, white material was precipitated. Lesser amounts were precipitated in the following washings. These precipitates were filtered off by suction after each washing. When the water washing was added to the sodium hydroxide washings, a flocculent precipitate formed. This was filtered off and combined with the other precipitates.

The ether solution was allowed to evaporate to dryness and the residue dried and crystallized from ethyl alcohol. A yield of 0.17 gram of white, needle crystals melting at 169-169.5°C. was obtained. When the mother liquor was evaporated to dryness, a hard residue remained. This was crystallized from ethyl alcohol and 0.92 gram of white crystals also melting at 169-169.5°C. was obtained. The total yield was 1.09 grams or 35.3 per cent of the calculated theoretical.

The precipitates obtained during the sodium hydroxide washings of the ether solution of the methyl ester were assumed to be unreacted ursolic acid in the form of its sodium salt, which is insoluble in both ether and water. In order to verify this, the combined precipitates were suspended in hot water and concentrated hydrochloric acid added until the mixture was acid to litmus. The heavy precipitate which formed was filtered by suction, washed with water, and dried. The product melted at 283-283.5°C. A mixed melting point with a sample of known ursolic acid, m.p. 284-285°C., showed no depression.

In order to obtain a larger sample of methyl ursolate for subsequent work, the methylation was repeated. To 3 grams (0.0065 mole) of ursolic acid dissolved in 10 ml. of methyl alcohol was added 1.3 grams (0.0103 mole) of dimethyl sulfate and 75.8 ml. of 0.0868 N potassium hydroxide in methyl alcohol. The mixture was refluxed for 3 hours and then worked up as described above. Washing of the ether solution of the product with sodium hydroxide solution again precipitated unreacted sodium ursolate. Evaporation of the ether solution of the ester yielded 2.24 grams of white product (72.5 per cent of the calculated theoretical). This was crystallized from ethyl alcohol and the white, needle crystals obtained melted at 169.5-170°C. The mother liquor yielded more crystals melting at 169-169.5°C.

A third sample of methyl ursolate was prepared from 5.099 grams of ursolic acid by the second procedure described above. A yield of 2.4 grams of ester melting at 168-169°C. was obtained (50.1 per cent of the calculated theoretical).

The low yield obtained in the first run may be explained by the presence of too great an excess of potassium hydroxide and not great enough an excess of dimethyl sulfate. Excessive amounts of alkali either saponify the ester as rapidly as it is formed, or, since esters of the hindered carboxyl group in ursolic acid are hydrolysed only with difficulty, it may be that ester formation is simply retarded. The low yield in the third run is likewise probably a result of excess alkali. It was later found (see p. 132) that a longer period of refluxing combined with addition of more dimethyl sulfate at intervals during this period increased the yield of methyl ursolate.

Reduction of Methyl Ursolate to the Diol*

A solution of 2 grams of methyl ursolate in 120 ml. of Skellysolve "B" was placed in a three-necked, round-bottomed flask fitted with a reflux condenser, a mechanical stirrer with a mercury seal, and a dropping funnel. To the solution was added 1.2 grams of granulated sodium followed by 4.7 ml. of anhydrous n-butyl alcohol added dropwise from the funnel during one hour, the mixture being stirred and heated on a steam bath during this period. Since it was noted that the sodium in the reaction flask had become coated over, an additional 1 gram was added. The mixture was then refluxed for 1.5 hours longer. Enough 95 per cent ethyl alcohol was added until the solution became clear and the solution was refluxed one hour longer. This clear solution was then filtered and washed successively with a 100 ml. portion and a 50 ml. portion of 5 per cent aqueous sodium hydroxide solution in a separatory funnel. Approximately 100 ml. of ethyl

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*Method of Bleyberg and Ulrich, Berichte, <u>64B</u>, 2504 (1931).

ether was added to compensate for the volume of Skellysolve lost by evaporation. The ether-Skellysolve solution was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. A yield of 1.77 grams of crude residue melting at 210.5-215.5°C. was obtained. This represented a yield of 94.2 per cent of the theoretical calculated as the diol which would be formed by reduction of the ester grouping to the primary alcohol without reduction of the double bond.

Purification of the Crude Diol by Crystallization

The crude, white reduction product was crystallised from ethyl alcohol and the melting point rose to 221.5-223.5°C. (I). This was recrystallised from methyl alcohol and the crystals obtained melted at 224.5°C.(II). Concentration of the mother liquor from II gave crystals melting at 222.5-223°C. (III). Evaporation of the mother liquor from III to dryness gave crystals melting at 194-202°C. (IIIa). Fractions II and III were combined and recrystallised from methyl alcohol, the crystals obtained melting at 223-224°C. (IV). Concentration of the mother liquor from IV yielded another crop of crystals melting at 225-226.5°C. This was combined with fraction IV. A mixed melting point with some of the unknown alcohol, m.p. 226-228.5°C. (uncorr.), isolated from Uva Ursi by Feiertag was determined and the mixture gave a slight depression.

The mother liquor from I was concentrated and a crop of crystals melting at 150-166°C. (V) was obtained. This was recrystallised from methyl alcohol and crystals melting at 167.5-172.5°C. (VI) were obtained. The mother liquor from V was evaporated to dryness, the residue melting at 148-168°C.

Acetylation of Fraction IV of the Diol

A sample of 0.1 gram of fraction IV of the diol was dissolved in a mixture of 5 ml. of acetic anhydride and 10 ml. of anhydrous pyridine, refluxed on a steam bath for one hour, and then allowed to stand at room temperature for 48 hours. At the end of this period, the mixture was poured into water to decompose the excess acetic anhydride and a white precipitate formed. This was filtered off, washed with water, and dried. The crude product melted at 218.5-224.5°C. Recrystallization from methyl alcohol raised the melting point to 233-235°C. A mixed melting point with the starting material showed a depression of approximately 37°C. to 29°C. The filtrate from the crude product yielded more crystals melting at 150-160°C. upon standing. Recrystallization from ethyl alcohol raised the melting point to 200-208°C. When the remainder of the original filtrate was evaporated to dryness, a residue melting at 148-153.5°C. was obtained. Recrystallization from methyl alcohol changed the melting point to 153.6-154.5°C.

Attempted Purification of the Diol by Chromatographic Adsorption

Although the melting point of the diol is in fair agreement with that reported by Feiertag, an attempt at further purification by chromatographic adsorption methods was made.

A. A solution of 0.2761 gram of fraction V of the diol in 100 ml. of benzene was adsorbed on a column of "Hydralo", an activated aluminum oxide prepared by the J. T. Baker Chemical Co. For this work, and for succeeding chromatographic adsorption experiments, a pyrex tube 19 cm. long and 14 mm. in diameter was used as the adsorption tube. A perforated cork was placed in the lower end and the tube mounted on a suction flask. A small pledget of cotton was placed over the cork, the tube filled with the adsorbent to a depth of 7-8 cm., and another pledget of cotton placed on top of the column after the adsorbent had been packed.

The column was wetted with 10-15 ml. of benzene and the solution of the diol was drawn through the column by mild suction. The chromatogram was then developed with another 75 ml. of benzene. When viewed under ultra-violet light, the column showed no fluorescence, so it was extruded with a glass rod and arbitrarily cut into three segments with a spatula. Each of the segments was eluted with a 1:1 mixture of ether and methyl alcohol, the eluates evaporated to dryness, and the residues weighed.

The top segment yielded 0.0969 gram of white, needle crystals melting at 219-220°C. The center segment yielded 0.0965 gram of powdery solid melting at 220.5-221.5°C. The bottom segment yielded 0.0703 gram melting at 200-204°C. after softening at 95°C. and resolidifying at 130°C. The filtrate yielded 0.1085 gram of residue melting at 153.5-155.5°C. Total recovery was 0.2722 gram; 99 per cent.

B. The residues from the top and center segments in A, above, were combined making 0.1758 gram. This was dissolved in 75 ml. of benzene and chromatographically adsorbed on "Hydralo". After development of the chromatogram, the column was extruded, divided into two segments, each segment eluted with a 1:1 mixture of ether and methyl alcohol, and the eluates evaporated to dryness. The top segment yielded 0.1261 gram of needle crystals melting at 220-221°C. The bottom segment yielded 0.0437 gram of powdery solid melting at 219.5-220.5°C. The filtrate yielded no residue upon evaporation. Total recovery was 0.1698 gram; 96.6 per cent.

C. A sample of 0.0703 gram of the residue from the bottom segment in A, above, was dissolved in 50 ml. of benzene and chromatographically adsorbed on "Hydralo". After development of the chromatogram, the column was extruded, divided into two segments, each segment eluted with a 1:1 mixture of ether and methyl alcohol, and the eluates evaporated to dryness. The top segment yielded 0.0288 gram of white solid which softened at 73-100°C., resolidified at 100-105°C., and finally melted at 164.5-166.5°C. The bottom segment yielded 0.0353 gram melting at 214-216°C. The filtrate yielded 0.0063 gram, but no melting point was taken. Total recovery was 100 per cent.

Acetylation of the Chromatographed Fraction from E

The residues from the two segments of the chromatographic adsorption described under B, above, were combined and dissolved in 15 ml. of pyridine and 6 ml. of acetic anhydride. The solution was allowed to stand at 50-60°C. for 48 hours and at room temperature for an additional 72 hours. The mixture was poured into water, the precipitate filtered off, dried, and crystallized from methyl alcohol. The white, needle crystals obtained melted at 153-153.5°C.

The results obtained in the work up to this point indicated that the product prepared by reduction of methyl ursolate is very similar to the alcohol isolated from Uva Ursi by Feiertag. The melting points observed for the reduction product and its acetate are in fair agreement with those reported by Feiertag; however, rigorous proof of the identity of the synthetic compound with the natural one and proof that both compounds are diols was lacking. Since insufficient material for these proofs was available, the experiment was repeated and the work is described beginning on p. 22 .

Purification of the Crude Diol from Uva Ursi

In the course of this work it was decided to obtain a large enough sample of the unidentified triterpene diol from Uva Ursi with which to perform a characterization by comparison with the diol prepared by synthesis from ursolic acid. The source of the material was 44 grams of crude compound which Feiertag had isolated by extraction of powdered Uva Ursi leaves with Skellysolve "B". This material had precipitated out of the solvent in the extraction flask and was filtered off while the solvent was still hot. It was a light, fluffy, greenish-yellow powder which melted at 203-221°C.(uncorr.). No further work had been done on this material which was assumed to be the impure diol.

A sample of 10 grams of this crude alcohol was crystallized from ethyl alcohol, and white crystals melting at 220.5-221.5°C.

were obtained. A second crop of crystals was formed upon concentration of the mother liquor, but no melting point was taken. The two fractions were combined and refluxed for one hour with methyl alcohol. All of the material did not dissolve and the mixture was filtered while hot. The methyl alcohol-insoluble portion was crystallised from ethyl alcohol and crystals melting at 251.5-260°C. were obtained. Concentration of the mother liquor gave more crystals melting at 242.5-250.5°C. after previous softening at 232-235°C. Evaporation of the mother liquor to dryness yielded a residue melting at 227-228°C. The methyl alcohol-soluble portion was allowed to crystallize from the solvent and crystals melting at 222.5-235.5°C. were obtained. Concentration of the mother liquor gave more crystals melting at 213.5-218.5°C.

Since crystal crops with such a variation in melting points were obtained, it was assumed that there was present a mixture of the unidentified diol and ursolic acid. Since the sodium salt of ursolic acid is insoluble in both ether and water, it was decided to convert the contaminating acid to its sodium salt and then extract the unknown alcohol with ether.

The two crystal crops from the methyl alcohol-soluble portion were combined and refluxed for 15 minutes with an alcoholic sodium hydroxide solution. The solution was then diluted with three volumes of water and the precipitate extracted with ether. All of the precipitate did not dissolve and the insoluble portion was filtered off. The ether solution was dried over anhydrous sodium sulfate, filtered, and the ether removed by distillation. The residue was crystallised from ethyl alcohol, the crystals so obtained melting at 219.5-222°C. Concentration of the mother liquor gave more crystals melting at 217-220°C. The two crystal crops were combined and recrystallized from acetone, the melting point of the fine, white, needle crystals becoming sharp at 222-223°C. The optical rotation of these crystals was determined with the following result:

$[\alpha]_D^{24}$ + 68.57° (56 mg. in 2 ml. of chloroform α_D + 1.92°, l 1 dm.).

A sample was dried overnight at 108°C. in a vacuum oven and a macro carbon and hydrogen analysis was run with the following results:

Calculated for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 80.28, 80.41; H, 10.66, 10.56.

The low values obtained in the analyses are probably due to impurities still present in the sample. The presence of small amounts of ursolic acid, which contains three oxygen atoms, would produce low results. The results do indicate, however, that the compound probably possesses two oxygen atoms. Since the melting point of the sample was not as high as that obtained by Feiertag for the diol, it was decided to further purify the compound by acetylation and saponification of the diacetate.

Acetylation of the Unidentified Diol

The unidentified diol obtained above was acetylated by dissolving 176.5 mg. in 16 ml. of pyridine and 2 ml. of acetic anhydride and allowing the mixture to stand for 24 hours at room temperature (about 24°C.).* The ester was precipitated by dilution with three

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* Method of Drake and Shrader, J. Amer. Chem. Soc., 57, 1854. (1935).

parts of water, extracted with ether, and the ether solution washed successively with four 25 ml. portions of 10 per cent hydrochloric acid, three 25 ml. portions of saturated sodium carbonate solution, and three 25 ml. portions of water. The ether solution was then dried over anhydrous sodium sulfate, filtered, and the ether removed by distillation. The residue was crystallised from ethyl alcohol, a yield of 93 mg. of small, white crystals melting at 138-140°C. being obtained. Recrystallisation from ethyl alcohol gave needle crystals melting at 148.5-149°C. Concentration of the mother liquors gave crystals melting at 137-139°C. and 143-144°C. These two crops were combined and recrystallised from acetone and needle crystals melting at 147.5-148.5°C. were obtained.

The optical rotation of the diacetate was determined on the highest melting fraction (148.5-149.5°C.) with the following results:

$[\alpha]_D^{24}$ + 53.36° (52.1 mg. in 2 ml. of benzene α_D + 1.39°, l 1 dm.).

A sample of the highest melting diacetate was dried overnight at 105°C. in a vacuum oven and a macro carbon and hydrogen analysis was made with the following results;

Calculated for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.35; H, 10.49.

From these values, it may be concluded that the compound is a diacetate and that the compound from which it was prepared is, therefore, a diol.

Saponification of the Diacetate

A sample of the above diacetate was saponified by refluxing an alcoholic solution of it with several pellets of U.S.P. potassium hydroxide on a steam bath for 24 hours. The mixture was then diluted several times with water and the white precipitate extracted with ether. The ether solution was washed with 10 per cent hydrochloric acid and water, dried over anhydrous sodium sulfate, and the ether removed by distillation. The residue was crystallised from ethyl alcohol and two crystal crops were obtained. The first melted at 224-225°C. and the second at 222-223°C. These were combined and recrystallised from methyl alcohol. The crystals obtained melted at 225.5-226.5°C. The optical rotation was found to be as follows: $[\alpha]^{32}_{D} + 70.88°$ (44.3 mg. in 2 ml. of chloroform $\alpha_{D} + 1.57°$, l 1 dm.).

This rotation compares much more favorably with that observed by Ruzicka for his diol than does that of the diol melting 222-223°C. on which the first determination was made.

Repetition of the Synthesis of the Diol from Ursolic Acid

Since not enough material was available from the earlier synthesis to properly evaluate the results and to extend the work, the preparation of the diol was repeated and the results are described in the following.

Attempted Reduction of Methyl Ursolate

A solution of 2.4 grams of methyl ursolate in 150 ml. of Skellysolve "B" was placed in the apparatus previously described on p. 13 , 1.44 grams of granulated sodium added, and 5.6 ml. of anhydrous n-butyl alcohol dropped in from the funnel during one hour of refluxing on a steam bath.

An additional one gram of sodium was then added and the mixture refluxed for an additional 1.5 hours. At this time 95 per cent ethyl alcohol was added until the solution became clear and the mixture was refluxed one hour longer. The filtered solution was worked up as previously described. A crude yield of 2 grams was obtained (89.5 per cent of the calculated theoretical). Crystallization from ethyl alcohol gave a white, powdery solid melting at 193-211°C.

Acetylation of the Reduction Product

In order to purify the reduction product obtained above, it was decided to acetylate the material, purify the acetate, and saponify back to the diol. The product was refluxed for 4 hours with acetic anhydride and glacial acetic acid. The reaction mixture was poured into water, extracted with ether, the ether solution washed with sodium bicarbonate solution, dried, and ether removed by distillation. The product, after one crystallization from ethyl alcohol, melted at 229-233°C. Since this melting point was much higher than that previously observed for the diacetate of the diol obtained by reduction of methyl ursolate, it was assumed that reduction had probably not taken place. Methyl acetyl ursolate, which would result from acetylation of unreduced methyl ursolate, melts at 245-246°C. and the acetate obtained might probably be this compound.

Saponification of the Acetate

In order to verify the assumption that reduction had not taken place and that acetylation had produced methyl acetyl ursolate, the acetate was saponified by dissolving in ethyl alcohol, adding 5 or 6 pellets of U.S.P. potassium hydroxide, and refluxing for 2.5 hours on a steam bath. The reaction mixture was diluted with water, extracted with ether, and the ether evaporated. The product was crystallized from ethyl alcohol and the crystals melted at 165.5-166.5°C. Since the methyl group of methyl ursolate or its acetate is hydrolysed off only with great difficulty, this product would be expected to be methyl ursolate. A mixed melting point with a known sample of methyl ursolate showed no depression. This confirms the assumption that reduction of the starting methyl ursolate had failed.

Reduction of Methyl Ursolate

Since the above attempt at reduction of methyl ursolate to the diol had failed, the experiment was tried again and the conditions varied somewhat. A solution of 1.7 grams of methyl ursolate in 100 ml. of Skellysolve "B" was placed in the apparatus previously described and 13 grams of sodium, a ten mole excess, was added. Instead of butyl alcohol, which had been used previously, 4 ml. (about one mole equivalent) of absolute ethyl alcohol was dropped in from the funnel during 50 minutes of refluxing on a steam bath. The mixture was refluxed for another 40 minutes, 95 per cent ethyl alcohol added until the solution became clear, and the solution refluxed 50 minutes longer. It was then filtered, washed with three portions of water, one portion of 10 per cent hydrochloric acid, and finally with one portion of water. Ether was added to make up the volume of Skellysolve lost by evaporation and the solution dried over anhydrous sodium sulfate, filtered, and the solvent removed by distillation. The residue was crystallized from ethyl alcohol and 520 mg. of white, needle crystals melting at 221.5-223°C. was obtained. Concentration of the mother liquor gave a second crop of 476 mg. of crystals melting at 222-224.5°C. The total yield was 62.5 per cent of the calculated theoretical.

The two crops of crystals were combined and recrystallized from acetone, the crystals so obtained melting at 222-223°C. Recrystallization from methyl alcohol raised the melting point to 223-224°C. A mixed melting point of these crystals with the diol (m.p. 222-223°C.) from Uva Ursi which was described on p. 20 showed no depression. The optical rotation of the crystals was determined with the following result:

$[\alpha]_D^{24}$ + 68.80° (50 mg. in 2 ml. of chloroform α_D + 1.72°, l 1 dm.).

A sample of the purified product was dried overnight at 105°C. in a vacuum oven and a macro carbon and hydrogen analysis made with the following results:

Calculated for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 80.42, 80.41; H, 11.06, 10.78.

The low values obtained are probably due to small amounts of impurities. Although the values are somewhat low, a glance at Table I will show that they check quite closely with those observed for the diol obtained from Uva Ursi. The values for carbon are particularly close.

Acetylation of the Diol

A sample of 170 mg. of the diol melting at 223-224°C. was dissolved in 14 ml. of anhydrous pyridine, 2 ml. of acetic anhydride added in portions with shaking, and the mixture allowed to stand at about 24°C. for 24 hours. It was then diluted with three volumes of water, the precipitate which formed extracted with ether, the ether solution washed successively with four 25 ml. portions of 10 per cent hydrochloric acid, three 25 ml. portions of saturated sodium carbonate solution, three 25 ml. portions of water, and dried over anhydrous sodium sulfate. The ether solution was filtered and the solvent removed by distillation. The residue was crystallized from ethyl alcohol and 105 mg. of snow-white, needle crystals melting at 150.5-151.5°C. were obtained. A mixed melting point with a sample of the diacetate (m.p. 151-151.5°C.) of the triterpene diol from Uva Ursi prepared by Feiertag showed no depression. A mixed melting point with the diacetate (m.p. 148.5-149.5°C.)of the diol from Uva Ursi, the preparation of which was described on p. 20 , also showed no depression. Concentration of the mother liquor gave 25 mg. more of diacetate melting at 150.5-151.5°C. The total yield was 64.4 per cent of the calculated theoretical.

The optical rotation was determined on the diacetate with the following result:

$[\alpha]_D^{24}$ + 54.08° (51.4 mg. in 2 ml. of benzene α_D + 1.39°, l 1 dm.).

Not enough of the material was available for saponification to the diol in order to obtain a higher melting product than that originally purified.

