A PHYTOCHEMICAL STUDY OF CITRULLUS VULGARIS SCHRADER

and

A STUDY OF THE REACTION OF THEOPHYLLINE WITH BARBITURATES

By

Walter Mayo Higgins

A thesis submitted to the Graduate School
of the University of Wisconsin in partial
fulfillment of the requirements for the
degree of DOCTOR OF PHILOSOPHY

UNIVERSITY OF WISCONSIN

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I. A PHYTOCHEMICAL STUDY OF CITRULLUS VULGARIS SCHRADER

INTRODUCTION

In 1926, I. S. Barksdale obtained from watermelon seed a substance which he believed to be a saponin, and which possessed depressor activity. No attempt was made to characterize the material and no constants were given.

The original purpose of this investigation was to repeat Barksdale's work and attempt to isolate and identify the depressor substance. However, his results could not be duplicated.

Since watermelon seeds had been used medicinally by physicians and housewives for several centuries and since no detailed chemical examination has been reported, an investigation of the literature on the seeds was made and a phytochemical examination of watermelon seeds was undertaken.

SURVEY OF THE LITERATURE

Scientific Names and Synonyms of Citrullus Vulgaris Schrader

The small number of plants known and described by early botanical writers seldom showed sufficient similarity to require more than one name to distinguish them. However, where an author knew of two or more plants sufficiently similar that a mononomial could not be used to differentiate between them, a second distinguishing name was required. Among the early writers who were familiar with what is now known as Citrullus vulgaris Schrader, and who knew of no other closely related plant, were Bock(1), who in 1552 listed the plant as Citrullus, and Matthiolus(2) who listed it as Anguria in 1558. Fuchs(3), however, knew some other plant so closely related that a second distinguishing name was required, for in 1542 he listed the plant as Cucumis Citrullus. Other pre-Linnean names and their authors listed by C. Bauhin in his Pinax Theatri Botanici are as follows:

Anguria citrullus, C. Bauhin(4), in 1671

Cucumer citrullus, Dodonaeus(5), in 1559, and others(4)

Cucumer melon, Caesalpino(6), in 1583

Bauhin also states that this is the same plant as the Pepo of Dioscorides, Galen, and Pliny. However, the Latin translation by Ruellio(7) lists Pepo as Cucumis sativus Linne, the cucumber; the German translation by Berendes(8) lists Pepo as Cucumis Melo Linne, the muskmelon; and the English translation by Goodyer(9) lists Pepo as Cucurbita pepo Linne, the pumpkin.

J. Bauhin(10), in 1651 listed the same as Citrullus folio *colocynthidis, semina negro.*

Linne(11), in his Species Plantarum in 1753 in which he gave to each and every plant a binomial consisting of a genus name and a trivial name, used the name *Cucurbita citrullus* for what he had previously called *Cucurbita folis multipartis* in his Hortus Upsalensis(12) and his Materia Medica(13), and *Cucurbita seminum margine basi dililuto* in his Hortus Cliffortianus(14). Royen(15) had also used the latter distinguishing name given by Linne.

Since different investigators may have used different scientific names for the same plant, a search was made in Index Kewensis(16) for all the known synonyms of the plant. Index Kewensis recognises *Citrullus vulgaris* Schrader as the standard name. The following synonyms and authors occur in the publication.

Citrullus vulgaris, Schrader(17), in 1836?
Citrullus amarus, Schrader(17), in 1836?
Citrullus aquosus, Schur(18), in 1866.
Citrullus caffer, Schrader(19), in 1834.
Citrullus cafforum, Schrader(20), in 1838.
Citrullus chodospermus, Nal__ and Dunal(21), in 1836.
Citrullus edulis, Spach(22), in 1838.
Citrullus fistulosis, Stocks(23), in 1851.
Citrullus pasteca, Sageret(24), in 1826.
Cucurbita anguria, Duchesne(25), in 1786.
Cucurbita caffra, Ecklon and Zeyher(17), in 1836?
Cucurbita citrullus Linne(11), in 1753.
Cucurbita pinnatifida, Schrank(26), in 1824.

Cucumis amarissimus Schrader(27), in 1827.

Cucumis citrullus Seringe(28), in 1834.

Cucumis colocynthis Thunberg(29), in 1772-1775.

Cucumis dissectus DeCaisne(20), in 1834.

Cucumis laciniosus Eeklon(31), in 1840.

Anguria citrullus Miller(32), in 1768.

Common Names

Citrullus vulgaris Schrader is commonly known as Watermelon in the English speaking countries. In Germany it is known as Wassermelone and Arbuse(33), and in France as Melon d'eau and Pasteque(34). Common names in other languages are: Zandia(35), Sandia(36), Sandia O Zandia(34), (Spanish); Melancia(37), (Portuguese); Tarbuj, Tarmuj, Tarbus, Kalingar, Pitcha-pullum(38), (India); Neele, Niri, Kogai, Equsi, Guna(39), Sele(40), (Africa); Si-kwa, Han-kwa(33), (China); and Bathee, Batheca, and Battieh(41), (Arabia).

Etymology

The etymology of the various terms used to designate the generic and species names given to *Citrullus vulgaris* Schrader follows:

Citrullus- Diminutive of the Latin *citrus* meaning a small citrul or orange(42).

vulgaris- from the Latin *vulgarus*-- common(43).

Amarus- from the Latin *amarus*-- bitter(44).

Aquosus- from the Latin *aquosus*-- abounding in water(44).

Caffer and Cafforum- from the African region of Caffaria(45).

Chodospermus- from the greek σπερμα — seed(46). The origin of chodo could not be traced.

Edulis- from the Latin edulis-- edible(44).

Fistulosis- from the Latin fistulosus-- hollow one(44).

Pasteca- from the Arabic Battich(41).

Cucurbita- from the Latin cucurbita-- a gourd(43).

Caffra- see above.

Anguria- from the Greek ἀγγούριον — a small vessel(46).

Citrullus- see above.

Pinnatifida- from the Latin pinnatus — feathered, and findere — to split(44).

Cucumis- from the Latin cucumis-- a cucumber(43).

Amarissimus- superlative of amarus.