Table 1

Comparison of Properties of the Diols

		Uva Ursi Diol	Synthetic Diol	Ruzicka's Diol 3)	Uvaol 5,6,7)
Free diol, m.p.	:	222-223° 225.5-226.5° (No depression when mixed)	223-224°	223-226°	232-233°
$[\alpha]_D$	:	+68.57° ($CHCl_3$) 70.88° ($CHCl_3$)	+68.80° ($CHCl_3$)	+71.33° ($CHCl_3$)	+74.4° ($CHCl_3$)
Analysis	:	C, 80.28, 80.41 H, 10.66, 10.56	80.42, 80.41 11.06, 10.78	Not reported	81.33, 81.44 11.32, 11.34
Diacetate, m.p.	:	148.5-149.5° (No depression when mixed)	150.5-151.5°	150-151°	157-159°
$[\alpha]_D$	:	+53.36° (benzene)	+54.08° (benzene)	+53° (benzene)	Not reported
Analysis	:	C, 77.35 H, 10.49	------	Not reported	77.52, 77.06 10.33, 9.83

Conclusions

The results, as may be seen from Table I, show conclusively that the Uva Ursi diol is identical with that synthesised from ursolic acid and probably with Ruzicka's diol. It is very probable also that the diol isolated by Rusii and Ōsumi is the same, although the melting points reported are somewhat high. Since this diol and ursolic acid exist together in Uva Ursi, and since this work has shown the close relationship between the two, it is possible that the biogenesis of each is identical, one resulting from further reaction of the other.

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II. THE PREPARATION OF A TRITERPENE DIOL FROM OLEANOLIC ACID

Introduction

The main purpose of this work was to prepare a diol from oleanolic acid, as had been done from ursolic acid, in the hope of partially synthesising the natural diol isolated from Uva Ursi1). The close relationship between ursolic acid and oleanolic acid, which differ only in the position of the very unreactive double bond, is evident throughout the vast amount of literature on these two triterpene acids. Thus it was considered possible that the Uva Ursi diol might be a derivative of oleanolic acid rather than of ursolic acid and, if so, synthesis of the diol from oleanolic acid would provide the proof.

However, the behavior of the product obtained by the catalytic hydrogenation of methyl oleanolate was of such a nature as to make the task of identifying this product a problem in itself. Consequently, a large part of the experimental work described in the following is concerned with the purification and identification of the hydrogenation product. The diol which one would expect to obtain from oleanolic acid is "erythrodiol". This triterpene diol was first isolated in the form of its mono-stearate from the fruit of *Erythroxylon novogranatense* Morren, by Zimmermann2). Ruzicka3) has prepared erythrodiol synthetically as an incomplete reduction product in the preparation of β-amyrin from oleanolic acid by a Rosenmund reduction of the acid chloride to the aldehyde, followed by a Wolff-Kishner reduction of the semicarbazone. Erythrodiol differs in structure from oleanolic acid in that the tertiary carboxyl group of the latter has been reduced to the primary alcohol group. Further reduction of the hydroxy-methyl group of erythrodiol to a methyl group produces β-amyrin, a triterpene alcohol occurring fairly widely in nature.

Coincident with the chemical reduction of methyl urselate which was previously described, (p. 24), an analogous reduction of methyl oleanolate was carried out and is described below. All melting points are corrected.

Experimental

Preparation of Methyl Oleanolate

To 20 grams (0.044 mole) of oleanolic acid dissolved in 300 ml. of absolute methyl alcohol were added 2.9 grams of U.S.P. potassium hydroxide and 6.5 grams (0.052 mole) of dimethyl sulfate, and the mixture refluxed for a period of 4.5 hours. At the end of this time it was poured into water with the formation of a heavy, white precipitate. This was filtered off by suction, dried, dissolved in ether, and the ether solution washed three times with 100 ml. portions of 3 per cent aqueous sodium hydroxide solution, then once with water. During the first washing, there was precipitated from the ether solution a quantity of white, flocculent material, insoluble in both ether and water. The precipitate was filtered off, dried, and subjected to the methylation procedure again, the ether solution of the product being added, after washing, to the first ether solution of the ester. The combined solution was then allowed to evaporate to dryness, the residue dried, and the ester crystallized from ethyl alcohol. The first crop of crystals weighed 11.73 grams and melted at 201-202°C. Concentration of the mother liquor yielded a second crop of 4.09 grams of the ester with a melting point of 200°C., and a third crop, weighing 0.54 gram, melting at 198-199°C., making a total of 16.4 grams of ester. This represented a yield of 83 per cent of the calculated theoretical.

A second sample of methyl oleanolate was prepared from 16 grams of oleanolic acid by the same procedure using proportional quantities of reagents, with the exception that the mixture was refluxed for 5.3 hours. A yield of 9.95 grams of ester melting at 198-199°C. was obtained (60.6 per cent of the calculated theoretical).

A third sample of methyl oleanolate was prepared from 3 grams of oleanolic acid by the same procedure. A yield of 2.02 grams of ester melting at 200-201°C. was obtained (65.4 per cent of the calculated theoretical).

A fourth sample of methyl oleanolate was prepared by the same procedure from 6 grams of oleanolic acid. A yield of 1.615 grams of ester melting at 199-200°C. was obtained (27 per cent of the calculated theoretical). The much lower yield obtained in this run was probably due to an excess of potassium hydroxide in the reaction mixture. Although esters of the tertiary carboxyl group are saponified only with difficulty, the presence of excess potassium hydroxide may cause saponification of part of the ester as rapidly as it is formed or may, perhaps, merely retard formation of the ester.

Hydrogenation of Methyl Oleanolate to the Diol

A solution of 16.4 grams (0.035 mole) of methyl oleanolate in 200 ml. of dioxane was hydrogenated by Dr. Homer Adkins over a catalyst of 3 grams of copper chromite for six hours under 300 atmospheres of pressure at 260°C. The compound took up approximately 0.10 to 0.12 mole of hydrogen. The calculated amount of hydrogen necessary to reduce the ester linkage to an hydroxyl group without affecting the double bond is 0.07 mole.

The reduction product was separated from the catalyst by centrifuging and filtering through a Jena glass fritted funnel. The filtrate, upon evaporation of the dioxane, yielded a white residue. This was crystallized from ethyl alcohol and two fractions were obtained:

Fraction I. That which crystallized out of the alcohol upon cooling in an ice bath weighed 7.57 grams and melted at 215-222°C.

Fraction II. That which remained in the alcohol when the above had crystallized out was much more soluble and was obtained as a white residue upon evaporation of the mother liquor. It weighed 7.11 grams and melted not sharply at about 203°C.

There was obtained a total yield of 89.1 per cent of the theoretical calculated as the diol which would be expected to be formed by reduction of the ester grouping without reduction of the double bond.

As a means of purification, Fraction I was subjected to a series of crystallizations which is described below. Several of the fractions which were obtained in this manner were further purified by means of chromatographic adsorption, which procedure is also described in the following. Fraction II was also subjected to the chromatographic adsorption purification, this work being described following the description of the work with Fraction I.

Attempted Purification of Fraction I by Repeated Recrystallization

A portion of Fraction I was dissolved in ethyl alcohol and allowed to stand overnight in the ice box. A heavy, flocculent precipitate formed which was filtered off and dried. It was slippery in consistency and the melting point was not sharp at 219-226°C.(Ia). Another crystallization from ethyl alcohol raised its melting point to 231-232°C. (Ib). A further purification of this fraction by chromatographic adsorption is described under A and B on the pages following. Evaporation of mother liquor Ia to dryness gave a residue melting at 214.5-216.5°C. (Residue Ia). The further purification of this fraction by chromatographic adsorption is described under C on the pages following. Concentration of mother liquor Ib yielded more flocculent precipitate melting at 231.5-234.5°C. (Ic). The further purification of this fraction by chromatographic adsorption is described under D and E on the following pages.

The mother liquor Ic was evaporated to dryness and the residue crystallized from methyl alcohol. Two crops of crystals were obtained. The first crop melted at 206-210°C. (Id). After recrystallization from ethyl alcohol the melting point was unchanged. The second crop melted at 210.5-212°C. (Ie). This was recrystallized from ethyl alcohol and the melting point rose to 216-217.5°C. (If). Another crystallization from ethyl alcohol raised the melting point to 223.5-225.5°C. (Ig).

The mother liquor Ig upon evaporation yielded a residue melting at 221.5-222.5°C. which was combined with Ig and recrystallization from ethyl alcohol attempted, but crystallization did not occur. The solution was then evaporated to dryness, the residue melting at 217.5-220.5°C. (Ih).

The combined mother liquors Ie and If were evaporated to dryness and the residue recrystallized from methyl alcohol. The crystals softened at 189°C. and melted at 207-210.5°C. These were combined with fraction Id and recrystallized from ethyl alcohol. Two crops of crystals were obtained, the first melting at 207-209°C. (Ii) and the second melting at 203.5-207°C. (Ij).

Purification of Fraction I by Chromatographic Adsorption

A. A sample of 0.2036 gram of fraction Ib (m.p. 231-232°C.) obtained in the recrystallizations was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Alorco", an activated aluminum oxide prepared by the Aluminum Ore Co. After development of the chromatogram with 100 ml. of benzene, the column was extruded and cut into three equal segments with a spatula. Each segment was eluted with a 1:1 mixture of ether and methyl alcohol and the eluates evaporated to dryness. The top segment yielded 0.1395 gram of white solid which melted at 211-215.5°C. The center segment yielded 0.0576 gram which melted at 229.5-230.5°C. The bottom segment yielded 0.0064 gram which softened at 194°C. with some decomposition and finally melted at 215.5-220.5°C. The filtrate yielded no residue. Total recovery was 0.2035 gram; almost 100 per cent.

B. The product obtained from the center segment in A was combined with some of the starting material in A making 0.3619 gram. This was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Alorco". After development of the chromatogram, the column was extruded, divided into three equal segments, each segment eluted with a 70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness.

The top segment yielded 0.2328 gram of white solid which melted at 230.6-232.2°C. This was crystallized from methanol, after which the melting point was sharp at 231.7°C. Evaporation of the mother liquor yielded a residue melting at 226.5-227°C.

The center segment yielded 0.1084 gram which melted at 229.5-230.4°C. This was crystallized from methyl alcohol, after which the melting point became sharp at 231.7°C. A mixed melting point with the compound from the top segment having the same melting point showed no depression. Evaporation of the mother liquor yielded a residue melting at 229.5-231.5°C.

The bottom segment yielded a residue of 0.0034 gram and the filtrate gave 0.0028 gram, but no melting points were taken. Re-elution of the entire adsorbent yielded 0.010 gram of compound melting at 231.7°C. after recrystallization from methanol. Total recovery was 0.3574 gram; 99 per cent.

C. The residue Ia from the attempted purification by crystallization (p. 35), melting at 214.5-216.5°C. and weighing 0.3875 gram, was dissolved in 200 ml. of benzene and chromatographically adsorbed on "Alorco". After development of the chromatogram, the column was extruded, cut into three equal segments, each segment eluted with a

70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness.

The top segment yielded 0.4624 gram of white solid which melted at 213.7-215.5°C. Crystallization from ethyl alcohol raised the melting point to 223.5-225°C. Evaporation of the mother liquor gave a residue melting at 216.5-218°C. Recrystallization of the 223.5-225°C. fraction from methyl alcohol raised its melting point to 228.5-229°C. Evaporation of the mother liquor gave a residue melting at 219.5-228°C.

The center segment yielded 0.2282 gram melting at 217-219.5°C. Crystallization from methyl alcohol raised the melting point to 225-226.5°C. Another crystallization from methyl alcohol did not alter this melting point. Evaporation of the combined mother liquors gave a residue melting at 210.4-213.7°C.

The bottom segment yielded 0.0036 gram and the filtrate gave 0.0065 gram. No melting points were taken. Re-elution of the entire adsorbent gave 0.0700 gram of residue melting at 216.5-218.5°C. Total recovery was 0.7707 gram; 86.8 per cent.

2. A sample of 0.2096 gram of fraction Ic, m.p. 221.5-224.5°C. from the attempted purification by recrystallization was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Alorco". After development of the chromatogram, the column was extruded, cut into three equal segments, each segment eluted with a 1:1 mixture of ether and methanol, and the eluates evaporated to dryness. The top segment yielded 0.1915 gram of white solid which melted at 221.5-224.5°C. The center segment yielded 0.0050 gram melting at 210.5-212.5°C. The bottom segment and the filtrate yielded nothing. Total recovery was 0.1965 gram; 93.8 per cent.

E. Another portion of the sample used in D was combined with the remainder of fraction Ia, m.p. 219-226°C., obtained in the first crystallization (p. 35). This sample, weighing 0.5283 gram, was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Alorco". After development of the chromatogram, the column was extruded, cut into three equal segments, each segment eluted with a 70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness.

The top segment yielded 0.2316 gram of white solid which melted at 215-219.5°C. Crystallization from methyl alcohol raised the melting point to 221-223.5°C. Evaporation of the mother liquor gave a residue melting at 214.7-220.5°C. Recrystallization of this from methyl alcohol raised the melting point to 224.5-225.5°C.

The center segment yielded 0.2035 gram which melted at 215.5-219.5°C. Crystallization from ethyl alcohol gave two crops of crystals, one melting at 215.5-217°C. and the second melting at 220-221.5°C.

The bottom segment yielded 0.0612 gram which melted at 212.1-213°C. Evaporation of the filtrate gave 0.0070 gram. No melting point was taken. Total recovery was 0.5033 gram; 95.2 per cent.

Purification of Fraction II by Chromatographic Adsorption

Fraction II, m.p. 208°C., was recrystallized from ethyl alcohol, the melting point rising to 211-215.5°C. Evaporation of the mother liquor gave a syrupy mass which hardened on standing.

F. A sample of 0.200 gram of the crystals melting at 211-215.5°C. was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Hydralo", an activated aluminum oxide. After development of the chromatogram, the column was extruded, divided into three equal segments, each segment eluted with a 70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness. The top segment yielded 0.1332 gram of white solid melting at 218.5-222.5°C. Crystallization from methyl alcohol did not change the melting point. The center segment yielded 0.0504 gram melting at 143-150°C. The bottom segment yielded 0.0025 gram and the filtrate yielded 0.0041 gram. No melting points were taken. Total recovery was 0.1902 gram; 95.1 per cent.

G. A sample of 0.0883 gram of the top segment from F was dissolved in 50 ml. of benzene and chromatographically adsorbed on "Alorco". After development of the chromatogram, the column was extruded, cut into two segments, each segment eluted with a 70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness. The top segment yielded 0.0835 gram of white solid melting at 216.5-220.5°C. The bottom segment and the filtrate yielded nothing. Total recovery was 94.5 per cent.

H. Another sample of 0.8624 gram of the same material as used in F was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Alorco". After development of the chromatogram, the column was extruded, cut into three segments, each segment eluted with a 70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness.

The top segment yielded 0.2326 gram of white solid melting at 213.7-215°C. Crystallization from methyl alcohol raised the melting point to 216-217°C. The center segment yielded 0.2573 gram melting at 215-216.5°C. Crystallization from methyl alcohol gave two crops of crystals, both melting at 213.7-215.5°C. These were combined and recrystallized from methyl alcohol, the crystals so obtained melting at 216.5-217.5°C. The mother liquor from the first crystallization yielded a residue melting at 213.7-215.5°C. This was combined with the latter crystals and the mixture recrystallized from ethyl alcohol. The crystals melted at 213.1-215.5°C. The bottom segment yielded 0.1863 gram melting at 212.5-213.4°C. The filtrate yielded 0.0478 gram melting at 204.2-206.8°C. The entire adsorbent was re-eluted and 0.0420 gram of crystals melting at 212.1-213.3°C. was obtained. Total recovery was 0.7660 gram; 89 per cent.

Acetylation of Some Combined Fractions from the Chromatographic Adsorptions

Several fractions from the chromatographic adsorptions of both the original two fractions of the diol, all melting between 210°C. and 230°C. were combined. This mixture was acetylated by refluxing for 5 hours with 2 ml. of acetic anhydride and 5 ml. of glacial acetic acid. The reaction mixture was then poured into ice water to decompose the excess anhydride and to precipitate the ester. The material was extracted with ether, the ether solution washed with 5 per cent sodium bicarbonate solution twice and with water once, and then dried over anhydrous sodium sulfate, filtered, and evaporated. The residue weighed 0.43 gram. This was crystallized from methyl alcohol and a crop of white, needle crystals melting at 166.2-171.2°C. was obtained. Recrystallization from ethyl alcohol raised the melting point to 174.2-176.1°C. These needle crystals were then recrystallized from a mixture of ethyl acetate, ethyl alcohol, and water. White platelets melting sharply at 178-179°C. were obtained. Concentration of the mother liquors gave more needle crystals melting at 166.2-171.2°C. The fraction of diacetate melting at 178-179°C. was used to determine the optical rotation with the following result:

$[\alpha]^{25}_{D}$ + 62.09° (49.6 mg. in 2 ml. of chloroform α_D + 1.54°, l 1 dm.).

Saponification of the Diacetate

The diacetate melting at 178-179°C. was saponified by refluxing with alcoholic potassium hydroxide for 2.75 hours. The reaction mixture was acidified and extracted with ether and the ether solution dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The free diol was recrystallized from ethyl alcohol containing a little water. White crystals melting at 224.5-226.5°C. were obtained. Recrystallization from methyl alcohol raised the melting point to 230-231°C. The optical rotation was determined with the following result:

$[\alpha]^{26.5}_{D}$ + 63.49° (31.5 mg. in 2 ml. of dioxane α_D + 1.00°, l 1 dm.).

Repetition of the Preparation of a Diol from Oleanolic Acid

Because of the somewhat inconclusive results obtained in the attempts to purify and characterize the diol obtained by catalytic hydrogenation of methyl oleanolate, it was decided to repeat the work using different methods of purification of the crude hydrogenation product. It had been noted in the literature that the acetates of the triterpene alcohols lend themselves more easily to purification than do the free alcohols. Consequently this procedure was adopted.

The details are reported in the following description of the experimental work.

Hydrogenation of Methyl Oleanolate to the Diol

A sample of 9.95 grams (0.021 mole) of methyl oleanolate was dissolved in dioxane and hydrogenated over 3 grams of a copper-chromium oxide catalyst under a pressure of 300 atmospheres at 260°C. for ten hours. The reaction was carried out by Dorothy Swift under the direction of Dr. Homer Adkins. The compound took up approximately 0.06-0.07 mole of hydrogen. The amount of hydrogen calculated for reduction of the ester grouping to the alcohol without affecting the double bond is 0.042 mole.

The reduction product was separated from the catalyst by centrifuging and filtering through a Jena glass fritted funnel. The solvent was removed by evaporation on a hot plate and the residue dried. The yield of crude product was 9.4 grams; 94.5 per cent of the calculated theoretical. The material melted at 204.8-210.7°C. Crystallization of this product from ethyl alcohol yielded 3.72 grams of white solid melting at 210.7-217.5°C. Concentration of the mother liquor gave an additional 3.57 grams which melted at 203-207.7°C. This mother liquor was evaporated to dryness and 1.7 grams of residue melting at 188-196°C. was obtained. The fraction melting at 210.7-217.5°C. was recrystallized from methyl alcohol. This yielded 1.81 grams of compound melting at 218.5-223.8°C. Concentration of the mother liquor gave 0.92 gram of compound melting at 212.7-217.8°C. Evaporation of this mother liquor yielded 0.47 gram of residue melting at 188-203.9°C.

Acetylation of the Fraction Melting at 218.5-222.5°C.

One gram of the impure diol melting at 218.5-222.5°C. was mixed with 4 ml. of acetic anhydride and 10 ml. of glacial acetic acid and refluxed on a steam bath for four hours. At the end of this period the mixture was poured into ice water. The mixture was extracted with ether, the ether solution being washed with sodium carbonate solution and with water, and dried over anhydrous sodium sulfate. Evaporation of the ether yielded 1.02 grams of product, 85.7 per cent of the calculated theoretical. Crystallization from ethyl alcohol gave 0.678 gram of crystals melting at 178.5-180.5°C. After recrystallization from methyl alcohol the compound melted sharply at 181-181.5°C. During the recrystallization not all of the compound dissolved readily in the solvent and so it was filtered off. Recrystallization of this material from fresh methyl alcohol gave crystals melting sharply at 182.5-183°C. Concentration of the mother liquor yielded more crystals melting at 183°C. The optical rotation was determined on the 182.5-183°C. fraction with the following result:

$[\alpha]_D^{25.5}$ + 56.61° (54.4 mg. in 2 ml. of chloroform α_D + 1.54°, l 1 dm.).

Another determination of the optical rotation was carried out with the following result:

$[\alpha]_D^{25.5}$ + 56.48° (51.7 mg. in 2 ml. of chloroform α_D + 1.46°, l 1 dm.).

Saponification of the Diacetate

A sample of 0.403 gram of the diacetate described above was saponified by refluxing for 5 hours with methyl alcoholic potassium hydroxide. At the end of this period the mixture was poured into water, acidified with dilute hydrochloric acid, and extracted with ether. The ether solution was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. A yield of 0.396 gram of the free diol was obtained, which, after crystallization from methyl alcohol, melted at 230.5-231.5°C. Concentration of the mother liquor gave more crystals melting at 228.5-229.5°C. These were recrystallised from methyl alcohol, the melting point rising to 230.5-231.5°C. The optical rotation was determined with the following result:

$[\alpha]_D^{27.5}$ $+ 66.66^\circ$ (50.1 mg. in 2 ml. of dioxane $\alpha_D + 1.67^\circ$, l 1 dm.).

Acetylation of the Fractions Melting at 188-202.9°C.

Two fractions of the lower melting reduction product from this second hydrogenation, one melting at 188-196°C. and the other at 188-202.9°C., were combined, making a total of 2.107 grams. This mixture was acetylated by refluxing on a steam bath for 8 hours with 8 ml. of acetic anhydride and 20 ml. of glacial acetic acid. At the end of this period the mixture, which was pinkish in color, was poured into water, the precipitated diacetate extracted with ether, and the other solution washed twice with 5 per cent sodium bicarbonate solution and once with water. The color was for the most part removed by stirring the ether solution with charcoal and filtering. The ether solution was dried and ether removed by evaporation, a residue remaining. This residue was crystallized from ethyl alcohol and two crops of crystals were obtained. The first melted at 164.3-169.2°C. and the second melted at 89.5-99.5°C. The latter was again crystallized from ethyl alcohol and the melting point rose to 168.3-173.2°C. This was combined with the first crop above and recrystallized from acetone. The crystals obtained melted at 175-177°C. Another crystallization, this time from methyl alcohol, caused the melting point to become sharp at 177-178°C. Although this melting point was sharp, it is still lower than that obtained by acetylation of a higher melting fraction of the hydrogenation product. Presumably it is the same as the higher melting acetate, but not as fully purified. Not enough material was available for further purification, however.

Acetylation of the Fraction Melting at 203-207.7°C.

The fraction of the impure diol obtained by crystallization of the crude product and melting at 203-207.7°C. was acetylated by refluxing on a steam bath with 10 ml. of acetic anhydride and 20 ml. of glacial acetic acid. The product was worked up as described above and the crude diacetate was crystallized from methyl alcohol. When placed in the oven to dry at 100°C., the crystals melted. This residue was recrystallized from ethyl alcohol, the crystals so obtained melting at 175.5-177°C. This acetate very probably is identical with the 182.5-183°C. acetate prepared on p. 44 , but in a more impure condition. The saponification of a similar lower-melting diacetate (p. 42) yielded a diol with the same melting point as that of the diol from the high melting fraction.

Determination of the Saponification Equivalent of the Diacetate*

The above diacetate was used to determine the saponification equivalent in order to prove the presence of two hydroxyl groups in the hydrogenation product. Each sample was saponified by refluxing on a steam bath with 20 ml. of 0.1029 N alcoholic potassium hydroxide for 2 hours. At the end of this period the excess potassium hydroxide was titrated with tenth-normal hydrochloric acid using phenolphthalein as the indicator.

	I	II
Wt. of Sample	0.2082 Gm.	0.1637 Gm.
Ml. of KOH (0.1029 N)	20.00	20.00
Ml. of HCl (0.1000N)	12.75	13.80
Saponification equiv.	266	282.1

Assuming the presence of one hydroxyl group in the molecule, the molecular weight of the acetate would be the same as the saponification equivalent. If it is assumed that two hydroxyl groups are present, the molecular weight of the diacetate would be twice the value obtained above. The diol which would be expected to form by reduction of methyl oleanolate would have a molecular weight of 526. The result obtained with the first sample, multiplied by 2, is in fair agreement with this value. The second sample apparently was not saponified for a long enough period, which accounts for the high value obtained. The molecular weight of acetyl methyl oleanolate itself is 512 and that of the compound which would be obtained by reduction of the ester linkage to a methyl group is 468. Also, the melting point of the former is 221-222°C. and that of the latter, β-amyrin, is 198-199°C.

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*Method of Shriner and Fuson, "The Systematic Identification of Organic Compounds", 2nd Ed., p. 118 (1940).

Therefore, it is not probable that the hydrogenation product is either of these.

Acetylation of Some Combined Fractions of the Diol

Several fractions of the diol from both hydrogenation experiments were combined, making a sample of 946 mg. This was acetylated by refluxing with 10 ml. of glacial acetic acid and 4 ml. of acetic anhydride for 4 hours. A crude yield of 1.193 grams of yellow, oily material was obtained (94.3 per cent of the calculated theoretical). Crystallization from ethyl alcohol gave 330 mg. of white, needle crystals melting at 176-178°C. Concentration of the mother liquor gave a second crop of 121 mg. melting at 168.2-171.2°C. These were combined and recrystallized from methyl alcohol, giving 368 mg. of needle crystals melting at 177.1-178.7°C. Another crystallization from methyl alcohol gave 288 mg. melting at 181-182°C. The optical rotation of these crystals was found to be:

$[\alpha]_D^{28.5}$ + 60.43° (41.7 mg. in 2 ml. of chloroform α_D + 1.26°, l 1 dm.).

A macro carbon and hydrogen analysis was carried out on this diacetate, after drying at 105°C. in a vacuum oven overnight, and the following results were obtained:

Calculated for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found; C, 76.70; H, 10.16.

The somewhat low values obtained are probably due to the fact that the compound was not completely pure, its melting point being 1-2° lower than that previously obtained. The analysis does, however, confirm the assumption that this compound is a diacetate and that the original hydrogenation product is, therefore, a diol.

Saponification of the Diacetate

A portion of the above diacetate and the residues from the combined mother liquors were dissolved in 25 ml. of methyl alcohol, 3 or 4 pellets of U.S.P. potassium hydroxide added, and the solution refluxed on a steam bath for 18 hours. It was then poured into water, the precipitate extracted with ether, the ether solution dried over anhydrous sodium sulfate, filtered, and solvent removed by distillation. Crystallization of the crude residue from ethyl alcohol gave crystals melting at 226.5-227.5°C. Recrystallization from methyl alcohol raised the melting point to 229.5-230.5°C. The optical rotation of these crystals was found to be:

$[\alpha]^{26.5}_{D}$ + 69.29° (38.1 mg. in 2ml. of chloroform α_D + 1.32°, l 1 dm.).

Formylation of the Diol (I)

A diformate of the fraction of the diol melting at 215.5-222.5°C. (p. 43) was prepared by refluxing 0.50 gram with 85 per cent formic acid for ten minutes. The diol dissolved completely and a precipitate formed after five minutes of heating. At the end of the reflux period the mixture was poured into water, the precipitated product filtered off by suction, washed with sodium carbonate solution to remove any excess formic acid, and then washed with water and dried. The white product was crystallized from methyl alcohol and the fine, white platelets obtained melted at 190-193°C. Recrystallization from ethyl alcohol raised the melting point to 195-198°C. These crystals were then recrystallised from acetone, the product melting at 198.5-201°C. When this was recrystallized from acetone, the melting point rose to 215-219°C.

Concentration of the alcoholic mother liquors yielded crystals melting at 176.1-183°C. The acetone mother liquor of the 215-219°C. fraction was concentrated with the formation of crystals melting sharply at 194.5-195.5°C. Recrystallization of these from acetone raised the melting point to 203-210°C. Since the melting point became higher and distributed over a wider range with each crystallization, it seems likely that the formate was being hydrolysed back to the diol or otherwise decomposed. The fact that the products were dried at 100°C. for five or ten minutes may have contributed to the decomposition.

Formylation of the Diol (II)

The formylation was repeated using the procedure previously described on 0.4 gram of the same sample that was used in the first trial. Crystallization of the crude product from ethyl alcohol yielded crystals melting at 181-183°C. Another crystallization from the same solvent raised the melting point to 193-197°C. All fractions were then combined and recrystallized from acetone, the crystals so obtained melting at 198-201°C. This behavior on recrystallization corresponds with that observed previously.

Attempted Oxidation of the Diol to a Dicarbonyl Compound and Preparation of the Monoxime

Zimmermann[4)] has shown that erythrodiol contains one primary and one secondary hydroxyl group. This was evidenced by the ease of formation of the monoxime of the dicarbonyl compound obtained by chromic acid oxidation of the diol. The monoxime, which melted at 375°C., could be prepared by brief warming of the alcoholic solution of the dicarbonyl compound with hydroxylamine acetate, whereas the dioxime, which melted at 263°C., required much longer heating at a higher temperature.

It was decided to try this reaction on the diol obtained in this work in order to obtain further proof of its identity. Accordingly, 0.3 gram of the 210.7-217.8°C. fraction of the diol was dissolved in 20 ml. of warm glacial acetic acid and to this was added dropwise during 30 minutes a solution of 0.1 gram of chromic oxide in some water and 5 ml. of glacial acetic acid at a temperature of 40-45°C. The mixture was allowed to stand at this temperature for 1/2 hour with frequent stirring. The solution was then poured into water and the crude product precipitated. This was filtered off by suction, washed with water, and dried. The dried residue was dissolved in ether and the ether solution washed with 5 per cent sodium bicarbonate solution. The ether was removed by distillation and the residue dissolved in ethyl alcohol. The solution was warmed with a solution of hydroxylamine acetate for twenty minutes at 40°C. The product which precipitated on cooling melted at 241.6-247.7°C. Crystallisation from methyl alcohol raised the melting point to

248.7-251°C. This very small amount of product was used up in an attempt to test for the presence of nitrogen. No trace of this element was found.

Repetition of the Oxidation of the Diol

A sample of 474 mg. of the diol obtained by combining several fractions, all melting between 228°C. and 231°C., from both hydrogenation experiments was placed in a round-bottomed flask equipped with dropping funnel and mechanical stirrer. To this was added 33 ml. of glacial acetic acid and the mixture was warmed to 40-45°C. on a water bath. A solution of 158 mg. of chromic oxide in a few drops of water and 6.5 ml. of glacial acetic acid was added dropwise with stirring during 20 minutes. After all had been added, the mixture was stirred and kept at 40-45°C. for an additional 30 minutes. At the end of this time it was poured into water, the precipitate which formed being filtered off, washed with water, and dried. The white solid was dissolved in ether, the ether solution washed with sodium carbonate solution and water, dried over anhydrous sodium sulfate, filtered, and the ether removed by distillation. A yield of 396 mg. of colorless residue was obtained.

The crude product was dissolved in about 10 ml. of ethyl alcohol, 11 ml. of an alcoholic solution containing approximately 0.4 gram of hydroxylamine acetate was added, and the solution was warmed to 40°C. for 20 minutes on a water bath. A few drops of water were then added and the solution cooled in an ice bath. A crop of 158 mg. of buff-colored, powdery crystals melting at 222.5-230°C. with decomposition was obtained. Concentration of the mother liquor gave 101 mg. of white crystals melting at 220.5-223.5°C.(decomp.). A qualitative test for nitrogen* was positive. The two crystal crops were combined and recrystallized from a mixture of ethyl alcohol and benzene. The produce melted at 252.5-259.5°C.(decomp.). Two more fractions were obtained from the mother liquor and they both melted at 232.5-242.5°C.(decomp.). These three fractions were combined and recrystallized from methyl alcohol-benzene, the crystals so obtained melting at 253.5-254.5°C.(decomp.). Another crystallization from methyl alcohol-benzene raised the melting point to 259.5-261.5°C.(decomp.). Recrystallization from ethyl alcohol-benzene produced 37 mg. of crystals melting at 261.5-263°C.(decomp.). A qualitative test for nitrogen was positive.

Since the highest melting crystals of the oxime which were obtained melted at 261.5-263°C., it seems probable that the dioxime had formed rather than the monoxime, which Zimmerman reported as forming under these conditions. Not enough of the material was available for analysis to further substantiate this assumption.

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* Method "C" of Shriner and Fuson, "The Systematic Identification of Organic Compounds", 2nd ed., p. 114 (1940).

TABLE I

Comparison of the Diol with Erythrodiol

	Diol	Erythrodiol Zimmermann 2,4)	 Ruzicka 3)
Free Diol, m.p.	231-233°	231°	232-233°
$[\alpha]_D$	+69.29° ($CHCl_3$) +66.66° (dioxane)	+75.38° ($CHCl_3$)	+74.6° ($CHCl_3$)
Diacetate, m.p.	182.5-183°	186°	184-185°
$[\alpha]_D$	+60.43° ($CHCl_3$) +56.54° ($CHCl_3$)	+59.41° ($CHCl_3$)	+60° ($CHCl_3$)
Analysis	C, 76.70 H, 10.16	C, 77.26, 77.79 H, 10.62 10.19	C, 77.54 H, 10.18
Diformate, m.p.	194.5-195.5°	195°	
Oxidation product			
Monoxime	———	273°	
Dioxime	261.5-263°	263°	

Examination of the results listed in the above table indicate that the diol prepared from oleanolic acid very likely is identical with erythrodiol. A search of the literature revealed that this compound has not previously been prepared from oleanolic acid by the method reported in this dissertation.

Attempted Reduction of Methyl Oleanolate by Chemical Means*

A sample of 1.5 grams of methyl oleanolate was dissolved in 120 ml. of Skellysolve "B" and the solution placed in a three-necked, round-

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* Method of Bleyberg and Ulrich, Berichte 64B, 2504 (1931).

bottomed flask fitted with a reflux condenser, a mechanical stirrer with a mercury seal, and a dropping funnel. To this was added 1.2 grams of granulated sodium and 4.5 ml. of n-butyl alcohol, added dropwise from the funnel during one hour, the mixture being stirred and heated on a steam bath during this period. An additional 1 gram of sodium and 3 ml. of butyl alcohol were added during 30 minutes in order to insure the presence of an excess of hydrogen. The mixture was then refluxed for about two more hours. At the end of this time, 95 per cent ethyl alcohol was added until the solution became clear and the mixture refluxed for an additional hour. It was then filtered through a fluted filter paper and washed successively with a 100 ml. and a 50 ml. portion of 5 per cent aqueous sodium hydroxide solution in a separatory funnel. Ether was added to compensate for losses of Skellysolve by evaporation. The ether-Skellysolve solution was then dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. A crude residue weighing 1.29 grams was obtained. This represented a yield of 91.5 per cent of the calculated theoretical of diol. After crystallization from methyl alcohol it melted at 199-201.5°C. A mixed melting point of this product with the starting material showed a depression of 15°C.

Attempted Purification of the Reduction Product by Chromatographic Adsorption

A sample of 0.2034 gram of the reduction product was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Hydralo". After development of the chromatogram, the column was extruded, cut in half, each segment eluted with a 70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness. The top segment yielded 0.1047 gram of white solid melting at 210.5-213°C. The bottom segment yielded 0.0611 gram which softened at 145°C. and finally melted at about 160°C. The filtrate yielded no residue. Total recovery was 0.1658 gram; 82 per cent.

A sample of 0.1026 gram of the residue from the top segment in the above was dissolved in 70 ml. of benzene and rechromatographed on "Alorco". The column was again cut in half, after development of the chromatogram, and each segment eluted as above. The top segment yielded 0.090 gram melting at 205-209.5°C. The bottom segment yielded 0.0050 gram, but no melting point was taken. The filtrate yielded nothing. Total recovery was 93 per cent.

Repetition of the Chemical Reduction of Methyl Oleanolate

In view of the inconclusive results obtained in the first attempt to reduce methyl oleanolate by chemical means, it was deemed advisable to repeat the work and to purify the product in a different manner.

A sample of 1.04 grams of methyl oleanolate was dissolved in 200 ml. of purified petroleum ether (B.P. 66°C.), placed in the apparatus previously described, and 8 grams of granulated sodium added (a ten-mole excess). To this mixture 3 ml. of absolute ethyl alcohol was added dropwise over a period of 30 minutes and the mixture then refluxed for another 50 minutes. After this time sufficient 95 per cent ethyl alcohol was added to decompose the excess sodium and the mixture was allowed to stand overnight. It was then refluxed 50 minutes longer and allowed to stand for four days, during which time it acquired an orange color.

The mixture was then filtered and washed with water in a separatory funnel. Ethyl ether was added and the solution washed with two portions of 5 per cent sodium hydroxide solution. The ether-petroleum ether solution was then dried over anhydrous sodium sulfate, filtered, and the solvent removed by distillation to a volume of about 25 ml. When cooled in an ice bath, 724 mg. of crystals formed. They melted at 220-224°C. Evaporation of the remainder of the mother liquor gave 126 mg. of orange, powdery material. This was crystallized from ethyl alcohol and decolorized with Norite. The white crystals obtained were combined with the first 724 mg. and recrystallized from ethyl alcohol. This yielded 255 mg. of crystals melting at 219-222°C. Concentration of the mother liquor gave 136 mg. melting at 223-224.5°C. When this mother liquor was evaporated to dryness, 93 mg. more of crystals melting at 217.5-220.5°C. was obtained.

Acetylation of the Reduction Product

All of the above fractions were combined making a total of 583 mg. This sample was dissolved in 50 ml. of dry pyridine and 8 ml. of acetic anhydride. The solution was allowed to stand overnight at 21°C. It was then diluted with three volumes of water and the precipitated product extracted with 200 ml. of ether. The ether solution was washed with three portions each of 10 per cent hydrochloric acid, saturated sodium carbonate solution, and water. The ether solution was dried over anhydrous sodium sulfate, filtered and the solvent removed by distillation. The crude yield of acetate was 581 mg., 83.7 per cent of the calculated theoretical. It melted at 180-188°C. This was crystallized from ethyl alcohol and 392 mg. of fine, white, lustrous crystals melting at 194.5-197°C. was obtained. Concentration of the mother liquor gave crystals melting at 172.2-176.2°C. All of the fractions of the acetate were combined and recrystallized from petroleum ether, the melting point rising to 200-204.8°C.

Saponification of the Acetate

The final batch of crystals of the above acetate was saponified by refluxing for 3 hours with alcoholic potassium hydroxide. The mixture was diluted and extracted with ether. The ether was evaporated off and the residue crystallized from ethyl alcohol. The crystals obtained melted at 232.5-233.5°C. The identity of this material was not further investigated. Its melting point is 8-9°C. lower than the catalytic reduction product, erythrodiol, however it may be that the material was not completely pure in spite of the short melting range. Such behavior is not uncommon among the triterpene derivatives.

References

1) Parks, L. M., "A Phytochemical Study of Arctostaphylos Uva-ursi", Ph.D. Thesis, University of Wisconsin (1938).

2) Zimmermann, J., Rec. trav. chim. 51, 1200 (1932).

3) Ruzicka, L. and Schellenberg, H., Helv. Chim. Acta 20, 1553 (1937).

4) Zimmermann, J., Helv. Chim. Acta 19, 247 (1936).

III. CHARACTERIZATION OF A TRITERPENE ACID FROM *THYMUS VULGARIS* LINNÉ

Introduction

In 1941, Rogofsky[1] reported the isolation of several grams of white, needle-like crystals from *Thymus vulgaris* Linné. These crystals were obtained from the ether extract of the powdered leaves and flowering tops. The crude extract, after removal of the ether, was "a light green, readily pulverizeble material", which was found to be slightly soluble in the common organic solvents. This material was partially purified by crystallization from ethyl alcohol using Norite to remove the greenish color. Fractions of crystals melting at 278-281°C., 275-277°C., 272-275°C., and 270-271°C. were obtained.

Also isolated was oleanolic acid (caryophyllin) which was identified through its acetate. Neither this compound nor any other, such as the one above, had previously been isolated from thyme.

The purpose of the work described in the following was to characterize the compound from the ether extract. Rogofsky had successfully prepared from it a potassium salt which was insoluble in ether and water. This behavior, along with the high melting point, suggested the probability that the compound was of high molecular weight and possibly that it might be a triterpene derivative, as is oleanolic acid.

Experimental*

Preliminary Examination of the Crystals

The white crystals obtained from thyme for which Rogofsky reported a melting point of 278-281°C., were found to melt at 276-279.5°C. The compound was found to be insoluble in water, 5 per cent hydrochloric acid, 5 per cent sodium hydroxide solution, and only moderately soluble in ether. It dissolved in concentrated sulfuric acid producing a yellow solution. It was moderately soluble in ethyl alcohol, methyl alcohol, and chloroform at room temperature.

Attempted Purification by Recrystallization

A sample of the material was crystallized from 95 per cent ethyl alcohol, after which the melting point was 277.5-279.5°C. (Fraction 1). Recrystallization from methyl alcohol raised this melting point to 279-282°C.(2). When the mother liquor from fraction 2 was chilled in an ice bath, a second crop of crystals melting at 280-281.5°C. was obtained (3). The mother liquor from fraction 1 was evaporated to dryness, the residue melting at 268-272°C.(1a). The mother liquor from fraction 3 was likewise evaporated to dryness, the residue melting at 267-271°C. This was combined with fraction 1a.

Fraction 2 was recrystallized from methyl alcohol and the crystals obtained melted at 279-282°C. as before (2a). Concentration of the mother liquor gave more crystals melting at 279-283°C.(2b). The mother liquor was evaporated to dryness, the residue melting at

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*All melting points are corrected unless otherwise indicated.

279-281°C.(2s). All three fractions were combined since their melting points were practically the same.

Attempted Acetylation of Fraction 2

Since recrystallization had not effected any significant change in the melting point, it was decided to attempt a purification through some derivative of the compound. The appearance, solubility behavior, high melting point, formation of a potassium salt, and method of isolation suggested the possibility that the compound might be a member of the group of compounds commonly known as "triterpene acids", all of which contain hydroxyl groups in addition to carboxyl groups. If so, the compound should form an acetate derivative.

A small quantity of the crystals from fraction 2 was refluxed for 30 minutes with acetic anhydride. The reaction mixture was then poured into water, a heavy white precipitate forming. This was filtered off and dried in an oven at 110°C. for 15 minutes. The crude product melted at 181.5-188°C. It was crystallized from methyl alcohol, after which it melted at 185.7-192°C., resolidified at about 204-206°C., and finally melted indefinitely above 280°C. This behavior is suggestive of that of the "diacetate" of ursolic acid. Although it has but one hydroxyl group, ursolic acid forms a "diacetate" which breaks down on heating to the monoacetate. Riviere and Pichard[2] proposed the theory that it was a mixed anhydride of monoacetyl ursolic acid and acetic acid, which, when heated above its melting point, lost acetic acid and formed monoacetyl ursolic acid anhydride melting above 300°C. Van der Haar[3,4,5] maintained that this "diacetate" was diacetyl ursolic acid anhydride with one molecule of acetic anhydride of crystallization, which, when similarly heated, lost acetic anhydride to give the high melting product. This compound, when heated with alcohol, was split to form monoacetyl ursolic acid. From the fact that the melting point of the original crystals from thyme is only a few degrees lower than that of ursolic acid and from the behavior of the acetate, it was assumed that the compound very possibly might be ursolic acid.

Acetylation of the Original Crystals from Thyme (I)

A sample of 1.5 grams of the crystals obtained by Rogofsky was refluxed for one hour with 25 ml. of acetic anhydride. The reaction mixture was then poured into ice water and the precipitate filtered off and dried at 105°C. for 24 hours. The crude product weighed 1.8 grams and melted at 166-172°C., resolidified at 212°C., and finally melted completely at 308°C. The material was crystallised from methyl alcohol and the crystals obtained melted at 246-255°C. without resolidification after further heating. Another crystallisation from the same solvent raised the melting point to 255-261°C. It was again crystallised from the same solvent and the melting point rose to 270-275°C. Another crystallisation from the same solvent did not change the melting point.