Colocynthis- from the Latin colocynthis and the Greek κολοκυνθίς meaning the colocynth, which probably came from καλοκύντη — the round gourd or pumpkin(43).

Dissectus- from the Latin dissectus-- to cut(44).

Laciniosus- from the Latin lacinia-- a flap(44).

Description of the plant

Citrullus vulgaris Schrader is a tendril-bearing, prostrate or climbing succulent vine. Wood(47) gives the following description:

"An annual plant with a prostrate slender, hairy stem with branching tendrils. Leaves palmately five lobed, very glaucous beneath, lobes mostly sinuate-pinnatifid, all of the segments obtuse. Flowers yellow, solitary, on hairy peduncles, bracted at the base. Calyx tubular-campanulate, five-toothed, corolla deeply five-parted; filaments of the male flower, three; pistils of the female, three cleft; style short; stigmas three, thick, two lobed; fruit, oval or elliptical, smooth, discolored, indehiscent, subligneous, light or dark-green externally, beneath which is a white juicy substance, in the center a red or yellow edible pulp, sweet and juicy; it is from three to five celled, and contains many obovate, smooth, compressed seeds, thickened at the margin, and of a black or yellowish-white color."

Stille and Maisch(48) give the following description of the seeds:

"The seeds are about 1/2 inch long, blackish or brown and marbled, broadly ovate, flat, blunt on the edge, near the pointed end with two thin converging ridges."

Habitat

The exact origin of Citrullus vulgaris Schrader is unknown, and there is some variation of ideas among the early authors. Seringe(28) says it is indigenous to Africa and India; Linne(11) says "Apulia, Calabria, Sicilia". However, De Candolle(41) says that no one pretends to have found it wild in any country and that "L'indication faite bien legerement par Linne, de la Pouille, la Calabre, la Sicile, est copiee de Matthiole qui dit simplement que les meilleures pasteque sont de ces provinces".

Hehn and Sallybrass(49) believe that it originated in Africa and India and postulated its course of migration as follows:

"The watermelon migrated to Persia when communication with India opened either during the Arabian or Mongolian dominion, to Greece in company with the Turk, to Russia from the Tartar kingdom of Astrakhan and Kasan."

As lines of communication advanced and civilization migrated, watermelons spread; to Britain, the West Indies, the Americas, the

Islands of the Pacific and finally to New Zealand and Australia(50). Now watermelons are grown in nearly all tropical and temperate countries.

Medicinal History

The seeds of Citrullus vulgaris Schrader have been used in European medicine for centuries. That the seeds were actually used medicinally at an early date is evidenced by the fact that they were listed in an inventory of a pharmacy in Frankfort-on-Main(51), Germany about 1450 and were listed in the Dispensatory of Valerius Cordus(52) in 1546 as one of the Four Greater Cold Seeds. The "Four Greater Cold Seeds" were the seeds of watermelons, gourds, cucumbers, and muskmelons.

Salmon(53) in the Pharmacopoeia Londonensis of 1707 described the action of watermelon seeds as being "opening, cleansing, diuretic". He adds that they "allay sharpness and heat of the reins, bladder, and blood, quench thirst in agues, and temper the sharpness of choller". Quincy(54) in 1726 ascribed emolient and aperient action to them and noted that they were prescribed "when choler and acrimony abound".

Linne(55) in 1772 listed the seeds as useful in fever, stranguria, agrypnia, and nephritis.

Hook(56) in 1850, having failed to produce diuresis with accepted remedies, tried a tea prepared from the seeds because "it hadbbeen used by the common people for various affections of the urinary organs". Excellent results were obtained within three days.

King(57) in 1875, stated that the seeds were mucilaginous and diuretic and of service in strangury and other urinary diseases.

In 1904, Peckolt(58) reported that in a Pharmacy in Brazil, the kernels of the seed were mixed with sugar and sold as "orchata de melancia" (orgeat of watermelon) which when mixed with water was used as a substitute for almond emulsion.

Barksdale(59) in 1926, obtained from the seed a substance, believed to be a saponin, which produced a lowering of blood pressure as evidenced by both laboratory and clinical experiments. These experiments were confirmed by Althausen and Kerr(60) in 1929. However, in 1931, Gargill and Eady(61) found that only a small per cent of the patients showed a great reduction in blood pressure and that the effects lasted only a short while.

There is now on the market a preparation, Citrin(62), described as a saponin glucoside, which is an "aqueous extract of the active principle" of watermelon seed "and free from proteoses, peptones, and histamin". Its action is "hypotensive and thus decreases irritability, dizziness and dyspnea, symptoms associated with arterial hypertension".

The seed have been official in many Pharmacopoeias. The national Pharmacopoeias in which they have appeared, according to Brunts and Jaloux(63), are listed below.*

France:

Codex Medicamentarius sive Pharmacopoia Gallica, p. 283, (1818).

Codex, Pharmacopee Francaise, p. 438, (1837).

Spain:

Pharmacopoea Hispana, p._____, (1794)

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* All of these, except the two without page numbers have been personally verified.

Pharmacopoea Hispana, p. 4, (1797).

Pharmacopoea Hispana, p. 19, (1803).

Pharmacopoea Hispana, p. 28, (1817).

Pharmacopea Oficial Espanola, p. 91, (1884).

Mexico:

Nueva Farmacopea Mexicana, p. 192, (1874).

Nueva Farmacopea Mexicana, p. 96, (1884).

Nueva Farmacopea Mexicana, p. ____, (1896).

Nueva Farmacopea Mexicana, p. 278, (1904).

Denmark:

Pharmacopoea Danica, p. 63, (1772).

Pharmacopoea Danica, p. 32, (1805).

Portugal:

Pharmacopea Portugueza, p. 266, (1876).

The seed have never been official in the United States Pharmacopoeia or National Formulary, but they have received considerable attention in various American dispensatories from 1876 to 1937, such as the Eclectic Dispensatory, 1st ed., American Dispensatory, 10th and 16th eds., National Dispensatory, 3rd ed., National Standard Dispensatory, 2nd ed., and the United States Dispensatory, 3rd through 22nd eds.

Chronological Survey of the Constituents

Popow(63) in 1888, reported the presence of resin, albumin, and dextrose in the seeds. In 1890, Nardini(65) reported that the seeds were composed of 49.6 per cent water, 12.4 per cent ether soluble material, 10.4 per cent total nitrogenous material, 4.7 per cent protein, and 14.7 per cent crude fiber.

The oil, from seeds obtained from various geographical locations and extracted by different methods, has been investigated by Woinarowskaja and Naumowa(66), Wijs(67), Heering and Grimme(68), Piemaerts(40), The Imperial Institute(39), Power and Salway(69), Beljajew(70), Iwanow and Kuretschkina(71), and Nolte and Loesecke(72). The results of their investigations are found in Table I. Probably the most reliable analysis of the oil was made in 1939 by Nolte and Loesecke.

TABLE I -- OIL ANALYSES

	Woinarowskaja and Naumowa	Wijs	Power and Salway			Hearing and Grimme	Pieraerts	Imperial Institute							Beljajew		Iwanow and Kurotechkina	Nolte and Loesecke
Date of Report	1902	1903	1910			1911	1917	1925							1929		1930	1939
Source of Seeds	Russia		United States			S.W. Africa	Belgian Congo[a]	North Africa	North Africa	Western Africa	Seirra Leone	Nigeria b	c		Russia		Russia	Florida
Source of Oil	W.S.	W.S.	Kernels	Kernels	Shells	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	Kernels	W.S.	W.S.
Method of Extracting Oil	d	P.E.	Express	P.E.	P.E.	Ether	Boiling Water	P.E.	P.E.	P.E.	P.E.	P.E.	P.E.	Express	d	d	d	P.E.
Freezing Point	-20°C.					-5°C.												
Melting Point						-2°C.												
Density	0.925	0.916	0.9233 20°C.	0.9219 20°C.	0.9361 20°C.	0.9143 15°C.	0.9231 15°C.	0.9232 15°C.	0.9836 15°C.	0.9230 15°C.	0.9218 15°C.	0.9234 15°C.	0.9234 15°C.	0.9266 15°C.	0.9246 15°C.	0.9215 15°C.		0.9197
Refractive Index						1.4728 20°C.	1.4716	1.4670 40°C.	1.4670 40°C.	1.4670 40°C.	1.4645 40°C.	1.4650 40°C.	1.4650 40°C.					1.4669
Acid Value	0.4[f]	1.2	3.9	3.6		1.8	1.34	1.3	3.2	1.8	17.8[e]	1.3	3.8					0.42
Ester Value						196.4	5.3											7.5

TABLE I -- OIL ANALYSES (continued)

	Woinarowskaja and Naumowa	Wijs	Power and Salway			Heering and Grimme	Pieraerts	Imperial Institute							Beljajew		Iwanow and Kurotechkina	Nolte and Loesecke
Source of Oil	W.S.	W.S.	Kernels	Kernels	Shells	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	W.S.	Kernels	W.S.	W.S.
Method of Extracting Oil	d	P.E.	Express	P.E.	P.E.	Ether	Boiling Water	P.E.	P.E.	P.E.	P.E.	P.E.	P.E.	Express	d	d	d	P.E.
Total Fatty Acids (continued)																		
Refractive Index						1.4632 40°C.												
Iodine Value		122.7				112.3	102.0							106.0				
Neutralization Equivalent		197.1				196.2	180.9							215.7				
Average Molecular Weight		284.1				286.2	310											
Total Solid Fatty Acids %																		14.56
Freezing Point							57.5-57°C.											
Melting Point							58.5-59°C.											
Iodine Value							2.18											
[illegible]							229.0											

The report of Power and Salway(69) in 1910 is the earliest recorded detailed chemical investigation of watermelon seed. The seeds were separated into kernels and shells and each analysed separately. The results of the oil analyses are summarised in Table I. After saponification of the expressed oil, there was isolated, in addition to the fatty acids, a small amount of a phytosterol, $C_{20}H_{34}O$ (m.p. 163-164°C.). In the "press cake", besides residual oil were found soluble proteins, sugars, and a resinous material. From the resin, a small amount of a phytosterol, m.p. 158-159°C., apparently identical with the one obtained from the expressed oil, was isolated, and a compound, $C_{24}H_{40}O_4$, thought to be a new dihydric alcohol (m.p. 260°C. decomp.) to which the name Cucurbitol was given. The acetyl derivative melted at 150°C.

The shells, amounting to 48.7 per cent of the total weight of the seed, contained a fatty oil from which was isolated after saponification, in addition to the fatty acids listed in Table I, another phytosterol, $C_{20}H_{34}O$ (m.p. 141-142°C.). The residue of shells contained a trace of copper and a resinous material from which was isolated more of the phytosterol $C_{20}H_{34}O$ (m.p. 141-142°C.) and more cucurbitol. In a later investigation, the same authors(73) obtained evidence which indicated that cucurbitol was a phytosterol glycoside rather than a dihydric alcohol. No evidence could be obtained for the presence of alkaloids in any part of the seed. The investigation of the Imperial Institute also gave no indication of alkaloids or cyanogenetic glucosides.

Barksdale(59) in 1926, obtained a substance from the seeds by extraction with water which he reported to give saponin tests and which he called *Cucurbitin*. No constants or analyses were given for the substance.

Enzymes which have been detected are catalase, nuclease, and lipase by Jono(74) in 1931; citricodehydrase by Watanabe(75) in 1936; and urease by Damodaran and Sivaramakrishnan(76) in 1937.

Krishnan and Krishnaswamy(77) in 1939 made a detailed analysis of the nitrogenous constituents of watermelon seeds. The nitrogenous constituents are made up of glutelin, globulin, water soluble protein, proteoses and peptones. No canavanine or citrulline was found, and only traces of arginine.

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EXPERIMENTAL

Preliminary Examination of Watermelon Seed

The material used in this examination was from a lot of 25 pounds of watermelon seed obtained from S. B. Penick and Company and stated to be from the 1939 crop and of good quality.