Acetylation of the Original Crystals (II)

Since the melting point obtained for the above acetate was neither sharp nor as high as that reported for acetyl ursolic acid, it was decided to vary the method in the hope of obtaining a higher melting product. A sample of 0.5 grams of the original crystals from thyme was acetylated by the method of Shriner and Fuson.[*] It was dissolved in 5 ml. of pyridine and 2 ml. of acetic anhydride, refluxed for 5 minutes, and set aside at room temperature for 48 hours. At the end of this period, the mixture was poured into cold water, the precipitate which formed extracted with ether, and the ether solution washed with several portions of 5 per cent sodium bicarbonate solution. The bicarbonate washings were extracted with ether and the ether added to the main ether solution. This was dried over anhydrous sodium sulfate, filtered, and the solvent evaporated. The residue still had the odor of pyridine so it was redissolved in ether and washed with several portions of 5 per cent hydrochloric acid and then with water. The ether was removed as before and the residue dried in a vacuum oven. It melted at 261-269°C. Crystallization of this residue from methyl alcohol changed the melting point to 263.5-266°C. Another crystallization from absolute ethyl alcohol did not change the melting point.

Acetylation of the Original Crystals (III)

A third method of acetylation was tried on a sample of 0.5 gram of the original crystals from thyme. This sample was dissolved in 15 ml. of glacial acetic acid and 5 ml. of acetic anhydride and the solution refluxed for 3.5 hours, then allowed to stand overnight. The reaction mixture was poured into water, the precipitate extracted with ether, and the ether solution washed with saturated sodium

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[*] Shriner and Fuson, "The Systematic Identification of Organic Compounds", 2nd Ed. (1940), p. 138.

bicarbonate solution until effervescence ceased. The ether solution was dried over anhydrous sodium sulfate, filtered, and ether removed by distillation. The dried residue melted at 264-275°C. with some decomposition. Crystallization from ethyl alcohol changed the melting point to 263-274°C. with some decomposition. A mixed melting point with some of the acetate from II (m.p. 263.5-266°C.) showed no lowering. A mixed melting point with the starting material showed a lowering of about 45°C. Recrystallization from ethyl alcohol did not alter the melting point.

Chromatographic Adsorption of the Acetate

Since recrystallization of the acetates did not seem to raise the melting point or shorten its range to any great extent, it was decided to attempt a purification by chromatographic adsorption. The acetates from runs II and III were combined making a sample of 0.897 gram. This was dissolved in 150 ml. of benzene and adsorbed on a column of "Hydralo" 5.25 cm. in depth. After development of the chromatogram with 125 ml. of benzene, the column was extruded, cut into three segments, each segment eluted with 95 per cent ethyl alcohol, and the eluates evaporated to dryness.

The top segment yielded 0.161 gram of white solid, which, after crystallization from ethyl alcohol, melted at 262-268°C.(uncorr.) after turning slightly brown at 245°C.(uncorr.). The center segment yielded 0.148 gram of white residue, which, after crystallization from ethyl alcohol, melted at 270.5-272°C.(uncorr.) with a slight amount of decomposition. The bottom segment yielded 0.114 gram of residue which melted at 260-270°C.(uncorr.) after crystallization from ethyl alcohol. The filtrate yielded no residue upon evaporation. The total eluate amounted to only about half the weight of material adsorbed, so the entire adsorbent was re-eluted with 100 ml. of 2 per cent ethyl alcohol in benzene. The residue, after evaporation of the solvent, weighed 0.097 gram. No melting point was taken. Total recovery was 0.520 gram; 58 per cent.

Saponification of the Chromatographed Acetate

The highest melting crystals obtained in the chromatographic adsorption experiment, those from the center segment, were saponified by dissolving in absolute methyl alcohol containing three pellets of U.S.P. potassium hydroxide and refluxing for 3 hours on a steam bath. At the end of this time, the reaction mixture was poured into water, the precipitate which formed extracted with ether and the ether removed by evaporation. The dried residue was crystallized from ethyl alcohol and the fine, needle crystals obtained melted at 280.5-282.5°C. A mixed melting point with a known sample of ursolic acid (m.p. 284-286°C.) showed no depression.

Acetylation of the Original Crystals (IV)

In order to obtain a larger sample of the acetate of the unknown compound from thyme, the acetylation procedure described under III was repeated on 2 grams of the original crystals. The only alteration made in the above procedure was a lengthening of the reflux period from three to eight hours. The product was worked up as previously described and a crude yield of 2 grams was obtained, 91.7 per cent of the theoretical calculated as acetyl ursolic acid. The product was crystallised from ethyl alcohol and a crop of fine, white, needle crystals melting at 270-277°C.(uncorr.) was obtained. Concentration of the mother liquor gave a second crop of crystals melting at 239.6-249.7°C. Recrystallization of the first crop from methyl alcohol yielded two crops of material, the first, a mass of fine, needle crystals, melting at 280-282°C.(uncorr.), and the second, which had a powdery consistency, melting at 264-273°C.(uncorr.). Concentration of the mother liquor gave a third drop melting at 266-271°C.(uncorr.), which was combined with the second crop. The mother liquor was evaporated to dryness and the residue melted at 175.2-186°C. The 280-283°C. fraction of crystals was recrystallised from methyl alcohol and the melting point rose to 283-286°C.(uncorr.). Another crystallization, this time from ethyl alcohol, did not alter the melting point. A mixed melting point of these crystals with a sample of acetyl ursolic acid (m.p. 285-286°C.) showed no depression.

Attempted Preparation of an Acid Chloride of the Acetate

Since a fairly high melting acetyl derivative had been obtained in the last acetylation, above, it was desirable to prepare another derivative of the unknown compound from thyme. There was on hand a sample of acetyl ursolyl chloride with which a comparison could be made, so it was decided to attempt the preparation of the acid chloride from the acetyl compound already prepared.

Approximately 0.2 gram of the acetyl derivative from IV melting at 283-286°C.(uncorr.) was placed in a flask and 1 ml. of thionyl chloride was added dropwise. The acetate dissolved immediately.

The solution was allowed to stand overnight and the excess thionyl chloride then removed by distillation under reduced pressure. An attempt at crystallization from a mixture of benzene and petroleum ether failed and the solvent was evaporated off. The residue was then crystallized from heptane, from which was obtained fine, white, needle crystals melting at 299°C.(uncorr.) after softening at 215-220°C.(uncorr.). The behavior of this melting point indicated that a mixture was present, probably of unreacted acetate and acid chloride. Further crystallization did not seem to purify the product to any extent.

Acetylation of the Original Crystals (V)

Another acetylation of the original crystals from thyme was run on a 2 gram sample by the same procedure as used in IV with a reflux period of 4.5 hours. The product was worked up as before and a yield of 1.9 grams was obtained (87.2 per cent of the calculated theoretical). The crude material was crystallized from ethyl alcohol, the crystals obtained melting at 273-278°C.(uncorr.). After recrystallization from methyl alcohol the melting point rose to 288-290°C.(uncorr.). Another crystallization from methyl alcohol did not alter this melting point. A mixed melting point with a sample of acetyl ursolic acid (m.p. 290-291.5°C.(uncorr.)) showed no depression.

The mother liquors from the above were combined and concentrated yielding a crop of crystals melting at 287-290°C.(uncorr.). This was crystallized from a dioxane and water mixture, the melting point falling to 280-288°C.(uncorr.). Concentration of this mother liquor gave more crystals melting at 226.6-288.7°C. The mother liquor was evaporated to dryness and the residue melted at 149-161.5°C.

Preparation of an Acid Chloride of the Acetate

The preparation of the acid chloride of the acetate was repeated using a 0.2 gram sample of the above acetate melting at 288-290°C. (uncorr.). It was dissolved in 1 ml. of thionyl chloride and allowed to stand for 2 hours. It was then refluxed on a steam bath for half an hour. At the end of this time, the excess thionyl chloride was removed by vacuum distillation. The residue was crystallized from a mixture of benzene and petroleum ether, the brownish needles obtained melting at 191-195°C. Recrystallization from heptane gave white, needle crystals melting at 217.5°C. after softening at 210.7-215.5°C. Another crystallization, this time from Skellysolve "B", raised the melting point to 222°C. A mixed melting point with a known sample of acetyl ursolyl chloride (m.p. 223°C.) showed no depression.

Saponification of the Acetyl Derivative

Approximately 013 gram of the acetyl derivative from V melting at 288-290°C.(uncorr.) was saponified by dissolving in methyl alcohol, to which three pellets of U.S.P. potassium hydroxide had been added, and refluxing on a steam bath for 3 hours. The mixture was then acidified and poured into water. The precipitate which formed was filtered off and crystallised from methyl alcohol. The crystals obtained melted at 263-268°C.(uncorr.). Recrystallization from ethyl alcohol gave one crop of crystals melting at 281-284°C.(uncorr.) and another melting at 266-283°C.(uncorr.). The first crop was recrystallized from methyl alcohol, but the melting point did not change.

Acetylation of the Original Crystals VI

Although the acetate derivative of the crystals isolated from thyme was prepared successfully as described above, it was decided to attempt the purification of a large sample by acetylation and fractional crystallization of the acetate. For this run, 10 grams of the compound was placed in an acetylation flask and refluxed for 8.5 hours with 50 ml. of acetic anhydride and 100 ml. of glacial acetic acid. At the end of this time, the mixture was poured into water, the precipitate which formed filtered off and washed with sodium bicarbonate solution, and the material dried in a vacuum oven.

A series of crystallizations was then carried out on this dried acetate. It was first crystallized from ethyl alcohol, after which it melted at 271-281°C.(uncorr.)(Fraction 1). This was then recrystallized from methyl alcohol, the melting point rising to 279-284°C.(uncorr.)(2). Another crystallization from methyl alcohol raised the melting point to 283-287°C.(uncorr.)(3). Recrystallization of these crystals from methyl alcohol changed the melting point to 287-290°C.(uncorr.)(4). After another crystallization from methyl alcohol, the acetate melted at 286-289°C.(uncorr.)(5). Finally, crystallization from acetone produced little change, the crystals melting at 288-290°C.(uncorr.)(6).

The mother liquors from these crystallizations were all concentrated and more crystals obtained. The mother liquor from fraction 1 upon concentration gave crystals melting at 231.5-251.5°C.(1a).

Further concentration of the mother liquor gave more crystals melting at 228.5-244.4°C.(1b). The remaining mother liquor was then evaporated to dryness, the residue melting at 212.7-242.6°C.(1c). The further purification of fractions 1a and 1b by chromatographic adsorption is described on p. 73 .

Concentration of the mother liquor from fraction 2 gave crystals which melted at 275-280°C.(uncorr.)(2a). The rest of this mother liquor was combined with the mother liquor from fraction 3 and the mixture concentrated. Crystals melting at 283-287°C.(uncorr.)(3a) were obtained. This fraction was recrystallized from methyl alcohol and the melting point became 282-284°C.(uncorr.)(3b).

The mother liquor from fraction 4 was concentrated and crystals melting at 282-285°C.(uncorr.)(4a) obtained. The further purification of fractions 3b and 4a by chromatographic adsorption is described on p. 74 . The mother liquor from fraction 5 was evaporated to dryness and the residue crystallized from acetone. The crystals obtained melted at 285-287°C.(uncorr.)(5a). The mother liquor from fraction 6 upon concentration gave crystals melting at 288.5-290.5°C.(uncorr.)(6a).

Saponification of Fraction 5a

Fraction 5a of acetate VI was saponified by refluxing its methyl alcoholic solution with four pellets of U.S.P. potassium hydroxide for 3 hours. The product was then worked up in the usual manner and the crude product crystallized from methyl alcohol. The crystals obtained melted at 282.5-284°C.(uncorr.). A mixed melting point with a known sample of ursolic acid (m.p. 282-284°C.) showed no depression. A mixed melting point with some unsaponified fraction 5a showed a depression of 30-37°C. The optical rotation of these crystals was determined with the following results:

$[\alpha]_D^{24.5}$ + 62.1° (45.7 mg. in 2 ml. of 0.09 N methyl alcoholic potassium hydroxide α_D + 1.42°, l 1 dm.).

The optical rotation of a known sample of ursolic acid was determined at the same time with the following result:

$[\alpha]_D^{24.5}$ + 62.6° (40.6 mg. in 2 ml. of 0.09 N methyl alcoholic potassium hydroxide α_D + 1.27°, l 1 dm.).

Attempted Purification of Fractions Ia and Ib of the Acetate by Chromatographic Adsorption

Fractions Ia and Ib of acetate VI were combined making a sample of 1.180 grams. This was dissolved in 200 ml. of benzene and chromatographically adsorbed on "Hydralo". After development of the chromatogram, the column was extruded, cut into three segments, each segment eluted with ether, and the eluates evaporated to dryness. The top segment yielded 0.093 gram of white solid which melted at 181-206°C., resolidified at about 220°C., and finally melted at 260-268°C.(uncorr.). The center segment yielded 0.097 gram melting at 181-204°C., resolidifying at 220°C., and finally melting at 258-266°C.(uncorr.). The bottom segment yielded 0.058 gram which melted at 260-268°C.(uncorr.) after first melting at 193-203°C. and resolidifying at 218-220°C.

The top segment was re-eluted with a 70:30 mixture of ether and methyl alcohol, and 0.083 gram of residue melting at 221-234°C. was obtained. Recrystallization from methyl alcohol raised the melting point to 240-250°C. The bottom two segments were combined and also re-eluted with ether-methyl alcohol. A yield of 0.043 gram melting at 188-199°C. was obtained. Recrystallization from methyl alcohol raised the melting point to 232.5-235.5°C. The entire adsorbent was re-eluted with a 50:50 mixture of ethyl alcohol and ether and 0.215 gram of white solid melting at 269-275°C.(uncorr.) was obtained. The total recovery was 0.589 gram; 50 per cent.

Attempted Purification of Fractions 3b and 4a by Chromatographic Adsorption

Fractions 3b and 4a of acetate VI were combined making 0.812 gram. This sample was dissolved in 100 ml. of benzene and chromatographically adsorbed on "Alorco". After development of the chromatogram, the column was extruded, cut into three segments, each segment eluted with a 70:30 mixture of ether and methyl alcohol, and the eluates evaporated to dryness. The top segment yielded 0.083 gram melting at 285-288°C.(uncorr.). The center segment yielded 0.060 gram melting at 284-286°C.(uncorr.). The bottom segment yielded 0.037 gram melting at 284-287°C.(uncorr.). The filtrate gave 0.156 gram of residue melting at 285-288°C.(uncorr.). Re-elution of the entire adsorbent gave 0.324 gram more of compound. No melting point was taken. Total recovery was 0.660 gram; 81.3 per cent.

Preparation of the Acid Chloride of Fraction 8a

The acid chloride of fraction 8a of acetate VI was prepared by refluxing a sample dissolved in purified thionyl chloride on a steam bath for one hour and then allowing to stand at room temperature for 48 hours. The excess thionyl chloride was then removed by distillation and the residue crystallized from petroleum ether. The crystals obtained melted at 219-221°C. This melting point, although somewhat lower, compares favorably with that reported in the literature[6] for acetyl ursolyl chloride and also with that of the acid chloride described on p. 70 .

Attempted Preparation of the Methyl Ester of the Acetate

In order to obtain a third derivative of the compound from thyme, it was decided to prepare the methyl ester of the acetyl derivative. The reaction was carried out by refluxing a sample of the above acid chloride with methyl alcohol for six hours. At the end of this time the mixture was poured into water, the precipitate extracted with ether, the ether solution dried over anhydrous sodium sulfate, and the solvent removed by distillation. The residue was crystallized from methyl alcohol, after which it melted between 110°C. and 120°C., resolidified, and then melted sharply at 169°C. This is the melting point of methyl ursolate and it was assumed that the acetyl group might have been split off in some way yielding the methyl ester instead of the acetyl methyl ester. The assumption was confirmed by a mixed melting point with a known sample of methyl ursolate which gave no depression. Further proof of the fact that the acetyl group was split off was provided when a sample of known acetyl ursolyl chloride was refluxed with methyl alcohol as described above. The product obtained melted at 170°C. and gave no depression of melting point when mixed with the compound above melting at 169°C. This behavior of the acetyl acid chloride when refluxed with methyl alcohol has not been reported in the literature. In an unpublished report, Kremers, Sell, and Stockey report the preparation of acetyl ethyl ursolate by refluxing the acetyl acid chloride with absolute ethyl alcohol. They stated that the product was reacylated, but do not give a reason for this step.

Attempted Benzoylation of the Crystals from Thyme (I)

A sample of 1 gram of the original crystals isolated from thyme was dissolved in 5 ml. of anhydrous pyridine, 1 ml. of benzoyl chloride added, and the mixture warmed gently on a steam bath for a few minutes. It was then poured into water, the precipitate which formed filtered off, and washed with sodium carbonate solution, dilute hydrochloric acid, and water. The solid material was crystallized from methyl alcohol and the crystals obtained melted at 270-274°C.(uncorr.). A mixed melting point with the starting material showed a depression of 30°C. The substance was then refluxed with methyl alcohol, but not all dissolved. The insoluble portion was filtered off and crystallised from acetone. It melted at 247-270°C.(uncorr.). The soluble portion was allowed to crystallise and the fine, white needles obtained melted at 273-276°C.(uncorr.). Concentration of this mother liquor gave more crystals melting at 270-273°C.(uncorr.).

The fraction melting at 247-270°C. was washed with about 15 ml. of Skellysolve "A" and the insoluble portion filtered off. It melted at 276-281°C.(uncorr.). A mixed melting point with the starting material showed a depression of 35°C. Recrystallization from methyl alcohol caused the melting point to become sharp at 282-284°C.(uncorr.). A mixed melting point with a known sample of ursolic acid showed a depression of 36-37°C.

The identity of the benzoylated material could not be ascertained. It was evident from the mixed melting points that some reaction had taken place, but what it was is not known. The melting point of ursolic acid benzoate has been reported as being 214°C.[7,8] If the compound from thyme is ursolic acid, as is strongly indicated by the melting point of the free acid, the acetate, and the methyl ester, then some other compound has formed. It is possible also that the melting point reported for the benzoate is in error.

Attempted Purification Through the Benzoate (II)

Since the results of the benzoylation described above were indefinite, the experiment was repeated with the idea in mind of obtaining enough compound to be used in purifying the original crystals as well as obtaining a derivative for identification purposes. The procedure was varied somewhat from the above. A sample of 5 grams of the original crystals from thyme was refluxed over a small flame with 2 ml. of benzoyl chloride in a mixture of pyridine and benzene for 3.5 hours. At the end of this period, the mixture was poured into water, the precipitate which formed extracted with ether, and the ether solution washed with five portions each of 5 per cent hydrochloric acid, 5 per cent sodium bicarbonate solution, and distilled water. The ether solution was then dried, the solvent evaporated, and the residue crystallized from methyl alcohol. A crop of 1.3 grams of powdery crystals melting at 183-198°C. was obtained. A second crop of 1.1 gram with the same melting point was obtained by concentration of the mother liquor. It was combined with the first crop (Fraction 1).

The combined material was washed with 50 ml. of boiling Skellysolve "A".

The insoluble portion weighed 2.3 grams and melted at 206-211°C.(2). This fraction was treated with 75 ml. of boiling Skellysolve "A" and 1.5 grams of insoluble material melting at 215-222°C. was obtained(3). The treatment was repeated and 1.2 grams of insoluble material melting at 222-227°C. was obtained(4). When this material was crystallised from methyl alcohol not all dissolved in the solvent. The insoluble material melted at 245-260°C.(5). The soluble portion crystallised out and melted at 237-250°C.(5a). It was combined with fraction 5 and treated with 50 ml. of hot Skellysolve "A". The insoluble material melted at 240-250°C.(6). This was crystallised from methyl alcohol, after which it melted at 244-250°C.(7). Evaporation of the mother liquor gave a residue melting at 240-265°C.(7a).

The mother liquor from fraction 5a was concentrated and crystals melting at 224-228°C. were obtained (5b). This was treated with Skellysolve "A" as above, after which it melted at 228-231°C.(5c). This fraction was combined with fractions 7 and 7a and the mixture treated with hot Skellysolve "A". The insoluble portion melted at 243-250°C.(8). Crystallisation from ethyl alcohol raised the melting point to 273-278°C.(9). Another crystallisation from ethyl alcohol raised the melting point to 275-280°C.(10). Evaporation of the combined mother liquors from 9 and 10 gave a residue melting at 265-270°C.(9a).

Attempted Benzoylation (III)

Since no fraction of constant or sharp melting point was obtained in the first two attempts at benzoylation, the reaction was run again on a 2 gram sample of the original crystals from thyme. It was dissolved in 10 ml. of anhydrous pyridine and 0.7 ml. of benzoyl chloride and refluxed over a small flame for 2.3 hours. The product was worked up as described above. A quantity of material, 0.564 gram, was insoluble in the ether used to dissolve the product. It melted at 221.5-235.6°C. This material was washed with two portions of hot petroleum ether, after which it melted at 236-243°C. The washing was repeated and the melting point became 236-243.5°C. This material was then crystallised from acetone and two crops of crystals were obtained. The first melted at 234-236.5°C., and the second melted at 230.5-235.5°C. The two crops were combined and recrystallised from methyl alcohol, the crystals so obtained melting at 237.6-241°C. A second crop melted at 228-239.5°C. These two crops were combined and recrystallised from methyl alcohol and two crops were again obtained. The first crop melted at 232.5-236.5°C., and the second melted at 239-240.5°C.