Repetition of Barksdale's(1) Experiment

Two hundred grams of whole seeds were ground as finely as possible by means of a meat grinder and macerated overnight in 600 cc. of distilled water that had been made slightly alkaline with ammonium hydroxide and heated to 60°C. The resulting solution was strained through cheesecloth and then filtered until clear. This solution stood for several days and fermentation set in. The solution was then concentrated to a thin syrup on a steam bath, filtered, and an excess of lead subacetate added. The solution was filtered and the precipitate suspended in water and decomposed with hydrogen sulfide. This solution was filtered, concentrated to half its volume, filtered again, treated with hydrogen sulfide, and filtered until clear. To this clear solution was added in excess, a mixture of equal parts of diethyl ether and absolute alcohol. A white precipitate formed but there was not enough to collect. Since Barksdale had obtained considerable precipitate by this process, it was believed that possibly the fermentation had lowered the yield. The experiment was repeated using 50 grams of the drug and fermentation was not allowed to start. On the addition of the ether-alcohol mixture, a white flocculent precipitate formed. This precipitate was filtered off and on drying was found to be a white, very light powder which gave the following tests:

1. Soluble in water.
2. Insoluble in alcohol and ether.
3. Leaves no ash on ignition.
4. Did not melt below 330°C. but gradually darkened.
5. Gave no soapy appearance when shaken with water.
6. A little of the precipitate was sprinkled on about 5 cc. of concentrated sulfuric acid. The precipitate was soluble. There were no color reactions.
7. A small amount of the unknown was rubbed with concentrated sulfuric acid. There was no color display. Saponins give a play of colors. On rubbing saponins with Fröhde's reagent, a blue violet color forms which changes to green and gray. The test was negative with the precipitate obtained. This is Ruhle's saponin test(2).
8. Equal amounts of the precipitate were placed in each of 2 test tubes. About 3 cc. of water were added to each tube and to tube A was added 2 drops of hydrochloric acid. Both tubes were heated for 15 minutes in a boiling water bath. To each tube then was added 1 cc. of Fehling's solution and both tubes returned to the boiling water bath. After about 5 minutes, tube A contained a red precipitate while tube B remained clear.
9. A little of the precipitate was added to an aqueous solution of potassium ferricyanide containing a trace of ferric chloride and warmed. A dark Turnbull blue precipitate

formed. The experiment was repeated omitting the sample being tested. No precipitate formed. This is a positive Kobert(3) test for saponins. However, other reducing agents will give a positive test.

10. With nickel and cobalt salts, saponins give yellow colors. The test with the unknown was negative. A mercuric chloride solution is reduced to a calomel when heated with saponins, recognized by blackening with alkali. The test with the unknown was negative. These tests are also Kobert(3) tests for saponins.

11. A sample of the unknown was mixed with nitric acid and 1 drop of sulfuric acid added. There was no color. In the presence of nitrate, saponins give a red color on addition of 1 drop of sulfuric acid. This is Mitchell's test for saponins(4).

12. On adding a little of a mixture of equal parts of alcohol and concentrated sulfuric acid to a sample of a saponin, heating, and then adding a drop of ferric chloride solution, a blue green color develops. The test with the unknown was negative. This is Lapon's test for saponins(5).

13. On rubbing a little saponin with 2 drops of acetic anhydride, then allowing a drop of concentrated sulfuric acid to run in, a more or less intense red band forms at the point of contact. The test with the unknown was negative. This is Sagel's test for saponins(6).

14. The unknown did not hemolyze blood.

The filtrate from the lead subacetate treatment was treated with hydrogen sulfide to remove all the lead. The solution was filtered and the filtrate evaporated to dryness on a steam bath. Both before and during evaporation, a positive test was obtained with Fehling's solution.

Barksdale's work was repeated 3 times and in no case could a saponin be found.

Selective Solvent Experiments

In order to gain some knowledge of the soluble constituents, the seeds were subjected to a selective solvent investigation by the Dragendorff method. Furthermore, it was of interest to ascertain the action of selective solvents on the whole seed, the kernels alone, and the shells alone. For this purpose 100 grams of seed were crushed by means of pliers and carefully separated into kernels and shells by hand. The shells were ground to a fine powder in a small coffee mill while the kernels were ground to a fine powder by means of a mortar and pestle. A yield of 55.8 grams of the shells and 38.1 grams of the kernels was obtained. A quantity of whole seed was ground to as fine a powder as possible with the aid of a meat grinder.

Each of these three samples was then placed in a Soxhlet type continuous extractor and exhaustively extracted, successively, with solvents in the following order: petroleum ether (b.p. 60° to 70°C.), ether, chloroform, alcohol, water.

At the completion of each extraction, the extracts were concentrated to about 100 cc., placed in tared beakers, and the solvent allowed to evaporate completely at a temperature of about 35°C. The following data were obtained:

Table II

	Shells		Kernels		Whole Seed	
	Weight (Gms.)	Per cent	Weight (Gms.)	Per cent	Weight (Gms.)	Per cent
Weight of Sample used	55.8	100	38.1	100	50.0	100
P. E. extract	0.3	0.54	17.5	45.93	8.7	17.40
Ether extract	0.4	0.71	0.2	0.52	0.3	0.60
Chloroform extract	0.1	0.17	0.2	0.52	0.1	0.20
Alcohol extract	1.5	2.68	2.1	5.51	2.1	4.20
Aqueous extract	2.6	4.68	2.2	5.77	1.6	3.20
Total extraction	4.9	8.76	22.2	58.26	12.8	25.60
Marc	46.7	83.69	10.6	27.82	33.1	66.20
Total Recovery	51.6	92.44	32.8	86.07	45.9	91.80

Description of the Extracts

Petroleum ether extracts - The extract of the shells was a pale yellow, translucent, semi-solid material while those of the kernels and whole seeds were clear yellow oils at room temperature, containing small amounts of flocculent precipitates on the bottom. All possessed pleasant aromatic odors.

Ether extracts - The ether extracts in each case were pale yellow to reddish-yellow, semi-solid to solid, substances having the same pleasant aromatic odor of the petroleum ether extracts.

Chloroform extracts - The extract of the shells was a dark brown solid, that of the kernels a grayish solid, and that of the whole seeds a yellowish-brown solid. All possessed the same pleasant aromatic odor of the preceding extracts.

Alcohol extracts - The extract of the shells consisted of a mixture of reddish crystals and a red oily substance. That of the kernels was a light red solid containing some white amorphous material. The extract of the whole seed was a dark red material.

The crystals in the extract of the shells were separated from the oily substance by means of warm alcohol, in which the oil was readily soluble. The undissolved crystals were then dissolved in an excess of boiling alcohol and on recrystallization were obtained in fine white needles, which had the following properties:

1. Very soluble in water.
2. Soluble in hot alcohol.
3. Insoluble in ether.
4. Burn completely upon ignition, leaving no ash.
5. M.P., sharply at 163°C. (uncorr.).

This material was later identified as mannitol.

Aqueous extracts - The extracts of the shells and whole seeds were reddish brown solids while that of the kernels was a gray solid. They were all odorless.

Direct Alcoholic Extraction of the Shells

A quantity of watermelon seeds was cracked in a granulating machine and about 400 grams of the shells were separated and extracted in a continuous extractor with 95 per cent alcohol. After extraction was complete, the solvent was removed at a temperature of about 35°C. The residue was washed successively, until washings were colorless, with ether, alcohol at room temperature, and alcohol warmed to 45°C. The residue was then extracted with boiling alcohol. Upon cooling the boiling alcohol extract, white bead-like masses appeared on the bottom of the container. These were recrystallized from 95 per cent alcohol to a constant melting point. The final product was a white crystalline substance melting at 164° to 166.5°C. (corrected)* It was later identified as mannitol.

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* The thermometer used was standardized against a Bureau of Standards set of Anschutz thermometers.

Chemical Examination of Watermelon Seed

The material used in this examination was from the same lot as that used in the preliminary examination.

Solvent Extractions

On the basis of the small amount of material extracted with ether and chloroform in the preliminary examination, it was decided to omit those extractions in this examination. Since no satisfactory method had been found for separating the shells from the kernels, the extractions were carried out on the whole seed only.

Twenty pounds of seed were ground as finely as possible (about 20 mesh) by first crushing them in a granulating machine and then further grinding in a power mill. This powder was then placed in a muslin bag in a stainless steel percolator arranged for Soxhlet extraction and exhaustively extracted successively with petroleum ether (b.p. 60 to 70°C.), 95 per cent alcohol, and 50 per cent alcohol. The solvents were removed from the petroleum ether and 95 per cent alcohol extracts by distillation under an atmosphere of carbon dioxide. The solvent was removed from the 50 per cent alcohol extract under reduced pressure at 40°C. The following data were obtained:

	Weight (grams)	Per cent of sample
Sample	9080	100
P.E. extract	1630	17.95
95% Alcohol extract	409	4.5
50% Alcohol extract	49.5	0.54
Marc	6132	67.5
Total recovery	8220.5	90.5

After removal of the solvents, the extracts were stored under a carbon dioxide atmosphere until used.

Description of the Extracts

Petroleum ether extract - The extract was a clear reddish-orange oil with a slight flocculent precipitate. It had a pleasant, slightly aromatic odor resembling ginger.

Alcohol extract - The extract was a dark, reddish-brown oil containing suspended solid material. It had an aromatic odor resembling ginger.

Fifty per cent alcohol extract - The extract was a dark reddish-brown solid with a caramel-like odor and a disagreeable bitter taste.

Examination of the Petroleum Ether Extract

In order to ascertain how much alkali was needed to saponify the oil, the saponification value of this material was determined and found to be as follows:

Sample I	177.09
Sample II	174.4
Sample III	176.63
Average	176.03

Saponification

Five hundred grams of the petroleum ether extract was saponified for 14 hours in 500 cc. of 20 per cent alcoholic potassium hydroxide solution. After saponification the alcohol was removed under reduced pressure and the residue taken up in the least possible amount of water. This aqueous solution was extracted with seven 300 cc. portions of ether and the ether solution reserved for the preliminary experiments on non-saponifiable matter found on page 45.

Preparation of Lead Soaps

The aqueous solution of potassium soaps was then heated on a steam bath and treated with a hot concentrated aqueous solution of lead acetate to precipitate the lead soaps. Continuous stirring was necessary during this operation to keep the gummy lead soaps broken up as much as possible. After cooling, the lead soaps were filtered off and the filtrate set aside. The lead soaps were washed with several portions of water and the washings added to the above filtrate.

Separation of the Saturated from the Unsaturated Acids

The lead soaps were then dissolved in hot alcohol with the aid of a small amount of glacial acetic acid. After solution was effected, the alcohol was allowed to cool in the refrigerator for 12 hours. The precipitate of lead soaps was filtered, redissolved in alcohol and recrystallized thrice more as described above.

In order to determine if the separation had reached a satisfactory point, a portion of the precipitate of lead soaps was suspended in ether and converted to the acids. The ether solution of the acids was washed with distilled water until the washings were neutral to litmus. It was then dried over anhydrous sodium sulfate, filtered, and the solvent evaporated on a steam bath. The iodine value was determined and found to be 5.36. This low iodine value indicated that most of the unsaturated fatty acids had been removed from the saturated acids.

The precipitate of lead soaps was washed with several portions of alcohol in a Büchner funnel. After drying, the soaps were converted to acids as described above.

Esterification of the Saturated Fatty Acids

The saturated fatty acids were placed in a 500 cc. round-bottomed flask and esterified by refluxing with 300 cc. of methanol and 30 cc. of concentrated sulfuric acid for 12 hours. To the resulting solution was added about 4 times its volume of distilled water precipitating the methyl esters as an oily layer on the surface. The aqueous layer was drawn off from a separatory funnel and washed with several portions of ether to remove any suspended esters. The ether washings were added to the separated methyl esters and sufficient ether added to effect complete solution. The ether solution was washed with water until neutral to litmus, dried over anhydrous sodium sulfate and the solvent removed by evaporation on a steam bath.