The solvent was evaporated from the ether-soluble portion and the residue was crystallised from ethyl alcohol. A crop of 0.41 gram of powdery material precipitated from the solvent. It melted at 200-206°C. after previous softening at 190°C. Concentration of the mother liquor gave 0.21 gram more melting at 190-197°C. The mother liquor was evaporated to dryness and 0.26 gram of material was obtained, but no melting point was taken. All these fractions were combined, recrystallised from acetone, and two crops of crystals were obtained. The first melted at 217.5-222.5°C., and the second melted at 206.7-213.5°C. These two crops were combined and recrystallized from ethyl acetate, the crystals obtained melting at 237.6-253.7°C. after softening at 234°C.

Attempted Benzoylation of Ursolic Acid

At this point it was decided to try to prepare a benzoate of a known sample of ursolic acid for comparison purposes. A sample of 1 gram of ursolic acid was used and the procedure described in III followed. The crude material was crystallized from ethyl alcohol, but part of the material did not dissolve. This insoluble material was filtered off and found to melt at 279-281°C.(uncorr.). Upon standing the solvent yielded a crop of crystals melting at 280-284°C.(uncorr.). Concentration of the mother liquor gave another crop which melted at 267-272°C.(uncorr.). The mother liquor was evaporated to dryness and the residue melted at 180-212.7°C. A mixed melting point of the 280-284°C. fraction with the original ursolic acid showed a depression of 70-75°C. A mixed melting point with some of the benzoylated thyme crystals (m.p. 283-284°C.) showed a depression of 73-79°C. The identity of the product of benzoylation was not determined, however, from its melting point, or variety of melting points, it is assumed that it could not be benzoyl ursolic acid.

Table I

Comparison of Thyme Crystals with Ursolic Acid

	Thyme Crystals	Ursolic Acid
Compound, m.p.	282.5-284.5°C.	282-284°C.* 284-285°C.(9,10)
	No depression when mixed.	
$[\alpha]_D$	+62.1°	+62.6°*
Acetate, m.p.	288.5-290.5°C.	289-290°C.(9) 285-286°C.*
	No depression when mixed.	
Acetyl-acid chloride, m.p.	222°C.	223°C.* 224-225°C.(6)
	No depression when mixed.	
Methyl Ester	169°C.	170°C.*
	No depression when mixed.	

Summary

From the results as listed in Table I, it may be concluded that the compound isolated from the ether extract of *Thymus vulgaris* Linné, by Rogofsky is identical with ursolic acid. The existence of this triterpene acid along with oleanolic acid in the same plant is unusual while not particularly surprising in view of the close relationship between the two. It is possible that the repeated failure to raise the melting point to that reported for ursolic acid is due to the presence of small quantities of oleanolic acid, which differs from ursolic acid only in the location of the double bond. Because of this isomerism it is difficult to separate mixtures of the two.

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* Material on hand.

References

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IV. AN INVESTIGATION OF THE TRITERPENE CONSTITUENTS OF THE BUDS OF *CARYOPHYLLUS AROMATICUS* LINNÉ

Introduction

The existence of two or more triterpene constituents occurring together in a single plant has been observed in several instances. For example, ursolic acid occurs along with a triterpene diol in Uva Ursi, while oleanolic acid and ursolic acid are both found in *Thymus vulgaris* L. Clove buds (*Caryophyllus aromaticus* L.), from which oleanolic acid (caryophyllin) was first isolated[1] has been found to be an excellent sourse of this triterpene acid. However, to date, no other triterpene derivative has been obtained from this plant. It was thought to be possible that another such compound might reasonably be present as either a precursor or a reaction product of oleanolic acid, or possibly as an isomer.

The work which is described in the following was undertaken with the purpose in mind of attempting to isolate and identify such a compound or compounds. The drug was extracted only with petroleum ether since this solvent has been found to satisfactorily remove triterpene constituents from other plants.

Experimental*

Extraction of Clove Buds with Petroleum Ether

The material which was extracted consisted of 3.78 Kg. of "Clove Buds, U.S.P." obtained from S. B. Penick and Co. The drug was ground moderately coarse in a power mill and packed in two large

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*All melting points herein reported are corrected unless otherwise indicated.

glass percolators arranged for continuous extraction over two-liter flasks. Skellysolve "A" (b.p. 40-60°C.) was added through the condensers and the drug extracted for four days, fresh solvent being added from time to time to compensate for evaporation losses. At the end of this time, the marc in both percolators was allowed to drain and the extracts combined and set aside (Fraction A). The last portions of the percolate were tested for completeness of extraction by evaporation to dryness. A residue remained indicating incomplete extraction.

The marc was removed from the percolators, allowed to dry, and repacked as before. More petroleum ether was added and percolation continued for four days. A test, carried out as described above, indicated incomplete extraction. The marcs were allowed to drain and the extractives combined and set aside (Fraction B).

The marc was again removed from the percolators, allowed to dry, and the percolators repacked. Fresh solvent was added and percolation continued for three days. After the first day, the solvent in the flasks acquired a milky-white appearance. At the end of the third day, when percolation was discontinued and the marc allowed to drain, the flasks under each percolator contained a large quantity of white solid material. The contents of both flasks were combined and set aside (Fraction C).

The marc was removed, dried, repacked as before, and fresh solvent added. The drug was then extracted for a period of 3.5 days. It was necessary to stop this extraction twice because of lack of solvent. Part of the fresh solvent used was high test gasoline which had been washed with sulfuric acid until the washings were colorless, washed with water, dried over calcium chloride, and distilled. The fraction distilling between 60°C. and 70°C. was used. At the end of the extraction period 10 ml. of percolate was evaporated to dryness and the residue left indicated incomplete extraction. A heavy crop of white solid was present in each flask as before. The combined extracts were set aside (Fraction D).

The marc was removed, dried, repacked, fresh petroleum ether added, and extraction allowed to continue for ten days. Although a very slight residue was left when 10 ml. of fresh percolate was evaporated to dryness, the amount of solid material present in the extraction flasks had not materially increased during the last seven days. The extracts from both flasks were combined and set aside (Fraction E).

The total extraction period was 24.5 days.

Fraction A

After having stood for a few days, fraction A contained a brownish residue which appeared to be solid. The mixture was filtered and the light brown substance left on the filter paper had a semi-solid consistency. After drying for a week at room temperature, the material remained soft. It was then dried at 105°C. for three hours and feathery crystals were noted throughout the salve-like mixture. However, upon standing overnight, they redissolved in the mixture. A total of 9.5 grams of this brown material was obtained (Fraction A-1).

The filtrate from fraction A-1 was distilled under carbon dioxide on a steam bath. After four hours, no more petroleum ether distilled over and a residue of 740 ml. of dark brown liquid having the characteristic odor of eugenol remained (Fraction A-2). This fraction was stored under carbon dioxide overnight and then steam distilled until no more oil came over. A yield of 45 ml. of colorless, volatile oil of cloves was obtained. The residue remaining in the distilling flask, although mixed with water, was very cloudy and viscid in nature. An attempt to filter off what appeared to be solid matter was unsuccessful.

Saponification of Fraction A-2

To the liquid residue from the steam distillation was added one liter of 17 per cent sodium hydroxide solution and the mixture refluxed for 33 hours on a steam bath. The saponified mixture was then set aside for two months, after which time it was extracted with ether until the extracts were colorless and left no residue upon evaporation. The ether was removed by distillation and there remained a residue of about 75 ml. of dark brown liquid possessing the characteristic odor of eugenol.

Isolation of a Hydrocarbon

It was observed that when methyl alcohol was added to the non-saponifiable fraction, a mass of yellow solid precipitated. A portion of the non-saponifiable fraction was therefore treated with methyl alcohol until no more solid precipitated. This was filtered off by suction and the material obtained was of an oily, semisolid nature and melted below 100°C. It was crystallized from acetone, after which it was more solid in consistency but still retaining its yellow color. This material was soluble in concentrated sulfuric acid, it decolorized a solution of bromine in carbon tetrachloride, and it reduced a 2 per cent solution of potassium permanganate to a slight extent.

All of the compound was then recrystallized from acetone. The crystals obtained were dissolved in benzene and the yellow solution passed through a column of activated aluminum oxide ("Alorco"). The filtrate, after development of the chromatogram, possessed the same yellow color as the starting solution. There was little color on the adsorbent which was eluted with petroleum ether containing 2 per cent of ethyl alcohol. No residue was left after evaporation of the eluate. The yellow filtrate was evaporated to dryness and a yellow residue having the consistency of wax was obtained. It still retained a faint odor of eugenol. It melted at 60.5-62°C. A small amount, when dissolved in carbon tetrachloride, did not decolorize 3 drops of 5 per cent bromine in carbon tetrachloride, nor did it reduce a solution of potassium permanganate. An elementary analysis for halogen, sulfur, and nitrogen was negative.* The material was found to be insoluble in water, 5 per cent sodium hydroxide solution, and 5 per cent hydrochloric acid. It was soluble in concentrated sulfuric acid. This solubility behavior placed the compound in Class I of the scheme of Shriner and Fuson.** This observation and the fact that the substance was not adsorbed on aluminum oxide indicated that it was probably a hydrocarbon.

A sample of 1.666 grams of this substance was dissolved in purified petroleum ether and shaken with powdered, activated aluminum oxide. After standing overnight, the mixture was filtered and the

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* Shriner and Fuson, "The Systematic Identification of Organic Compounds", 2nd Ed. (1940), p. 112.

**Ibid., p. 7.

solvent removed by distillation. The residue was a colorless liquid which solidified to a pure white product on cooling. This residue was crystallized from chloroform, after which it melted at 67-68°C. The compound was silvery-white and of a waxy consistency. A sample was dried overnight in a vacuum oven at 35°C. and a macro carbon and hydrogen analysis was run with the following results:

Calculated for $C_{30}H_{62}$: C, 85.22; H, 14.78. Found: C, 84.94; H, 14.39. Total: 99.33.

From the melting point, analysis, and chemical behavior, it seems probable that the hydrocarbon described above may be either n-triacontane (m.p. 66°C.)[2] or n-hentriacontane (m.p. 68.4°C.)[2] or a mixture of the two. A search of the literature revealed that no hydrocarbon of this description has previously been isolated from cloves.

Isolation of More Hydrocarbon

Another larger portion of fraction A-3 was refluxed for 30-40 minutes with 20 per cent sodium hydroxide solution in order to remove the eugenol present. The mixture was then extracted with Skellysolve "B", the Skellysolve solution dried, filtered, and solvent removed by distillation. The residue was a pale yellow liquid which solidified on cooling. This material was crystallized from acetone and the white, waxy crystals obtained melted at 61-64°C. Recrystallization from methyl ethyl ketone raised the melting point to 65.5-66.5°C. The mother liquors, upon evaporation, yielded further small quantities of the hydrocarbon.

Isolation of Caryophyllene (?)

The combined portions of fraction A-2 from which the hydrocarbon had been precipitated by addition of methyl alcohol were refluxed with 20 per cent sodium hydroxide solution for one hour and then extracted with petroleum ether. The petroleum ether solution was dried, filtered, and the solvent removed by distillation. The residue was a brown oil which did not solidify when chilled in an ice bath. The addition of methyl alcohol did not precipitate any more hydrocarbon. This oily material, which measured 37 ml., was fractionally distilled through a Widmer column using an electrically heated oil bath.

Fraction 1 distilled over at 63-71°C. and was entirely methyl alcohol. Since no more material came over up to 195°C., a Wood's metal bath was substituted for the oil.

Fraction 2 distilled over at 250-258°C. and consisted of 16.5 ml. of colorless, syrupy liquid which had a pleasant, aromatic odor. This liquid was found to be insoluble in 5 per cent sodium hydroxide solution and in 5 per cent hydrochloric acid. It dissolved in concentrated sulfuric acid forming a red-violet solution with the evolution of heat. A small portion, dissolved in carbon tetrachloride, rapidly reduced a 2 per cent solution of potassium permanganate. Another portion also rapidly decolorized a 5 per cent solution of bromine in carbon tetrachloride with the evolution of hydrogen bromide. A few drops of the liquid was mixed with concentrated sulfuric acid containing a crystal of vanillin* and a violet-red color resulted.

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*Pharm. Acta Helv. 17, 101 (1942).

The tests with bromine, permanganate, and vanillin in sulfuric acid indicated that the compound was unsaturated.

When tested with ferric chloride solution, Schiff's reagent, and Fehling's solution, the material gave no reaction. With Tollen's reagent, a black precipitate formed after a short time. The substance was perfectly miscible with acetyl chloride, but no heat was produced. When water was added to the mixture, a second layer formed, but the odor was little if any different from that of the original material. A micro boiling point* was determined and found to be 259°C.

The properties observed for this liquid are suggestive of caryophyllene, a sesquiterpene, which is reported to boil at 258-259°C. at 753 mm. Hg. by Erdmann.3) Wallach,4) who first isolated the compound from cloves, reported that it distilled between 250°C. and 260°C. at atmospheric pressure. As isolated from plant sources, caryophyllene is a mixture of three hydrocarbons, α-, β-, and γ- (or iso) caryophyllene. It is very possible that the material isolated in this investigation is a mixture of the three isomers.

Fraction 3, the residue from the fractional distillation, was a dark-brown, viscous liquid of about the consistency of molasses. This material was diluted with acetone and a heavy precipitate of yellowish-brown substance was formed on cooling. This precipitate was filtered off, dissolved in purified petroleum ether, Norite added, and the mixture boiled for ten minutes. The charcoal was filtered off, the solvent evaporated, and a yellowish residue obtained. This was

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*Method C of Shriner and Fuson, "The Systematic Identification of Organic Compounds", 2nd Ed. (1940), p. 93.

dissolved in benzene and shaken with activated aluminum oxide, allowed to stand, the adsorbent filtered off, and the solvent evaporated. The residue was crystallized from acetone and a crop of snow-white crystals melting at 54-56°C. was obtained. Recrystallization from chloroform was attempted, but no crystals formed. Recrystallization from acetone gave crystals melting at 60-62.5°C. This material had a waxy consistency and probably was more of the hydrocarbon previously described.

Fraction B

Fraction B was filtered and 10.4 grams of light yellow or buff-colored powder was left on the filter paper (Fraction B-1). It was dried in the vacuum oven at 100°C., after which it was dissolved in 500 ml. of 95 per cent ethyl alcohol. The yellow solution was refluxed for 10 minutes with Norite, filtered, and the slightly yellow filtrate allowed to crystallize. A yield of 7 grams of pure white crystals melting at 307-309.5°C.(uncorr.) was obtained. The mother liquor was again refluxed with charcoal for 10 minutes, filtered, and concentrated. Upon cooling, a second crop of 1.5 grams of crystals melting at 305.5-308°C.(uncorr.) was obtained. A mixed melting point of the 307-309.5°C. fraction with a known sample of oleanolic acid (m.p. 307.5-309.5°C.) showed no depression.

The filtrate from fraction B-1 was distilled under carbon dioxide on a steam bath. Distillation was continued until the volume of solution remaining in the flask measured about 200 ml. This was transferred to a beaker and heated on a steam bath under a stream of carbon dioxide until boiling ceased. The residue was a dark-brown liquid possessing the characteristic odor of eugenol (Fraction B-2).

Fraction C

Fraction C was filtered and 23.8 grams of pale-yellowish or buff-colored solid was left on the filter paper (Fraction C-1). It still retained the odor of eugenol. The material was dried in the vacuum oven at 100°C. and a sample of 2 grams of the dried substance was crystallized from absolute methyl alcohol. Crystals melting at 295-308°C.(uncorr.) with slight decomposition were obtained. The mother liquor was greenish in color. The crystals were again crystallized from absolute methyl alcohol, the melting point rising to 300-308°C.(uncorr.) with slight decomposition. A third crystallization from methyl alcohol had no effect on this melting point. Recrystallization from absolute ethyl alcohol raised the melting point to 308-309°C. (uncorr.). A mixed melting point with a sample of oleanolic acid (m.p. 308-310°C.) showed no depression. The greenish mother liquor from the 295-308°C. fraction of crystals deposited more crystals upon standing. They melted at 303-308°C.(uncorr.).

The filtrate from fraction C-1 was distilled under carbon dioxide in the same manner as described under fraction B. The residue consisted of a dark brown liquid possessing the odor of eugenol (Fraction C-2).

Preparation of a Methyl Ester

The entire 0.661 gram of crystals from fraction C-1 melting at 308-309°C. was esterified. The sample was dissolved in 50 ml. of absolute methyl alcohol, 0.1 gram of U.S.P. potassium hydroxide and 0.25 ml. of dimethyl sulfate added, and the mixture refluxed for 5 hours on a steam bath. At the end of this time, it was poured into water with the formation of a white precipitate. This was extracted with ether, and the ether solution washed three times with 30 ml. portions of 3 per cent aqueous sodium hydroxide solution. During the first washing, there was precipitated from the ether solution a quantity of white, flocculent material, insoluble in either of the solvents. This behavior is characteristic of high molecular weight, polycyclic acids such as oleanolic acid. The sodium salts of these acids are insoluble in both water and ether, therefore, when crude esterification products are worked up and the ether solutions are washed with sodium hydroxide solution, any free acid remaining is precipitated as the sodium salt.

The precipitated material was filtered off, the ether solution washed with water, dried over anhydrous sodium sulfate, and solvent removed by evaporation. The residue was crystallized from ethyl alcohol and the white, needle crystals obtained melted sharply at 198-199°C. A mixed melting point with a known sample of methyl oleanolate (m.p. 199-200°C.) showed no depression.

Attempted Saponification of the Methyl Ester

An attempt was made to saponify the methyl ester by refluxing a methyl alcoholic solution of it with several pellets of potassium hydroxide for 3 hours. The product was worked up in the usual way and after crystallization from methyl alcohol it melted at 199.5-200.5°C. (uncorr.). This indicated that saponification had not taken place. This difficulty of saponification is characteristic of esters of the triterpene acids where the tertiary carboxyl group is strongly hindered.

Fraction D

Fraction D was filtered and 20.3 grams of buff-colored, crystalline solid was left on the filter paper (Fraction D-1). A portion of this material was crystallized from ethyl alcohol after decolorizing with Norite. A crop of pure white, needle crystals melting at 308-311°C.(uncorr.) was obtained (Fraction D-1a). Two more crops, obtained by concentration of the mother liquor, were combined and recrystallized from ethyl alcohol, two fractions of crystals being obtained. The first melted at 308-310°C.(uncorr.)(D-1b) and the second at 308.5-309.5°C.(uncorr.)(D-1c). The mother liquors were combined and concentrated, three fractions of crystals being obtained. The first melted at 298-304°C.(uncorr.)(D-1d), the second at 302-305°C. (uncorr.)(D-1e), and the third, obtained by evaporation of the mother liquor to dryness, at 283-293°C.(uncorr.)(D-1f). This latter fraction was recrystallized from methyl alcohol, the melting point rising to 298-304°C.(uncorr.)(D-1g). The mother liquor was evaporated to dryness and the residue melted at 280-290°C.(uncorr.)(D-1h).

Attempted Purification by the Method of Dodge[5)]

A further purification of the crystals melting at 308-310°C. (fraction D-1b) was attempted by the method of Dodge. Although this material had as high a melting point as any obtained from other fractions, this experiment was tried in an attempt to obtain an even higher melting compound by removing any minute amounts of impurities which might be present. Dodge had found that oleanolic acid could be freed from small amounts of contaminating ursolic acid by taking advantage of the lesser solubility of the potassium salt of oleanolic acid in alcohol. There was no reason to believe that ursolic acid might be contaminating the material, but the method was applied as a general one to remove any impurities whether of acidic nature or not.

A sample of 5 grams of the crystals was refluxed with 50 ml. of 0.5 N alcoholic potassium hydroxide solution and 25 ml. of water for one hour. The solution was filtered while hot and the filtrate allowed to cool slowly. Large needle crystals of the potassium salt formed. They were filtered off, dissolved in 3 parts of cold methyl alcohol, the solution filtered, and the salt reprecipitated by the addition of water. The snow-white precipitate was filtered off, dissolved in 10 parts of cold methyl alcohol, and a slight excess of concentrated hydrochloric acid added. The entire mixture solidified into a mass of white crystals. The mixture was diluted with more methyl alcohol and the crystals filtered off and dried. The material was then crystallized from ethyl alcohol and 3.2 grams of white, needle crystals melting at 308-310°C.(uncorr.) was obtained. Concentration of the mother liquor gave a second crop of crystals melting at 307.5-309.5°C.

(uncorr.). The mother liquor was evaporated to dryness and the residue melted at 305-308°C.(uncorr.). From the results it appears that no other substance was present, at least in detectable amounts.

Preparation of an Acetate

A sample of 1 gram of fraction D-1a was acetylated by dissolving in 5 ml. of acetic anhydride and 10 ml. of glacial acetic acid and warming on a steam bath for 3 hours. At the end of this period, the mixture was poured into water, the precipitate which formed filtered with suction, and washed successively with 5 per cent sodium bicarbonate solution and water. The dried product was crystallized from ethyl alcohol and the white, needle crystals obtained melted at 265.5-267°C.(uncorr.). A mixed melting point with a sample of acetyl oleanolic acid (m.p. 264-265°C.) showed no depression.

Fraction D-2

The filtrate from fraction D-1 was distilled under carbon dioxide on a steam bath and the residue consisted of about 25 ml. of dark-brown liquid possessing the characteristic odor of eugenol (Fraction D-2).

Fraction E

Fraction E was filtered and 10 grams of brownish material was obtained which contained some of the powdered crude drug which accidentally spilled into the extraction flask (Fraction E-1). This fraction was divided into two parts. One part was dissolved in ether and filtered to remove the crude drug. The filtrate was evaporated to dryness and the greenish residue dissolved in a mixture of ethyl alcohol and benzene. The second portion of the fraction was refluxed in a mixture of ethyl alcohol and benzene, filtered while hot, and the filtrate added to the alcohol-benzene solution of the first portion. This solution was decolorized by boiling with Norite for 10 minutes and filtering. The filtrate, upon cooling, produced a large crop of snow-white crystals melting at 307-309°C.(uncorr.). Concentration of the mother liquor yielded more crystals melting at 302-307°C.(uncorr.). From the melting points, these two fractions were assumed to be oleanolic acid, the latter in less pure form.