Bromination of the Methyl Esters

Since the methyl esters obtained above could be expected to contain small amounts of methyl esters of unsaturated fatty acids, the material was brominated to reduce the volatility during distillation. The esters were dissolved in chloroform at 0°C. A solution of bromine in carbon tetrachloride was added dropwise until a slight excess of bromine was present as evidenced by the strong odor and the blue color produced on moistened starch-iodide test paper. An excess of 0.5 cc. of bromine solution was then added and the solution allowed to stand for 30 minutes to insure complete bromination. The chloroform solution was washed with a saturated solution of sodium bicarbonate to remove any excess bromine and then with water to remove the sodium bicarbonate. The chloroform was evaporated on a steam bath leaving a dark brown liquid.

Fractionation of Methyl Esters

The mixture of esters was placed in a 100 cc. flask packed to half its capacity with glass wool. Before distillation was started the esters were degassed first by applying vacuum without heat. The tenaciously held gases were removed by gradually heating the esters *in vacuo*. Before distillation was begun, a pressure of 0.08 mm. of mercury was attained.

The distillation was carried out under the following conditions: distilling temperature 117° to 150°C., a pressure of 0.08 to 0.25 mm. of mercury and an electrically heated oil bath at a temperature of 194° to 240°C. The distillation was stopped when the pressure began to rise steadily and sharply indicating decomposition.

About 5 minutes were required for replacing the receiver and evacuating the system when a fraction was cut. The esters were first separated into fractions with approximately 10 degree boiling ranges. Tabulated below is the distillation data of this fractionation. Four fractions resulted.

Table III

First Distillation of Esters

Time	Distilling Temp.	Bath Temp.	Jacket Temp.	Pressure mm. Hg.	Fraction
2:05	117	194	162	0.08	
2:20	125	196	170	0.08	
2:34	122	198	171	0.1	
2:45	120	196	170	0.1	
3:10	117	198	169	0.15	
3:24	121	195	177	0.15	
3:40	115	195	178	0.15	
3:50	113	195	178	0.17	I
4:00	126	202	186	0.15	
4:04	127	202	185	0.15	
4:14	134	207	188	0.3	
4:30	139	210	189	0.3	
4:40	140	211	204	0.25	II
4:43	140	211	205	0.25	
4:50	147	211	215	0.25	III
4:53	140	212	215	0.3	
5:25	135	220	235	0.3	IV

Each of these fractions was individually further separated into fractions with 5 degree distilling ranges. When a new fraction was placed in the distilling flask, it was necessary to break the vacuum, allow the jacket and the bath to cool, and again evacuate the system. This operation required about 15 minutes. The data on this distillation are found in Table IV.

Table IV

Redistillation of the First Fractions

Time	Distilling Temp.	Bath Temp.	Jacket Temp.	Pressure mm Hg	Fraction
		Fraction I			
8:20	105	216	122	0.08	
8:25	107	216	125	0.08	
8:32	109	218	127	0.08	
9:10	110	214	125	0.08	1
9:15	108	212	127	0.1	
9:20	114	209	127	0.1	
9:29	109	213	125	0.1	
9:45	115	224	127	0.1	2
9:50	118	227	127	0.1	
10:00	115	220	127	0.1	
10:05	112	235	130	0.1	
10:30	120	240	130	0.1	3

TABLE IV (continued)

Time	Distilling Temp.	Bath Temp.	Jacket Temp.	Pressure mm Hg	Fraction
			Fraction II		
11:15	117	180	185	0.15	
11:32	122	182	187	0.2	
11:45	125	185	196	0.2	4
11:50	125	185	196	0.2	
11:55	130	185	196	0.2	5
12:15	130	185	220	0.2	
12:25	135	185	222	0.2	6
			Fraction III		
1:00	145	195	210	0.6	
1:05	148	195	210	0.5	7
			Fraction IV		
1:30	135	210	220	0.2	
1:35	130	211	213	0.1	
1:45	128	211	213	0.08	
2:10	130	211	213	0.08	8

When distillation was complete all of the fractions were clear, colorless liquids which became white crystalline masses on cooling to room temperature. Fractions 5, 6, and 7 were too small to work up satisfactorily.

Saponification of the Methyl Esters

Each of the 5 fractions was saponified on a steam bath with 20 per cent excess of alcoholic sodium hydroxide solution for 1 hour. The excess alcohol was removed by distillation on a steam bath under reduced pressure. Each fraction was then dissolved in the minimum amount of warm water and the solution acidified with concentrated hydrochloric acid. The suspensions were cooled to room temperature and extracted with ether. The ether solutions were washed with water until neutral to litmus, dried over anhydrous sodium sulfate, and the ether evaporated on a steam bath. After 3 crystallizations from acetone, the neutralization equivalents were determined.

Determination of the Neutralization Equivalents

About 0.1 gram samples of the acids were accurately weighed, dissolved in 25 cc. of alcohol neutralized to phenolphthalein, and titrated with 0.05 normal sodium hydroxide. The following results were obtained:

		Neutralization Equivalent
Fraction 1	A.	261.6
	B.	259.0
	Average	260.3
Fraction 2	A.	291.7
	B.	289.3
	Average	290.5
Fraction 3	A.	260.2
	B.	259.5
	Average	259.8
Fraction 4	A.	259.1
	B.	294.0
	Average	276.5
Fraction 5	A.	284.2
	B.	287.0
	Average	285.6

Identification of Saturated Fatty Acids

Schuette and Vogel(7) have shown that it is possible to calculate the amount and character of acids, differing from each other by 2 carbon atoms, in a mixture by means of binary mixture freezing point curves. The observed mean molecular weight from the neutralization equivalent determines the proper curve as well as the composition of the mixture if the melting point falls at 2 or more places on the curve. Using the mol per cent composition found on the curve, the mean molecular weight is calculated. If this figure does not agree within experimental error with the neutralization equivalent, it may be assumed that the fraction is not a binary mixture. In such cases additional fractional crystallizations are often helpful.

The freezing points and mean molecular weights of the 5 fractions of fatty acids obtained from the watermelon seed oil were determined in the above manner. The results are summarized below.

Fraction 1: The neutralization equivalent placed this fraction in the C_{16}-C_{18} binary system. From the curve, the solidification point indicated a mixture of 93.3 mol per cent of the C_{16} acid and 6.7 mol per cent of the C_{18} acid. The mean molecular weight calculated from the neutralization equivalent was 260.3 and from the curve, 258.6. This difference is within experimental error and is an indication that the fraction consisted largely of palmitic acid with some stearic acid.