Preparation of an Acetate

The fraction of crystals above melting at 302-307°C. was acetylated by dissolving in 5 ml. of acetic anhydride and 15 ml. of glacial acetic acid and warming on a steam bath for 3 hours. The mixture was then poured into ice water, the precipitate filtered off, washed with 5 per cent sodium bicarbonate solution and water, and dried. The product was crystallized from ethyl alcohol and the white, needle crystals obtained melted at 252-257°C. Recrystallization from methyl alcohol raised the melting point to 262-264°C. A mixed melting point with a sample of acetyl oleanolic acid (m.p. 264-265°C.) showed no depression.

Fraction E-2

The filtrate from fraction E-1 was distilled under carbon dioxide on a steam bath and the residue consisted of about 20 ml. of brown, oily liquid such as was obtained from the other fractions (Fraction E-3).

Summary

The work described above has failed to show the presence of any triterpene derivatives in clove buds other than the previously isolated oleanolic acid. All fractions of solid material which precipitated during extraction were shown to be oleanolic acid, identified through its acetyl derivative and methyl ester. No other solid material was isolated from the non-saponifiable portion with the exception of a hydrocarbon, probably n-triacontane or n-hentriacontane, which has not previously been reported as present in cloves. Finally, a liquid product, which appeared to be caryophyllene, a previously isolated sesquiterpene, was also obtained.

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V. SOME DERIVATIVES OF TRITERPENOID COMPOUNDS

Introduction

The similarity between the sterols and the triterpene derivatives was pointed out on p. 2 . It was therefore interesting to determine whether certain sterol derivatives which had proved of value in isolations, purifications, and transformations could be applied to the triterpene alcohols and acid-alcohols. If so, such derivatives might prove of value in similar isolations, purifications, and transformations in this field. The work described in the following deals with the preparation and attempted preparation of some of these derivatives.

PART A

In 1932, Josephson[1] found that hydro-aromatic secondary alcohols could be distinguished from tertiary alcohols by their reaction with triphenylchloromethane in pyridine. The secondary alcohols formed trityl ethers readily while the tertiary alcohols did not react. He prepared the trityl ether of cholesterol and reported its properties. Girard and Sandulesco[2,3] were able to transform hydroxyl groups to carbonyl groups through the trityl ether. For example, trans-dehydroandrosterone could be converted to the trityl ether, reduced to the trityl ether of androstenediol, esterified to the 3-trityl ether-17-acetate, and the trityl group removed to yield testosterone acetate. Several patents[4,5] have been obtained for the preparation of compounds such as testosterone by the thermal decomposition of triaryl methyl ethers of members of the cyclopentano-polyhydrophenanthrene series.

It was reasoned that β-amyrin might possibly be converted to the keto compound through its trityl ether and it was with this idea in mind that the following preparation of β-amyrin triphenylmethyl ether was attempted.

Preparation of Triphenylchloromethane

A quantity of student-prepared triphenylmethyl alcohol was purified by crystallization from 85 per cent ethyl alcohol, the pure product melting at 160.4-161°C.* A sample of 20 grams of this compound was placed in a dry, round-bottomed flask and enough acetyl chloride

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*All melting points are corrected.

to make a paste (about 15 ml.) was added. After the initial vigorous reaction had ceased, the mixture was heated under reflux on a steam bath and enough additional acetyl chloride to dissolve the solid material (about 10-15 ml.) was added. Upon cooling in an ice bath, almost all of the mixture solidified. The small amount of supernatant liquid was poured off and the solid then dissolved in 20 ml. of a 3:1 mixture of dry petroleum ether and acetyl chloride. The solution was poured into an Erlenmeyer flask and chilled in an ice bath, small, white, cubical crystals of triphenylchloromethane crystallising from the solvent. The crystals were filtered off, and after drying in a vacuum desiccator for 30 minutes, were found to melt at 112-112.5°C. Concentration of the mother liquor gave 3.5 grams of crystals melting at 111-112°C. A total yield of 18.5 grams was obtained (86.4 per cent of the calculated theoretical).

Preparation of β-Amyrin

A sample of 1.5 grams of β-amyrin acetate (m.p. 238.5-240°C.) from bittersweet[6] was saponified by refluxing with alcoholic potassium hydroxide on a steam bath for 3.5 hours. About 10 ml. of benzene was added to facilitate solution of the acetate. After the reflux period, the mixture was poured into ice water in a separatory funnel and the precipitate which formed was extracted with three portions of ether. The ether solution was washed with water, dried over anhydrous sodium sulfate, filtered, and ether removed by distillation. The residue was dissolved in hot acetone and allowed to stand overnight, long, needle crystals forming in the solution. These weighed 0.7 gram and melted at 198-199.5°C. Concentration of the mother liquor yielded 0.6 gram of crystals melting at 187.5-190°C. Further quantities of β-amyrin were obtained in this same manner.

Attempted Tritylation of β-Amyrin (I)

For this experiment a 10 ml. Erlenmeyer flask was baked dry at 110°C. for 2 hours, fitted with a reflux condenser containing a calcium chloride tube, and placed on a steam bath. Dry pyridine was prepared by allowing to stand over potassium hydroxide for 24 hours, refluxing over Drierite for one hour, and then distilling, using an all glass apparatus, into a flask containing potassium hydroxide. A sample of 0.5 gram of β-amyrin dried at 110°C. in a vacuum oven for 3 hours was placed in the reaction flask and 0.5 gram of triphenylchloromethane and 2 ml. of dry pyridine were added. The mixture was then heated and the solids readily dissolved. Heating was then continued for a total of 7 hours, crystals forming in the solution after 4 hours. The yellow mixture was then diluted with ice water and the precipitate which formed extracted with 3 portions of ether. The ether solution was washed once with water, twice with 2 per cent hydrochloric acid, and again with water. These washings were combined and extracted once with ether, the ether solution being added to the main ether extract. This was then washed with half-saturated sodium bicarbonate solution and then with water. The solutions were kept cold by addition of ice at intervals. These washings were also extracted with ether and the ether solution added to the main ether extract. This extract was dried over anhydrous sodium sulfate, filtered, and ether removed by distillation. The residue was a yellowish-white solid weighing 0.780 gram (99 per cent of the calculated theoretical).

The crude product was crystallized from acetone and 228 mg. of long, needle crystals melting at 197-198°C. was obtained. A mixed melting point with β-amyrin showed no depression. Upon standing the mother liquor deposited 84 mg. more of β-amyrin melting at 196-197°C. Concentration of the mother liquor gave 175 mg. of substance melting at 150-155°C. A mixed melting point with triphenyl carbinol showed no depression. Evaporation of the mother liquor to dryness gave 200 mg. of brownish residue which was not further investigated.

Attempted Tritylation of β-Amyrin (II)

The tritylation was repeated on a sample of 505 mg. of β-amyrin dried in a vacuum oven at 115°C. The apparatus used was a side-armed, 6 inch test tube which was dried by passing a stream of dry hot air through it for 15 minutes. The sample was placed in the tube, 600 mg. of triphenylchloromethane and 2 ml. of dry pyridine added, the tube tightly stoppered, and the mixture warmed on a steam bath for 24 hours. The solid material all dissolved within 10 minutes, but after 3 hours some material came out of solution. At the end of the heating period, the mixture was light-brown and contained some crystals. This crude material was worked up as previously described. A yield of 1.003 grams of product was obtained (126 per cent of the calculated theoretical). This was crystallized from acetone and 158 mg. of crystals melting at 185-192°C. was obtained. Concentration of the mother liquor gave 143 mg. melting at 182-187°C. Concentration of this mother liquor gave 61 mg. melting at 192-198°C. Further concentration of the mother liquor gave two more crops of crystals weighing 96 mg. and 100 mg. and melting at 167-175°C. and 157-160°C. respectively. The mother liquor was evaporated to dryness and 378 mg. of brown, oily material was obtained. Only 68 mg. remained unaccounted for. From the melting points, the substances obtained appeared to be β-amyrin, triphenyl carbinol, and mixtures of the two.

Attempted Tritylation of β-Amyrin (III)

A third attempt to prepare a trityl ether of β-amyrin was made using a dried, stoppered, 50 ml. round-bottomed flask as the reaction vessel. A sample of 500 mg. of dry β-amyrin was placed in the flask with 600 mg. of triphenylchloromethane and 1 ml. of dry pyridine and the mixture heated on a steam bath for 73 hours. At the end of this time, the mixture, which had solidified, was refluxed over a low flame for a few minutes and it turned black. The reaction mixture was allowed to stand for 48 hours and was then worked up as before. A crude yield of 979 mg. of brownish-black, oily material was obtained (124 per cent of the calculated theoretical). This was crystallized from acetone after being partially decolorized by boiling with Norite for a few minutes. A crop of 141 mg. of white, needle crystals melting at 192-194°C. was obtained. Concentration of the mother liquor gave 300 mg. of solid melting at 177-184°C. Evaporation of the mother liquor to dryness yielded a dark-brown residue which was not further investigated. Again the melting points indicated recovery of β-amyrin in fairly pure form or mixed with triphenyl carbinol.

From the results of these three attempts to prepare a trityl ether of β-amyrin, it may be concluded that such an ether does not form under the conditions employed. It may be added that the conditions used in these attempts were somewhat more drastic than those used by Josephson. His reactions were carried out at room temperature, while these were run on a steam bath and for longer periods of time. Steric hindrance may also be a factor in the failure of this reaction. Since the hydroxyl group of β-amyrin is attached to a carbon atom adjacent to one holding a gem-dimethyl group, it is possible that the position is sufficiently hindered to prevent the entrance of a radical of the magnitude of triphenylmethyl.

PART B

Phillips,7) in an investigation of the Walden inversion, found that when the p-toluenesulfonates of certain optically active alcohols were refluxed with potassium carbonate in various alcohols, he was able to obtain ethers of opposite configuration. Stoll8) applied this procedure to aromatic sulfonates of some of the sterols and was able to obtain isomeric ethers. For example, cholesteryl-p-toluenesulfonate when boiled with methyl alcohol gave the normal cholesteryl methyl ether, but when potassium acetate in methyl alcohol was used, he obtained an isomeric ether first thought to be of the epi configuration. This was later shown by Wallis and his school9-12) to be the methyl ether of i-cholesterol, a fact confirmed by Heilbron et al.13)

Although the basic structure of the triterpenes is such as to preclude the formation of an i-compound, it was of interest to see if the normal methyl ether of β-amyrin or its epimer could be prepared through the p-toluenesulfonate. The possibility of the formation of an epi-acetate of β-amyrin by boiling the p-toluenesulfonate with potassium acetate in ethyl alcohol was also investigated.

Freudenberg and Hess14) found that secondary and tertiary hydroxyl groups could be differentiated by the reactions of their p-toluenesulfonyl derivatives. When reacted with hydrazine, aniline, toluidines, or diethyl aniline, p-toluenesulfonic acid was split out of the secondary alcohols with the formation of an unsaturated compound.15) Since β-amyrin possesses a secondary hydroxyl group, it was decided to attempt the preparation of an amyrilene through the p-toluenesulfonate by reaction with pyridine.

Preparation of Cholesteryl-p-toluenesulfonate

In order to test the reagents and apparatus to be used in preparing the p-toluenesulfonate of β-amyrin, the reaction was first run on cholesterol, since its p-toluenesulfonate is known. A sample of 481 mg. of cholesterol, previously dried in a vacuum desiccator for 2 hours, was placed in a 6 inch, side-armed test tube which had been dried by passing a stream of hot air through it. To this was added 501 mg. of p-toluenesulfonyl chloride and 1.2 ml. of dry pyridine. The tube was tightly stoppered and the contents warmed on a steam bath for a few minutes until all the solid material dissolved. It was then set aside at room temperature for 21.5 hours.

The reaction mixture was worked up by the same procedure that was used in working up the supposed trityl ethers described on p. 105. The crude product weighed 629 mg., 95.3 per cent of the calculated theoretical. Crystallization from acetone gave 500 mg. of crystals melting at 132-133°C. A mixed melting point with a known sample of cholesteryl p-toluenesulfonate (m.p. 132-132.5°C.) showed no depression.

Preparation of β-Amyrin-p-toluenesulfonate (I)

A sample of 504 mg. of β-amyrin, previously dried at 110°C. in a vacuum oven for one hour, was placed in the side-armed test tube described above. To this was added 518 mg. of p-toluenesulfonyl chloride and 1 ml. of dry pyridine and the tightly stoppered tube warmed on a steam bath for 15 minutes, the solids dissolving after 5 minutes. At the end of the warming period, two layers of liquid were present, but the entire mixture solidified after standing overnight. The reaction product was worked up in the manner previously described and a yield of 0.549 gram of large, hard, translucent crystals was obtained (80 per cent of the calculated theoretical). Crystallisation from a mixture of acetone, benzene, and ethyl alcohol gave 230 mg. of crystals melting at 124-127°C. with decomposition to a deep-red liquid. Concentration of the mother liquor gave an additional 79 mg. having the same melting point. These two crops were combined and recrystallised from benzene. The hard, translucent crystals obtained melted at 117-120°C. to a red liquid. Recrystallisation from acetone-benzene did not alter this melting point. Another crystallisation from acetone gave crystals melting at 114-115.5°C. to a red liquid. Concentration of the mother liquor gave more crystals with the same melting point. A qualitative test for sulfur* was positive.

Preparation of β-Amyrin-p-toluenesulfonate (II)

In order to obtain a larger sample of the p-toluenesulfonate of β-amyrin, the preparation was repeated on 1 gram of previously dried β-amyrin. The sample was placed in the side-armed test tube, 1 gram of p-toluenesulfonyl chloride added, and both solids dissolved in 2 ml. of dry pyridine by warming the stoppered tube on a steam bath for 15 minutes. The mixture was set aside at room temperature for 48 hours and worked up as before. During the ether extraction, a considerable quantity of solid did not dissolve. This was filtered off and 700 mg. of white, crystalline material melting at 129.5-130.5°C.

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*Shriner and Fuson, "The Systematic Identification of Organic Compounds", 2nd Ed. (1940), p. 113.

to a red liquid was obtained. The crude product obtained from the ether solution weighed 569 mg. The total yield was 1.269 grams or 93 per cent of the calculated theoretical.

The 569 mg. fraction, when crystallized from acetone-benzene, yielded 377 mg. of large, hard crystals which melted at 123-124.5°C. (decomp.). Concentration of the mother liquer gave 35 mg. of needle crystals melting at 127-128°C.(decomp.). Further concentration of the mother liquor gave 105 mg. melting at 130-132°C.(decomp.). The 700 mg. fraction was combined with the above three fractions, recrystallized from acetone-benzene, and two crops of hard, translucent crystals were obtained. The first crop of 914 mg. melted at 117-118°C. (decomp.) and the second crop of 191 mg. melted at 126-128°C.(decomp.). A mixed melting point of the first crop with p-toluenesulfonic acid (m.p. 103-106°C.) showed a depression of 1-4°C. However, the acid itself did not melt with decomposition. A qualitative test for sulfur on the 117-118°C. fraction was positive. A sample of this fraction was dried at 70°C. in a vacuum oven for 3 hours and overnight in a vacuum desiccator and a macro carbon and hydrogen analysis made with the following results:

Calculated for $C_{37}H_{56}O_3S$: C, 76.50; H, 9.72. Found: C, 76.48; H, 9.57.

These results agree well with the calculated percentages and the compound was probably the desired ester. Although the mixed melting point of the product showed little depression when mixed with p-toluenesulfonic acid, the product could not have been identical with it, since the calculated carbon and hydrogen values for the acid are: C, 48.82; H, 4.68.

Preparation of β-Amyrin-p-toluenesulfonate (III)

A third sample of the p-toluenesulfonate was prepared in order to obtain another sample. A sample of 644 mg. of β-amyrin was reacted with 680 mg. of p-toluenesulfonyl chloride in 1.5 ml. of pyridine as previously described. A yield of 860 mg. of ether-insoluble material and 111 mg. of ether-soluble material was obtained (110 per cent of the calculated theoretical). Crystallization of the combined material from acetone-benzene gave hard, translucent crystals melting at 124-126°C. (decomp.).

Attempted Reaction of β-Amyrin-p-toluenesulfonate with Methyl Alcohol

A sample of 247 mg. of the p-toluenesulfonate from I was placed in a dried, round-bottomed flask fitted with condenser and calcium chloride tube, 30 ml. of anhydrous methyl alcohol added, and the mixture refluxed on a steam bath for 35.5 hours. Not all the sample had dissolved and part had turned reddish at the end of this period. This was filtered off and 155 mg. of compound melting at 112-114°C. (decomp.) was obtained. The methyl alcohol solution was concentrated, cooled, and 31 mg. of white crystals melting at 126-131°C. (decomp.) was obtained. This was shaken with 4 ml. of ether in the cold and filtered, a gray residue being left on the filter paper. The filtrate was evaporated to dryness and the residue crystallized from acetone. Hard, translucent crystals melting at 127-128°C. were obtained. Evaporation of the methyl alcohol mother liquor gave a residue of about 45 mg. of oily material which resisted attempts to crystallize it.

The results of this experiment indicated that the β-amyrin-p-toluenesulfonate had not reacted with methyl alcohol under the conditions employed.

Attempted Reaction of β-Amyrin-p-toluenesulfonate with Methyl Alcohol and Potassium Carbonate

Since the reaction with methyl alcohol at reflux temperature had failed, the reaction was tried again at a higher temperature and with potassium carbonate added. About 300 mg. of the p-toluenesulfonate melting at 117-118°C. from preparation II was placed in an 8 inch test tube with 300 mg. of anhydrous potassium carbonate and 10-15 ml. of anhydrous methyl alcohol. The tube was sealed off, placed in an iron pipe, and immersed in a boiling water bath (96°C.) for 2.5 hours. After standing overnight the tube contained some suspended solid matter and some purplish solid caked in the bottom. The tube was agitated to disperse the solid and heated for 8 hours. At the end of this time, the tube was opened and the contents poured into water. The precipitate was extracted with ether, the ether solution washed with water, two portions of 2 per cent hydrochloric acid, one portion of 5 per cent sodium bicarbonate solution, and water, dried over anhydrous sodium sulfate, filtered, and ether removed by distillation. The dried residue, weighing 220 mg., was crystallized from acetone-benzene and fine, needle crystals melting at 123.5-126°C. (decomp.) were obtained. A mixed melting point with the starting product showed no depression.

Attempted Reaction of β-Amyrin-p-toluenesulfonate with Pyridine

A sample of 191 mg. of β-amyrin-p-toluenesulfonate recovered from the methyl alcohol experiment was dried, dissolved in 8 ml. of dry pyridine in a stoppered test tube, and warmed on a steam bath for 48 hours. The brownish reaction product was then worked up in the same way as in the preparation of β-amyrin-p-toluenesulfonate. The crude product weighed 162 mg. It was crystallized from acetone-benzene and hard, translucent crystals melting at 126.5-128.5°C. (decomp.) were obtained. A qualitative test for nitrogen* was negative. A test for sulfur was positive. A mixed melting point with the starting material showed no depression. These results are somewhat surprising in view of the fact that a hydrocarbon, β-amyrilene II, had been prepared by Dieterle et al[16] by heating a mixture of β-amyrin, p-toluenesulfonyl chloride, and pyridine on a boiling water bath for 3 hours. In the reaction described above the p-toluenesulfonate was heated for 48 hours on a steam bath and the starting material was recovered unchanged although more drastic treatment was employed. Presumably, β-amyrin-p-toluenesulfonate was formed as an intermediate in Dieterle's reaction.

Attempted Preparation of Epi-β-Amyrin Acetate (I)

A sample of 0.5 gram of β-amyrin-p-toluenesulfonate was placed in a dried 25 ml. Erlenmeyer flask with 20 ml. of absolute ethyl alcohol, 0.35 gram of freshly fused potassium acetate added, and the

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*Method C of Shriner and Fuson, "The Systematic Identification of Organic Compounds", 2nd Ed. (1940), p. 114.

mixture refluxed on a steam bath for 82.5 hours. After 24 hours a quantity of solid material remained undissolved in the alcohol, but it eventually dissolved completely. At the end of the reflux period, the solution was poured into water, the white precipitate extracted with ether, the ether solution dried over anhydrous sodium sulfate, filtered, and the ether removed by distillation. The residue consisted of 337 mg. of a brownish oil which resisted all attempts at crystallization from ethyl alcohol-benzene, acetone, chloroform, petroleum ether, dioxane, benzene, acetone-benzene, and methyl alcohol.

Since all efforts at crystallization had failed, purification by chromatographic adsorption was attempted. The brownish, oily product was dissolved in 15 ml. of benzene and adsorbed on a column of 5 grams of aluminum oxide. After development of the chromatogram the column was extruded, cut into three segments, and each segment eluted with 30 ml. of 6 per cent ethyl alcohol in benzene. The top segment yielded 25 mg. of yellow, oily material, the center segment 7 mg. of material, and the bottom segment 3 mg. The filtrate yielded 133 mg. of colorless, oily residue which would not crystallize from alcohol-benzene. When the solvent was evaporated, a white solid melting at 78-80°C. was obtained. In attempting to recrystallize this material it was spilled and lost. From its melting point it appeared that some reaction had taken place, as the melting points of the starting product and epi-β-amyrin acetate are much higher than this.

Attempted Preparation of Epi- β -Amyrin Acetate (II)

In view of the inconclusive results obtained in the first attempt to prepare epi- β-amyrin acetate, the reaction was repeated. A sample of 0.5 gram of β-amyrin-p-toluenesulfonate was placed in a 50 ml. round-bottomed flask and 1 gram of freshly fused potassium acetate added. The solids were refluxed with 20 ml. of absolute ethyl alcohol for 46 hours. All the solid dissolved and the mixture had a slight odor of ethyl acetate at this time.