Fraction 2: The neutralization equivalent placed this fraction in the C_{18}-C_{20} binary system. However, the solidification point was far below the temperature range of this curve. Since this fraction was small, additional crystallizations were not feasible.

Fraction 3: The neutralization equivalent placed this fraction in the C_{16}-C_{18} binary system. From the curve, the solidification point indicated a mixture of 98 mol per cent of the C_{16} acid and 2.0 mol per cent of the C_{18} acid. The mean molecular weight calculated from the neutralization equivalent was 259.8 and from the curve, 256.7. This difference is within experimental error and is an indication that this fraction consisted largely of palmitic acid and a small amount of stearic acid.

Fraction 4: No checks were obtained for the neutralization equivalent and the solidification point was far below the range indicated by either of the neutralization equivalents. Since this fraction was small no further work was possible.

Fraction 5: The neutralization equivalent placed this fraction in the C_{18}-C_{20} binary system. From the curve, the solidification point indicated a mixture of 97.5 mol per cent of the C_{18} acid and

2.5 mol per cent of the C_{20} acid. The mean molecular weight calculated from the neutralization equivalent was 285.6 and from the curve, 284.8. These results are in good accord and indicate that the fraction consisted largely of stearic acid with a small amount of arachidic acid.

A summary of the data on the partial identification of the saturated fatty acids of watermelon seed is given in Table V.

Table V

	Methyl Esters			Acids				
Fract.	Dist. Range	Av. Pres. mm Hg	Weight (Gms.)	Weight (Gms.)	N. E. (Titr.)	F. P.	M. W. (Calc.)	Theoretical Composition (Per cent)
1	105-110	0.08	5.9	3.7	260	61.3	259	93.3 C_{16}; 6.7 C_{18}
2	108-115	0.1	5.9	2.8	290	58.2	?	?
3	115-120	0.1	4.6	1.4	260	59.6	257	98.0 C_{16}; 2.0 C_{18}
4	117-125	0.2	1.0	0.65	276	53.8	248	?
5	125-130	0.2	—	—	—	—	—	
6	130-135	0.2	—	—	—	—	—	
7	145-148	0.4	—	—	—	—	—	
8	128-135	0.1	6.9	5.2	286	67.8	285	97.5 C_{18}; 2.5 C_{20}

Isolation of the Mixed Unsaturated Fatty Acids

The various alcohol-acetic acid solutions, after removal of the saturated acids and containing the lead salts of the unsaturated fatty acids, were combined and acidified with concentrated hydrochloric acid. A heavy white precipitate of lead chloride formed in the solution. The whole was then diluted with four volumes of water causing the unsaturated fatty acids to separate as an oily layer on the surface. The unsaturated fatty acids were separated from as much water as possible and placed in a separatory funnel. The remainder of the aqueous layer was drawn off and the acids washed several times with distilled water. A portion of the acids was then dissolved in ether, the ethereal solution washed several times with water, and dried over anhydrous sodium sulfate.

Bromination of the Unsaturated Fatty Acids

The dried ethereal solution of the unsaturated fatty acids was placed in a 3-necked flask immersed in an ice-salt bath. Bromine was added to the cold ether solution through a dropping funnel keeping the solution agitated with a mechanical stirrer. When an excess of bromine had been added as evidenced by the color and strong odor of bromine, about 0.5 cc. additional bromine was added and the solution stirred for about 30 minutes longer. During this period a considerable amount of a heavy yellow precipitate formed in the reaction mixture.

Identification of Linoleic Tetrabromide

The yellow precipitate was removed by filtration and dried in the air. On drying, most of the yellow color disappeared due to the volatilization of adhering bromine. The dried precipitate was then recrystallized from petroleum ether, all of the material dissolving in the boiling solvent. On cooling, a white crystalline precipitate formed, which when filtered off and dried, melted at 114 to 115°C. (corrected). This corresponds to the reported melting point for linoleic tetrabromide. The fact that all of the material was soluble in petroleum ether, together with the sharp melting point of the recrystallized material, is evidence that acids more highly unsaturated than linoleic probably do not exist in watermelon seeds.

The mother liquor from the above crystallization was evaporated leaving a brown pasty mass, presumably a mixture of oleic dibromide and linoleic tetrabromide. Attempts to separate the 2 bromides by fractional crystallization from cold petroleum ether failed.

A second portion of the unsaturated acids was dissolved in an excess of 10 per cent aqueous sodium hydroxide solution and the barium salts precipitated by the addition of a concentrated aqueous solution of barium chloride. The barium salts were filtered off, washed with water until washings were neutral, and dried. Attempts to separate the barium oleate from barium linoleate by repeated crystallization from benzene according to the method of Lapworth, Pearson, and Mottram(8) failed.

Identification of Tetrahydroxy Stearic Acid

A third portion of the unsaturated fatty acid mixture was oxidized to the polyhydroxy acids according to the method of Lapworth and Mottram(9) as follows

Five grams of the unsaturated acids was dissolved in 400 cc. of a 1.25 per cent solution of sodium hydroxide. After solution was effected, 4000 cc. of ice water was added and the solution stirred and cooled to a temperature of 0 to 10°C. To this was added, in one portion, 500 cc. of a cold 1 per cent solution of potassium permanganate and the solution shaken vigorously for 5 minutes. The potassium permanganate was then decolorized with a saturated solution of sodium sulfite. The mixture was treated with 150 cc. of concentrated hydrochloric acid, and the precipitate of the hydroxy acids filtered off and dried. Repeated crystallizations from petroleum ether gave a product melting at 155 to 165°C. which was probably a mixture of the polyhydroxy stearic acids.

Repeated, careful fractional crystallization from 95 per cent alcohol yielded a small amount of material melting at 172 to 173°C. (corrected). However, most of the material melted at 155 to 165°C. The melting point of tetrahydroxy stearic acid is 173°C. This is further evidence of the presence of linoleic acid in watermelon seeds. No material could be isolated showing a melting point of dihydroxy stearic acid (132°C.), but it was probably this substance which gave the wide melting range to the majority of the material.