The product was worked up as before and 348 mg. of oily, yellow material was obtained. Recrystallization from acetone gave white, needle crystals melting at 141-144°C. Concentration of the mother liquor gave a second crop melting at 116-121°C. Further concentration of the mother liquor gave crystals melting at 126-128°C.(decomp.), probably unreacted starting material. No more crystals could be obtained from the mother liquor and evaporation to dryness gave a brown oil. Recrystallization of the 141-144°C. fraction from acetone gave crystals melting at 149-153°C. Concentration of the mother liquor gave crystals melting at 133-135°C. Recrystallization of the 116-121°C. fraction from acetone gave crystals melting at 127-128°C. Concentration of the mother liquor gave a small quantity of crystals melting at 106-110°C.

The water which had been used to dilute the crude reaction product during the working up procedure was tested for the presence of sulfur by heating a few mls. with 3 per cent hydrogen peroxide for 30 minutes. Addition of barium chloride T.S. produced a white turbidity indicating the presence of sulfate. This is evidence that p-toluenesulfonic acid had been split off the starting material. Epi-β-amyrin acetate is reported by Ruzicka and Wirz17) to melt at 128°C. It is possible that the 127-128°C. fraction obtained in this experiment is that compound, but not enough material was available for further identification. The small quantity of the 149-158°C. fraction also made purification and identification impracticable.

Attempted Preparation of Epi-β-Amyrin Acetate (III)

Because of the insignificant amount of reaction products obtained in the preceding run, the reaction was repeated on a larger sample, 3.00 grams, of β-amyrin-p-toluenesulfonate. It was placed in a round-bottomed flask with 4 grams of fused potassium acetate and 90 ml. of absolute ethyl alcohol and refluxed on a steam bath for 40.5 hours. All material had dissolved at this time. The mixture was then concentrated to 30 ml., transferred to an 8 inch test tube, and the tube sealed and heated in an iron pipe in a boiling water bath (100°C.) for 20 hours. At the end of this period the tube was cooled and a quantity of white, crystalline material formed. This was filtered off, the yield being 1.526 grams.

The solid material was washed with water to remove potassium acetate, the bulk decreasing materially, dried, and crystallized from acetone. A crop of 160 mg. of crystals melting at 143-153°C. was obtained. Concentration of the mother liquor gave 272 mg. melting at 108-115°C. The first fraction was recrystallized from acetone-benzene after which it melted at 160-164°C. Recrystallization from ethanol-benzene raised the melting point to 165-167°C. The optical rotation of this material was found to be:

$[\alpha]_D^{27.5}$ + 120.7° (29.5 mg. in 2 ml. of chloroform α_D + 1.78°, l 1 dm.).

This substance, dissolved in carbon tetrachloride, decolorized bromine in the same solvent with the evolution of hydrogen bromide. Another crystallization from ethanol-benzene raised the melting point of the fine, white needles to 167-168°C. A sample of 82 mg. was dried for 7 hours at 88°C. in a vacuum oven and overnight in a desiccator and a macro carbon and hydrogen analysis made with the following results:

Calculated for $C_{30}H_{48}$: C, 88.17; H, 11.84. Found: C, 87.97; H, 11.56.

This analysis indicates that p-toluenesulfonic acid must have been split out of the starting material with the formation of an unsaturated linkage. Such a reaction should produce one of the amyrilenes and the compound obtained corresponds most closely to β-amyrilene I. A comparison of the properties of the two is shown in the following table:

	Unknown Compound	β-Amyrilene I
Melting point	167-168°	170-175° (18)
$[\alpha]_D$	+120.7° ($CHCl_3$)	+112.9° (C_6H_6) (19)
Analysis	C, 87.97 H, 11.56	C, 88.17 H, 11.84

The filtrate of the original reaction mixture was poured into water, the precipitate extracted with ether, the ether solution dried, filtered, and ether removed by distillation. A crude residue of 720 mg. of a brown oil was obtained. Crystallization from acetone gave 135 mg.

of crystals melting at 103-111°C. This was combined with the 108-115°C. fraction from above and crystallized from acetone, after which the crystals melted at 110-113°C. Recrystallization from acetone-benzene raised the melting point to 127.5-130.5°C. A crystallization from ethanol-benzene gave two fractions of crystals. One consisted of shiny platelets melting at 133.5-134.5°C. The other was a white, crystalline powder melting at 123.5-125°C. A mixture of the two melted at 124-126°C. The optical rotation of the higher melting fraction was found to be:

$[\alpha]_D^{27} + 138.8°$ (24.2 mg. in 2 ml. of chloroform $\alpha_D + 1.68°$, l 1 dm.).

The optical rotation of the lower melting fraction was found to be:

$[\alpha]_D^{27} + 128.4°$ (21.9 mg. in 2 ml. of chloroform $\alpha_D + 1.41°$, l 1 dm.).

A sample of this lower melting fraction was dried at 88°C. in a vacuum oven for 4 hours and a macro carbon and hydrogen analysis made with the following results:

Calculated for $C_{30}H_{48}$: C, 88.17; H, 11.84. Found: C, 87.81; H, 11.57.

These results indicate that this compound also is a hydrocarbon corresponding to $C_{30}H_{48}$. That this compound is not the same as the 167-168°C. fraction is indicated by the fact that both were isolated from a crude product having an indefinite melting point. Also, the higher melting compound consisted of long, needle crystals while the lower melting one was a powdery material. It is possible, however, that the 123.5-125°C. fraction and the 133.5-134.5°C. fraction are the same (polymorphic forms), since a mixed melting point showed no depression and since the optical rotations were fairly close, although they differed in crystalline form. No analysis of the latter material could be made because of insufficient material.

It is possible that these compounds are all amyrilenes, hydrocarbons resulting from the loss of water from β-amyrin. The relationship of the 167-168°C. fraction to β-amyrilene I has already been pointed out. The two lower melting fractions correspond roughly to β-amyrilene II, which has been prepared by Dieterle et al[16] by heating β-amyrin with p-toluenesulfonyl chloride in pyridine on a boiling water bath. It has also been prepared by Winterstein and Stein[18] by pyrolysis of β-amyrin benzoate. A comparison of their properties is made below:

	Unknown Compounds	β-Amyrilene II
Melting point	123.5-125° 133.5-134.5°	147-148° (16) (137-138° impure)
$[\alpha]_D$	+128.4° ($CHCl_3$) + 128.8°	+139.2° ($CHCl_3$) (18)
Analysis	C, 87.81 H, 11.57	C, 87.81 (16) H, 11.63

At least three amyrilenes[16,18] have been prepared from β-amyrin by various means, but the structural differences between them have not been explained. From these amyrilenes, six amyrenes[18] have been prepared by reduction of one double bond, that in ring A. These likewise have not been structurally differentiated.

The fact that this reaction yielded a hydrocarbon instead of the desired epi-acetate is not particularly surprising in view of the work of Stoll.[20] He found that the p-toluenesulfonates of the saturated sterols having an epi-configuration produced unsaturated compounds and p-toluenesulfonic acid when heated with methyl alcohol for two hours. For example, when the p-toluenesulfonates of epi-cholestanol and epi-ergostanol were treated thusly, $\Delta^{2,3}$-cholestene and a new ergostene respectively were formed.

PART C

A derivative of the sterols which has proved of value as an intermediate in various transformations is the pyridonium steryl sulfate. Sobel and Spoerri[21)] have developed methods of preparing these sulfates and have suggested the possibility of using them in the isolation, thermal decomposition, and oxidation of sterols. Specifically, they prepared sulfates of cholesterol, dibromocholesterol, ergosterol, and lanosterol (a triterpene) and recorded their properties. They found that these pyridonium steryl sulfates could be easily prepared by condensing pyridine sulfur trioxide in the presence of an excess of the sterol. Quantitative conversion of the sterols to the pyridonium sulfates could be obtained by using an equal weight of pyridine sulfur trioxide in the presence of pyridine and acetic anhydride. One of the most interesting of the properties discovered in their investigation was the ease with which the pyridonium radical in the pyridonium sulfates could be replaced by the cations of various salts, and this was the basis for the preparation of a series of steryl sulfates.

Since the sterols and one triterpene, lanosterol, had successfully formed pyridonium sulfates, there seemed to be no reason why other triterpenes would not react similarly. If so, the derivatives might prove of value in transformations similar to those of the sterols. The attempted preparation of the pyridonium sulfates of β-amyrin, methyl ursolate, and methyl oleanolate is described in the following.

Preparation of Pyridonium β-Amyrin Sulfate (I)

A sample of 500 mg. of β-amyrin was dried at 105°C. for one hour and placed in a three-necked flask equipped with a stirrer, condenser, and calcium chloride tube. To this was added 6.4 ml. of dry benzene, 1 ml. of dry pyridine, 1 ml. of acetic anhydride, and 500 mg. of pyridine sulfur trioxide. The mixture was then heated to 55-60°C. in a water bath for 25 minutes. At the end of this time the material in the flask was a white, gelatinous mass. About 25 ml. of dry petroleum ether (b.p. 66°C.) was added to the cooled mixture and the gelatinous mass changed to a flaky precipitate. The material was chilled in the ice box for 3 hours, filtered with suction, and dried in a vacuum desiccator for half an hour. A yield of 565 mg. of white, powdery solid melting at 233.5-236.5°C. was obtained (82 per cent of the calculated theoretical).

The product was dissolved in 10 ml. of cold, dry chloroform to removė any unreacted pyridine sulfur trioxide which is insoluble in chloroform. However, all the material dissolved and chilling in an ice bath failed to precipitate anything, so four volumes of petroleum ether was added and a heavy, white, crystalline precipitate formed. This was filtered by suction, dried, and found to melt at 231-233.5°C. (493 mg.). A qualitative test for sulfur was positive, as was a test for nitrogen. A sample of this material was dried overnight at 105°C. in a vacuum oven and micro carbon and hydrogen analyses made with the following results:

Calculated for $C_{30}H_{49}SO_4C_5H_5NH$: C, 71.74; H, 9.46.

Found: C, 68.53, 68.21; H, 9.23, 9.13.

The low values obtained for carbon are explainable only by the assumption of the presence of impurities.

Preparation of Pyridonium β-Amyrin Sulfate (II)

In order to obtain more of the pyridonium sulfate for further reactions the preparation was repeated on a sample of 1.144 grams of β-amyrin,using the apparatus described above. To this was added 23 ml. of benzene, 1.8 ml. each of pyridine and acetic anhydride, and 1.14 grams of pyridine sulfur trioxide and the mixture heated to 55-60°C. on a water bath for 30 minutes. The product was worked up as before and 1.275 grams of white, crystalline powder melting at 230-231.5°C. was obtained (81 per cent of the calculated theoretical). This material was dissolved in cold chloroform, cooled in the ice box for an hour, filtered, and the filtrate diluted with several volumes of petroleum ether. The precipitated substance was filtered off and dried. A yield of 1.123 grams of white powder melting at 230.5-232.5°C. was obtained. Qualitative tests for both nitrogen and sulfur were positive.

Preparation of Sodium β-Amyrin Sulfate

A sample of 600 mg. of pyridonium β-amyrin sulfate was suspended in 12 ml. of water, 12 ml. of 10 per cent sodium chloride solution added, and the mixture vigorously shaken in a 50 ml. Erlenmeyer flask during 5 hours. At the end of this time the flask contained a foamy, white precipitate which rose to the surface. The mixture was allowed to stand overnight, filtered with suction, washed with water, and dried. The solid material was then washed with chloroform to remove any unreacted pyridonium salt and dried in a vacuum desiccator. The white, powdery solid obtained melted at 151-151.5°C. and weighed 480 mg. (88.7 per cent of the calculated theoretical). The compound gave a strong sodium flame and left a white residue when ignited. Evaporation of the chloroform washing gave 27 mg. of solid melting at 231.5-232.5°C. This evidently was unreacted starting material.

Preparation of the Pyridonium Sulfate of Some Low-Melting β-Amyrin

From the preparation of β-amyrin by saponification of its acetate similar to that described on p. 104, was obtained a quantity of white, needle crystals melting at 184-185°C. Crystallization from acetone gave a product melting sharply at 185-186°C. Concentration of the mother liquor gave more crystals melting at 184-185°C. This melting point is some 15°C. lower than that of pure β-amyrin. Since the product had been obtained from known β-amyrin acetate, it was decided to attempt a preparation of the pyridonium sulfate to see if β-amyrin with the proper melting point could be obtained through that derivative.

A sample of 500 mg. of this low-melting product was placed in the reaction flask used before and 1 ml. each of pyridine and acetic anhydride and 800 mg. of pyridine sulfur trioxide added. The procedure previously described was followed and a product melting at 228-229.5°C. was obtained. This was dissolved in cold chloroform, filtered, and reprecipitated with petroleum ether. The white, crystalline powder obtained melted at 231.5-233.5°C. A mixed melting point with the pyridonium β-amyrin sulfate prepared in II showed no depression.

In order to obtain a larger sample of this product, 2.462 grams of the low-melting product from β-amyrin acetate was reacted with 2.5 grams of pyridine sulfur trioxide in 3 ml. each of pyridine and acetic anhydride and 50 ml. of benzene. The crude product was worked up as before and 3.524 grams obtained (104.1 per cent of the theoretical calculated as pyridonium β-amyrin sulfate). It melted at 229-231°C. After dissolving in cold chloroform, filtering, and reprecipitating with petroleum ether, the compound melted at 232.5-236°C. and weighed 2.861 grams (84.5 per cent of the calculated theoretical). This product also did not depress the melting point of the pyridonium sulfate of pure β-amyrin. From this it seems that the low-melting product must have been only impure β-amyrin.

The benzene-petroleum ether mother liquor from this reaction upon evaporation to dryness yielded large, needle crystals melting at 234.5-238.5°C. Recrystallization from acetone-benzene gave 117 mg. of crystals melting at 239-239.5°C. A mixed melting point with β-amyrin acetate (m.p. 238.5-240°C.)showed no depression. This showed that some of the β-amyrin had been acetylated by the acetic anhydride in the reaction mixture.

Preparation of Sodium β-Amyrin Sulfate

In order to identify the low-melting product of β-amyrin acetate, the sodium sulfate derivative was prepared. A sample of 1.5 grams of the pyridonium sulfate from the above experiment was suspended in 30 ml. of water and 30 ml. of 10 per cent sodium chloride solution and the mixture vigorously shaken for several hours. The white precipitate which rose to the surface was filtered off with suction, washed with water, dried, washed with chloroform, and dried again. A yield of 1.303 grams of white powder melting at 180-180.5°C. was obtained (96.3 per cent of the theoretical calculated as sodium β-amyrin sulfate). This material was apparently identical with that prepared from β-amyrin of proper melting point.

Hydrolysis of Pyridonium β-Amyrin (Low-Melting) Sulfate

Since it appeared to be identical with known pyridonium β-amyrin sulfate, a portion of the pyridonium sulfate prepared from the low-melting β-amyrin was hydrolysed to determine whether purification had taken place. A sample of 500 mg. of the compound described on p. 127 was dissolved in 50 ml. of 70 per cent methyl alcohol made 2N with hydrochloric acid and refluxed on a steam bath for 20 minutes. At the end of this time a flocculent, white precipitate had formed. This was filtered off, dried, and crystallized from acetone, after which it melted at 184.5-186°C. Concentration of the mother liquor gave more compound melting at 184-186.5°C. Further concentration of the mother liquor gave crystals melting at 185.5-190°C. From this it appeared that little purification had taken place. Neither the identity of the contaminating material, if any, nor the reason for the low melting point of the saponification product of β-amyrin acetate could be determined. The melting points of the derivatives were identical with those of pure β-amyrin, so it seemed that the low-melting compound must be β-amyrin.

Attempted Thermal Decomposition of Sodium β-Amyrin Sulfate (I)

Sobel and Rosen[22] found that cholesterol could be dehydrated with the formation of 3,5-cholestadiene in quantitative yields by a thermal decomposition of potassium cholesteryl sulfate in octanol-2 containing sodium octan-2-oxide. The possibility of similarly dehydrating β-amyrin with the formation of one or more of the amyrilenes was therefore investigated.

To 60 mg. of sodium dissolved with warming in 12 ml. of octanol-2 (b.p. 179°C.) in a 25 ml. Erlenmeyer flask was added 300 mg. of sodium β-amyrin sulfate. The mixture was refluxed for one hour, allowed to stand overnight, and then refluxed for three more hours. The reaction mixture was cooled, transferred to a separatory funnel with ether, and the ether solution washed four times with water. The water was extracted once with ether and the extract added to the main solution which was dried over anhydrous sodium sulfate, filtered, and ether removed by distillation. The octanol-2 was removed by distillation at 22 mm. Hg., the alcohol distilling over between 120°C. and 135°C.

The crude product consisted of a viscous, dark-brown liquid which still possessed the odor of octanol. Attempts to crystallize the material from ethyl alcohol and from acetone failed. It was then dissolved in petroleum ether, shaken with 10 grams of activated aluminum oxide, filtered, and the pale yellow solution evaporated to dryness. The residue, a thin layer of almost colorless, clear material resisted attempts to crystallize it from acetone, benzene, petroleum ether, or ethyl acetate.

Attempted Thermal Decomposition of Sodium β-Amyrin Sulfate (II)

Since no crystalline material could be obtained from the first attempt, the thermal decomposition of sodium β-amyrin sulfate was repeated using a larger sample. To 200 mg. of sodium dissolved in 40 ml. of octanol-2 in a 100 ml. round-bottomed flask fitted with condenser and calcium chloride tube was added 1 gram of sodium β-amyrin sulfate. The mixture was refluxed for 1 hour and allowed to stand overnight. The solid had not all dissolved at this time and was jelly-like in consistency.

The product was worked up as before, but a quantity of substance did not dissolve in ether and so was filtered off. It was washed with ethyl alcohol, filtered, and 93 mg. of white material, which turned a violet color when dried, was left on the filter paper. The filtrate, upon standing, became partly filled with pure white platelets. These were filtered off and 125 mg. of crystals melting variously at 159-160°C., 152°C., 147°C., 146.5°C., 141.5°C., and 151.5°C., depending on the rate of heating, was obtained (Fraction 1). Concentration of the mother liquor gave 271 mg. of crystals melting at 153°C. (2). Another 46 mg. melting at 148-149°C. was obtained by further concentration of the mother liquor (3). Evaporation of the mother liquor to dryness gave an additional 47 mg. melting at 153-154°C. (4).

All four fractions were combined and recrystallized from methanol and 350 mg. of crystals melting at 151.7-152.2°C. was obtained (5). Concentration of the mother liquor gave 68 mg. melting at 151-152°C. (6).

Further concentration of the mother liquor gave a third crop of 42 mg. melting at 150-151.5°C. (7). Evaporation of the mother liquor to dryness gave 34 mg. of residue melting at 149.5-150°C. (8). A mixed melting point of fraction 5 with sodium β-amyrin sulfate showed no depression. A portion of fraction 5 was dried at 90°C. in a vacuum oven overnight and a macro carbon and hydrogen analysis made with the following results:

Calculated for $C_{30}H_{49}SO_4Na\cdot4H_2O$: C, 59.97; H, 9.56. Found: C, 59.97; H, 9.22.

The ether soluble portion of the reaction product amounted to 221 mg. of apparently crystalline material. This was taken up in ethyl alcohol and some water got into the solution precipitating some material which could not be filtered off. The precipitate was extracted with ether, the ether solution dried over anhydrous sodium sulfate, and the solvent removed by distillation. The residue could not be crystallised from ethyl alcohol or acetone. Crystallisation from methyl alcohol yielded some solid material melting at 163.4-166.3°C. After standing for several days, the mother liquor yielded a crop of crystals melting at 165-171°C. The two crops were combined and recrystallised from acetone, a very small amount of hard, brownish crystals melting at 180-190°C. being obtained. This material was not further investigated because of the small amount available. Nothing but a brownish, oily residue could be obtained from the acetone mother liquor.

Preparation of Methyl Ursolate

To 8.02 grams of ursolic acid dissolved in 250 ml. of methyl alcohol was added 41.3 ml. of 0.4258 N methyl alcoholic potassium hydroxide and 3.5 ml. of dimethyl sulfate. The mixture was refluxed on a steam bath for 11.5 hours, enough additional potassium hydroxide added to make the solution alkaline and 2 ml. more of dimethyl sulfate added, and the mixture refluxed 4.5 hours longer. The addition of base and dimethyl sulfate was then repeated and the mixture refluxed for 3 hours longer, making a total of 19 hours. The product was worked up as described on p. 11 and a crude yield of 6.3 grams of ester was obtained (76 per cent of the calculated theoretical). Recrystallization from ethyl alcohol gave white needle crystals of methyl ursolate melting at 168-169°C.

Preparation of Pyridonium Methyl Ursolyl Sulfate (I)

A sample of 1 gram of methyl ursolate was placed in the same apparatus used for preparing the pyridonium β-amyrin sulfate and dissolved in 25 ml. of benzene. To this was added 2 ml. each of pyridine and acetic anhydride and 1 gram of pyridine sulfur trioxide. The mixture was heated to 55-60°C. on a water bath for 25 minutes and the crude product worked up as previously described. A crop of white, needle crystals melting at 207.5-212°C. was obtained. More material was obtained from the reaction flask, not having been removed in the washing. The first crop was dissolved in cold chloroform, filtered, and reprecipitated with several volumes of petroleum ether. The precipitate was filtered off and 746 mg. of crystals melting at

145°C., resolidifying at about 160°C., and finally melting at 205-208°C. obtained. The second batch of solid obtained from the reaction flask was likewise treated with chloroform and petroleum ether and 449 mg. of crystals melting at 200.5-205.5°C. was obtained (Total yield: 90.2 per cent of the calculated theoretical). The two crops of product were combined and given two more treatments with chloroform and petroleum ether, the product melting at 144-154°C. Qualitative tests for sulfur and nitrogen were positive.

Preparation of Pyridonium Methyl Ursolyl Sulfate (II)

Because of the indefinite melting points of the products of the first reaction, the preparation of pyridonium methyl ursolyl sulfate was repeated. A 1 gram sample of methyl ursolate was reacted with one molar equivalent, 0.338 gram, of pyridine sulfur trioxide in 2 ml. each of pyridine and acetic anhydride. The product was worked up as before and after one treatment with chloroform and petroleum ether, 902 mg. of white solid melting at 145-155°C., resolidifying, and remelting at 205-207.5°C. was obtained. Evaporation of the chloroform-petroleum ether mixture gave 373 mg. of substance melting at 214-225°C. The two fractions were combined, dissolved in chloroform, filtered, and reprecipitated with petroleum ether, the product melting at 144-156°C. Evaporation of the mother liquor gave a residue melting at 218-234°C. This was recrystallized from methyl alcohol and white crystals melting at 236.5-241.5°C. were obtained. Recrystallization from ethyl alcohol changed the melting point to 237.5-240°C. A mixed melting point with a sample of acetyl methyl ursolate showed no depression. Evidently acetylation had taken place and it was this product which contaminated the pyridonium sulfate. After another treatment with chloroform and petroleum ether, the 144-156°C. fraction melted at 144-154°C.

Preparation of Sodium Methyl Ursolyl Sulfate

A sample of 820 mg. of pyridonium methyl ursolyl sulfate from I was suspended in 41 ml. of water, added to 41 ml. of 10 per cent sodium chloride solution, and vigorously shaken during one hour. The precipitate which rose to the surface was filtered off with suction and washed with water. Filtration was very slow and before the material was dry, chloroform was added forming an emulsion-like mixture. The solvent was evaporated and the residue consisted of fine, white platelets melting at 153-155°C. They gave a strong sodium flame and left a white residue on ignition. No further work was done with this compound.

Preparation of Pyridonium Methyl Oleanolyl Sulfate

A sample of 1 gram of methyl oleanolate (m.p. 198-199°C.), prepared by the method described on p. 32 , was dried at 100°C. in a vacuum oven for 2.5 hours and placed in the apparatus used for the preparation of pyridonium β-amyrin sulfate. To this was added 2 ml. of dry pyridine, 1.3 ml. of acetic anhydride, 1 gram of pyridine sulfur trioxide, and 25 ml. of benzene. The mixture was heated to 55-60°C. on a water bath for 25 minutes and the white, gelatinous product worked up as previously described. A yield of 1.709 grams of crude product melting at 231-236.5°C. was obtained. This was dissolved in cold chloroform, filtered, and reprecipitated with petroleum ether.

The white, powdery product melted at 233.5-237.5°C. and weighed 1.137 gram (85.1 per cent of the calculated theoretical). Another treatment with chloroform and petroleum ether did not change the melting point. A sample was dried at 105°C. in a vacuum oven for 3 hours and a macro carbon and hydrogen analysis made with the following results:

Calculated for $C_{31}H_{49}O_2SO_4C_5H_5NH$: C, 68.64; H, 8.80.
Found: C, 65.88; H, 8.29.

The low results are explainable only on the basis of the presence of impurities, perhaps some adsorbed chloroform.

Preparation of Sodium Methyl Oleanolyl Sulfate

A sample of 761 mg. of pyridonium methyl oleanolyl sulfate was suspended in 14.5 ml. of water, 14.5 ml. of a 10 per cent sodium chloride solution added, and the mixture vigorously shaken during two hours. When filtered off and dried, the product dissolved in chloroform and so the solution was evaporated to dryness. The residue was mixed with water and filtered. The white, powdery solid obtained melted at 153-159°C. After crystallization from ethyl alcohol, it melted at 144-152°C. The product gave a strong sodium flame and left a white residue when ignited. Its solubility in chloroform was puzzling since sodium was shown to be present. The compound was not further investigated.

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OLEANOLIC ACID

In 1825, Baget and Lodibert[1] independently isolated a white, crystalline material from the alcoholic extract of clove buds. Shortly afterward Bonastre[2] also isolated the material and "designated it provisionally under the name of caryophyllin or caryophilline". The compound was subsequently studied by Dumas,[3] Muspratt,[4] and Mylius.[5] Dumas proposed the formula $C_{10}H_{16}O$ for caryophyllin and regarded it as an isomeride of camphor, while Mylius suggested that $C_{20}H_{32}O_2$ agreed better with the high melting point. Muspratt and Martius[6] also gave some details as to the preparation and purification of the compound.

The first clue to the structure of caryophyllin was obtained by Hjelt,[7] who prepared a crystalline acetyl derivative. From a study of the chlorination products resulting from the action of phosphorus pentachloride, he deduced, as the most probable, the formula $C_{40}H_{64}O_4$. Meyer and Hönigschmid[8] found that caryophyllin melted at 295°C. and also confirmed the formula of Hjelt. They prepared an acetate and from its saponification value concluded that caryophyllin must be a tetratomic alcohol, and that the hydroxyl groups were primary and secondary. Herzog[9] also confirmed the formula of Hjelt, but stated that the acetyl derivative probably was a diacetate. He appears to have been the first to note that caryophyllin is capable of forming crystalline combinations with alkaline hydroxides, and he prepared the potassium and barium salts.

Dodge,[10] in 1918, made a thorough investigation of caryophyllin and concluded that it was an hydroxy-lactone, $C_{30}H_{48}O_3$. This conclusion was based on its neutrality in alcoholic solution, the tendency of the salts to hydrolyze, the precipitation by carbonic anhydride, the composition of the potassium salt, and the preparation of two acetates. His structure, however, could not explain the practically instantaneous neutralization of alkalies in alcoholic solution.

In 1908, Power and Tutin[11] isolated from olive leaves a crystalline compound melting at 303-304°C. They gave it the name oleanol, since their work indicated the presence of a phenolic hydroxyl group and an alcoholic hydroxyl group. However, van der Haar[12] showed that the compound was an hydroxy-acid and suggested that the name be changed to oleanolic acid. This name has now been accepted by all investigators. In 1927, van der Haar[13] proved that caryophyllin and the oleanol of Power were identical and isomeric with ursolic acid.*

As already pointed out, Baget and Lodibert isolated caryophyllin (oleanolic acid) from clove buds (*Caryophyllus aromaticus* L.), and Power and Tutin isolated it from olive leaves (*Olea europaea* L.). Ruccorini[14] reported its presence in the fruits of the olive also. In 1874, Scheibler[15] pointed out a substance in the plasma of sugar beet (*Beta vulgaris* L.) cells which he considered to be cholestin. Later, von Lippmann[16] isolated a substance which he called a phytosterol from the scum of sugar beet factory waste water. Kollrepp[17] found a substance in the sediment from carbonation and regarded it as iso-cholestin. The substance melted at 290°C. and was shown by van der Haar[13] to be oleanolic acid in 1927. Prelog[18,19] referred to this compound as the sugar beet sapogenin during his work on it.

In 1921, van Itallie[20] isolated a crystalline material from

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*For a review of ursolic acid see Parks, L. M., Ph.D. Thesis, University of Wisconsin (1938).

mistletoe (*Viscum album* L.) which he thought was ursolic acid. However, it is now clear that his product was impure, for Winterstein and Hämmerle[21] proved its identity with oleanolic acid. Van Itallie noted that more of the compound was obtainable from mistletoe from apple trees than that from poplar trees.

The Japanese workers, Kariyone and Matsushima,[22] in 1927, isolated a crystalline compound from the root of *Swertia japonica* Mak. and named it swertic acid. Kuwada and Matsukawa[23] later proved that this compound was oleanolic acid. In 1931, Wedekind and Schicke[24] reported the isolation of a sapogenin, $C_{30}H_{48}O_3$, from guaiac bark (*Guaiacum officinale* L.) and gave it the name guagenin. Later in the same year, Winterstein and Stein[25] showed that guagenin and oleanolic acid were identical.

Also in 1931, a sapogenin identified as oleanolic acid was isolated from the blossoms of marigold (*Calendula officinalis* L.) by Winterstein and Stein.[25] In 1932, the same investigators[26] reported that the leaves of *Aralia japonica* Thunbg. contain a saponin which is a mixture of the glucosides of oleanolic acid and hederagenin, a dihydroxy triterpene acid. The sapogenins obtained from various other Aralia species (araligenin, taraligenin, taragenin, panax sapogenin) are identical either with oleanolic acid or hederagenin according to these authors. Winterstein and Stein also established the identity of oleanolic acid from various sources (mistletoe, cloves, guaiac bark, sugar beet, and aralia leaves).

In 1935, oleanolic acid was isolated from *Momordica cochinchinensis* (Lour.) Sprengel by Kuwada and Fuma.[27] They called it momorgenin, but it was shown by them to be identical with oleanolic acid. In 1938, Markley, Sando, and Hendricks[28] reported that the ether extract of grape pomace (*Vitis vinifera* L.) consisted chiefly of oleanolic acid. Finally, in 1941, Rogofsky 29) isolated a crystalline compound from *Thymus vulgaris* L. which was shown by him to be oleanolic acid.

It is apparent from the foregoing that oleanolic acid is widely distributed and a natural product not typical of a single plant family. The following table lists the botanical sources of the acid, as determined thus far, arranged according to Engler's Syllabus.

Family	Genus, Species, and Author
Loranthaceae	Viscum album Linné
Chenopodiaceae	Beta vulgaris Linné
Leguminosae-Papilionatae	Glycine Soja Sieb.
	Soja hispida Moench
Zygophyllaceae	Guaiacum officinale Linné
Vitaceae	Vitis vinifera Linné
Myrtaceae	Caryophyllus aromaticus Linné
	Eugenia aromatica Baill.
	Eugenia caryophyllata Thunbg.
	Jambosa Caryophyllus Sprengel
Araliaceae	Aralia japonica Thunbg.
	Panax repens Maxim.
	Panax japonicus Mey.
Oleaceae	Olea europoea Linné
Gentianaceae	Swertia japonica Mak.
Labiatae	Thymus vulgaris Linné
Cucurbitaceae	Momordia cochinchinensis Spr.
Compositae	Calendula officinalis Linné

The general properties of pure oleanolic acid were noted by Dodge[10)] in 1916. It crystallized from boiling ethyl alcohol in white needles, very resinous to the touch. From dilute solutions, characteristic rosettes are slowly deposited. The crystals melt at 310°C. to a dark liquid without other evidence of decomposition. In a vacuum tube at 280-300°C. it sublimes in characteristic rosettes. It also sublimes with partial decomposition when heated in an open tube above 310°C. Dodge reported that it is practically insoluble in water, soluble in 35 parts of boiling 95 per cent ethyl alcohol and 106 parts at 20°C. It is soluble in 65 parts of ether, 118 parts of chloroform, 180 parts of acetone, and 235 parts of methyl alcohol. The alcoholic solutions are neutral to litmus. It is insoluble in aqueous alkalies, but on standing or digestion with alkaline hydroxide solutions, it is partly converted into the corresponding salt. It is readily soluble in 4 parts of 0.5 N methyl alcoholic potassium hydroxide.

Oleanolic acid gives a red-violet color changing to a blue and then to a green color with the Liebermann test. The constants of the acid and its common derivatives are listed in the table at the end of this discussion.

A major obstacle in the path of structural work on oleanolic acid is the difficulty of purifying the crude product. It is apparent from the constants reported that the older published methods for the isolation of oleanolic acid do not readily yield a pure product. This was confirmed by Dodge who found it practically impossible to obtain caryophyllin in satisfactory purity by simple recrystallization. The acid, as extracted with alcohol, ether, or petroleum ether, is always contaminated with amorphous matter according to Dodge. However, he found that the salts are easily purified, and from these a pure oleanolic acid can be prepared without difficulty.

As previously stated, elementary analyses by earlier workers indicated $C_{10}H_{16}O$, $C_{20}H_{32}O_2$, and $C_{40}H_{64}O_4$ for oleanolic acid. Much of the confusion was due to the inactivity of the functional groups. By titration Dodge found that the formula $C_{30}H_{48}O_3$ was the likely one. Winterstein and Stein in 1931, claimed that $C_{31}H_{50}O_3$ was the correct formula for the acid. However, a series of micro-titrations by Ruzicka 31) in 1932, definitely established the molecular formula as $C_{30}H_{48}O_3$.

The hydroxyl group was the first functional group established when Hjelt made his acetyl derivative. The fact that it was a secondary hydroxyl group was shown by the oxidation of methyl oleanolate to a ketone, methyl oleanonate. The position of the hydroxyl group was indicated by the isolation of 1,8-dimethyl-2-hydroxy picene from the dehydrogenation products of β-amyrin, a triterpene alcohol which differs from oleanolic acid only by the presence of a methyl group in place of the carboxyl group. A series of oxidation experiments by Kitasato et al 32-42) on hederagenin, a triterpene which differs from oleanolic acid only by the presence of a primary alcohol group in addition to the secondary alcohol group, definitely located the hydroxyl group at C_2 on the picene nucleus.

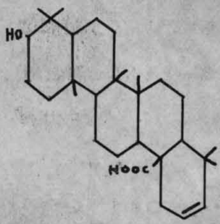
Early investigators believed that oleanolic acid was a dihydric alcohol since the carboxyl group appeared to be inert to the usual reagents. Dodge, proposed an hydroxy-lactone structure for the reasons pointed out before. However, van der Haar 12) in 1924, showed that oleanolic acid was a carboxylic acid and that the carboxyl group was attached to a tertiary carbon atom. This latter fact was indicated by the difficulty in saponification of esters of the group and by other reactions. The location of this carboxyl group is still not definitely settled.

The presence of at least one double bond in oleanolic acid was indicated by the fact that it gave a yellow color with tetranitromethane and the fact that crystalline bromo-lactones could be prepared. The double bond is, however, resistant to catalytic hydrogenation. The location of this unsaturated linkage is also controversial.

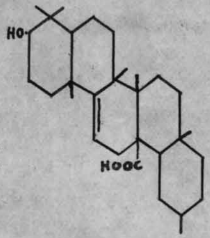
The chief contributors to the elucidation of the structure of oleanolic acid and its relatives have been Ruzicka and his school, Kitasato et al, Kon et al, Haworth, and Winterstein. For this work, two methods of approach were widely used, namely, dehydrogenation over selenium or palladium-charcoal and oxidative degradation. In 1929, Ruzicka[43-45] undertook a series of dehydrogenation experiments which led to the determination of the skeletal structure. From oleanolic acid and some dozen other triterpenoid compounds he obtained 1,2,3,4-tetramethyl benzene; 2,7-dimethyl naphthalene; 1,2,7-trimethyl naphthalene; 6-hydroxy-1,2,5-trimethyl naphthalene; 1,2,5,6-tetramethyl naphthalene, and 1,8-dimethyl picene. The isolation of these products led to the proposal of a perhydropicene structure which is confirmed by all the evidence.

Oxidative degradation has been the chief tool used for elucidation of the structural details of oleanolic acid. However, in spite of all the work thus far, no structure can be regarded as absolutely certain.

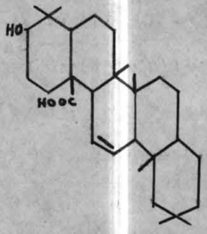
Many structures have been proposed, all formulated according to the isoprene rule, and several of these are shown below:



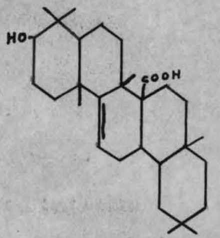
Spring (46)



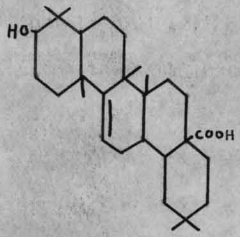
Ruzicka (47)



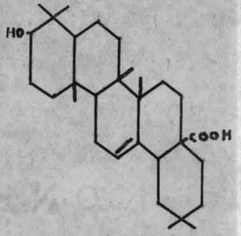
Kitasato (48)



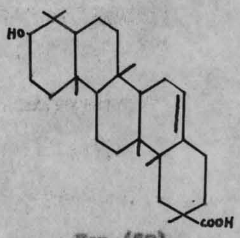
Spring (49)



Ruzicka (50)



Haworth (51)



Kon (52)

Kon's structure is now the generally accepted one since it seems to offer the best explanation of the properties and reactions of oleanolic acid. However, even it does not explain some of the dehydrogenation and pyrolysis products.

A large number of derivatives of oleanolic acid have been prepared. The reported derivatives of its functional groups are listed in the following table along with the references to the investigator reporting each.

Constants of Oleanolic Acid and its Derivatives as Recorded in the Literature

Oleanolic Acid	Acetyl	Methyl Ester	Acetyl-Methyl Ester	Benzoyl	Benzoyl-Methyl Ester	Author
M.P.	M.P.	M.P.	M.P.	M.P.	M.P.	
285°						4
295°	268-271°	187°	212-213°			8
303-304°	258°	194-95°	215°			11
310°	260-65°					10
287°						20
302°						19
306-308°	258-60°	196-98°				21
310°	268°	196°	220°			24
305°						32
310-10.5°	264.5-65.5°		221-22°			28
		194°	216-17°			53
				261°	269°	27
$[\alpha]_D$	$[\alpha]_D$	$[\alpha]_D$	$[\alpha]_D$			
+54.5° (EtOH)						10
+79.6° ($CHCl_3$)	+ 74.5°	+73.9°	+69.2° ($CHCl_3$)			24
+80.3° ($CHCl_3$)						32
	+74.9°	+76.1°				21

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ERYTHRODIOL

While examining the fruit of the coca bush (*Erythroxylon novagranatense* Morr.) for lycopene, Zimmermann[1)] isolated a substance which crystallised in fine, silvery, lustrous leaves. He concluded that this substance was the monostearate of a triterpene dialcohol. This conclusion was confirmed by the determination of the saponification number of the stearate as well as the diacetate, the gravimetric determination of the saponification product of the stearate, and the elementary analysis of the ester and the free alcohol. All the values obtained agreed within the limit of error with the empirical formula $C_{30}H_{50}O_2$ or $C_{30}H_{52}O_2$ for the free alcohol.

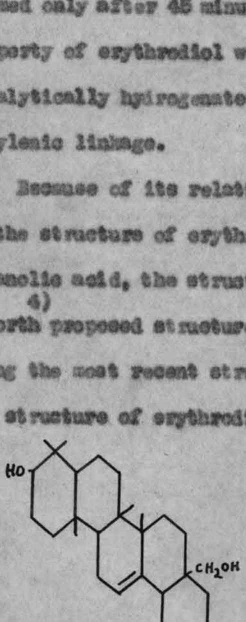
The free alcohol as well as the ester gave a weak yellow color with tetranitromethane indicating the presence of an ethylenic linkage. It gave a yellow color changing to orange and then to deep red with the Salkowski reaction. A Liebermann-Burchard reaction gave a rose-red color which quickly changed to violet.

The melting points of the diacetate and the free diol, and their specific rotations, as far as they are comparable, did not agree with any already known triterpene dialcohol. Therefore, the name "erythrodiol" was given to the free alcohol.

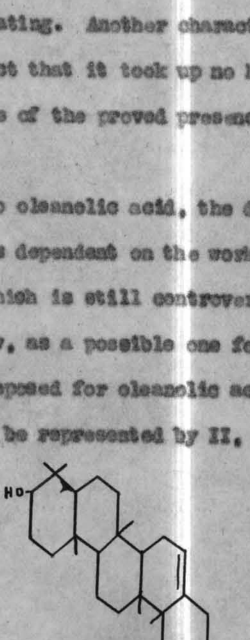
Zimmermann stated that erythrodiol consists of long, matted, silky, lustrous needles. It is soluble in most organic solvents, most easily in chloroform and most difficultly in petroleum ether. It melts at 231°C. and has an optical rotation of $[\alpha]_D + 75.38°$ ($CHCl_3$). These constants and those of the derivatives are reported in the table at the end of this discussion.

The carbon skeleton of erythrodiol[2)] was shown to be the same as that of oleanolic acid since oxidation of the primary alcohol group to a carboxyl group produced oleanolic acid. Confirmation of this observation was made by Ruzicka[3] who prepared erythrodiol from oleanolic acid by a Rosenmund reduction of the acid chloride followed by reduction of the semicarbazone of the aldehyde with sodium ethylate. β-Amyrin was also obtained in this transformation. One hydroxyl group was shown by Zimmermann to be primary by the ease of formation of the monoxime of the dicarbonyl compound obtained by oxidation of the diol with chromic acid. Only a short period of warming of the alcoholic solution of the oxidation product with hydroxylamine acetate was needed to form the monoxime, whereas the dioxime was formed only after 45 minutes of heating. Another characteristic property of erythrodiol was the fact that it took up no hydrogen when catalytically hydrogenated in spite of the proved presence of an ethylenic linkage.

Because of its relationship to oleanolic acid, the determination of the structure of erythrodiol was dependent on the work on oleanolic acid, the structure of which is still controversial. Haworth[4] proposed structure I, below, as a possible one for erythrodiol. Using the most recent structure proposed for oleanolic acid by Kon,[5] the structure of erythrodiol would be represented by II, below.



I



II

To date erythrodiol has not been isolated from any other sources. However, it is very likely that it will be found in other plants because of its close relationship to oleanolic acid which is widely distributed.

Physical Constants of Erythrodiol and Derivatives

	Zimmermann	**Ruzicka**
Erythrodiol, m.p.:	231°	232-233°
$[\alpha]_D$:	+75.38° ($CHCl_3$)	+74.6° ($CHCl_3$)
Diacetate, m.p.:	188°	184-185°
$[\alpha]_D$:	+59.41° ($CHCl_3$)	+60° ($CHCl_3$)
Monostearate, m.p.:	134°	
$[\alpha]_D$:	+49.90°	
Diformate, m.p. :	195°	
Mild oxidation product:		
Monoxime:	275°	
Dioxime:	263°	
Strong oxidation product:	231°	
Methyl ester:	181-182°	
Oxime:	281-282°	

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