Neither oleic acid nor any derivative of oleic acid could be isolated from the mixed unsaturated acids. However, from the pasty appearance of the mixed bromo acids and from the melting point behavior of the polyhydroxy acids, oleic acid is believed to be present, probably to a lesser extent than linoleic acid.

<u>Identification of Glycerol</u>

One hundred cc. of the aqueous liquor from the precipitation of the lead soaps of the fatty acids was evaporated to dryness on a steam bath. The residue remaining was extracted with a mixture of alcohol and ether, and the solvents were evaporated. A small amount of a thick syrup remained which had a sweet taste and was soluble in water. When heated with a small amount of potassium bisulfate, this syrup gave the characteristic pungent odor of acrolein, indicating the presence of glycerol.

The Non-Saponifiable Matter

Preliminary Investigation

The ether was removed by distillation from the non-saponifiable matter mentioned on page 30. The residue, oily in appearance, was again saponified by refluxing with 50 cc. of 20 per cent alcoholic sodium hydroxide and the solvent removed under reduced pressure. The residue was extracted with ether and the ether solution was dried over anhydrous sodium sulfate, filtered, and the solvent evaporated on a steam bath. The residue which weighed 1 gram was heated with 4 cc. of acetic anhydride and 10 cc. of glacial acetic acid on a steam bath for 2 hours. The solution was then poured into ice water, the water extracted with ether, and the ether evaporated. The residue, weighing 1.4 grams was dissolved in the minimum amount of hot 95 per cent alcohol. On cooling, the alcoholic solution deposited crystals which after 5 crystallizations from 95 per cent alcohol melted at 149 to 156°C. (corrected). Further crystallizations from 95 per cent alcohol failed to alter the melting point. Two crystallizations from methanol gave a product melting at 149 to 152.5°C. (corrected).

A portion of the acetate was saponified with alcoholic sodium hydroxide for 1 hour on a steam bath. The solution was then diluted with water, acidified with hydrochloric acid, and extracted with ether. The ether was evaporated and the residue, after 2 crystallizations from 95 per cent alcohol, had a melting point of 138 to 142°C. (corrected), and gave a positive Liebermann-Burchard test. The amount of material was too small for further work.

Detailed Examination

A 700 gram sample of the petroleum ether extract was saponified with a 10 per cent excess of a 20 per cent alcoholic sodium hydroxide solution on a steam bath for 14 hours. The alcohol and water were removed by distillation under reduced pressure and then by evaporation with continuous stirring. The residue was extracted 7 times with ether. The ether was removed by distillation and the oily residue again treated with alcoholic sodium hydroxide as above. After removing the alcohol and water and extracting with ether, the ether solution was washed with water to remove glycerol and any dissolved salts. Troublesome emulsions were encountered.

The ether solution was distilled and the residue heated on the steam bath until the last traces of water were removed. The residue was then extracted with ether. The ether-insoluble residue was dissolved in water and the aqueous solution extracted with ether, all of the ether solutions being combined and dried over anhydrous sodium sulfate. The ether solution was filtered and distilled, leaving a brown, oily residue weighing 4.824 grams.

The residue was dissolved in the minimum amount of boiling acetone. On cooling, the solution deposited crystals which after 4 crystallizations from acetone gave a positive Liebermann-Burchard test and melted at 140 to 148°C. (corrected), softening at 130°C. The mother liquor from the first crystallization was evaporated and the residue dissolved in the minimum amount of boiling 95 per cent alcohol. On cooling, the solution deposited more crystals melting at 140 to 148°C. (corrected). All of the crystalline material, weighing 0.417 grams, was acetylated by heating with 10 cc. of glacial acetic acid and 5 cc. of acetic anhydride under a reflux condensed on a steam bath for 5 hours. The solution was then poured onto crushed ice in a separatory funnel. After the ice had melted, the water was extracted twice with ether. The ether solution was washed with 5 per cent sodium bicarbonate until the washings were alkaline to litmus, then with water until the washings were neutral. The ether solution was dried over anhydrous sodium sulfate, filtered, and the ether removed by distillation from a tared flask, the last traces being removed under reduced pressure. The dried residue weighed 0.749 grams.

Two crystallizations from acetone gave a product melting at 164 to 170°C. (corrected) and further crystallizations from acetone did not alter the melting point. After 3 crystallizations from methanol, the product melted at 167.2 to 168.2°C. (corrected) with a previous softening at 160.4°C.

The mother liquors were combined, the solvent removed, and the residue (0.371 gram) dissolved in 75 cc. of petroleum ether containing 5 cc. of benzene to increase the solubility. This solution was chromatographed through a column of aluminum oxide 16 x 100 mm. The column was washed with 75 cc. of petroleum ether, extruded from the tube, and divided into 3 equal segments, each of which was eluted with acetone.

The top segment yielded 0.148 gram of crystalline material which, when crystallized twice from methanol, yielded 25 mg. of crystals (A) melting at 167.2 to 168.2°C. (corrected). These were temporarily set aside.

The center segment yielded 0.091 grams of crystalline material which, when crystallized from methanol, yielded in the first crop, 42 mg. of crystals (B) melting at 158 to 161°C. (corrected). These were temporarily set aside. From the mother liquor was obtained 12 mg. of crystals (C) which gave a positive Liebermann-Burchard test and melted at 123 to 127°C. (corrected). This latter material was too small for further work.

The bottom segment yielded 0.040 gram of material, and the filtrate yielded a residue of 0.020 gram. No crystalline material could be obtained from these residues by crystallization from methanol.

The remainder of the non-saponifiable matter (3.75 gram) was acetylated with 20 cc. of acetic anhydride and 40 cc. of glacial acetic acid and worked up as described on page 47. Since it had been found that the acetate could be crystallized from methanol, the acetylated material, weighing 3.88 grams, was dissolved in boiling methanol and on cooling, the solution deposited a small amount of crystals (D) melting at 157 to 162°C. (corrected). Since very little crystalline material could be obtained upon concentration of the mother liquor, the solvent was removed and the residue dissolved in 500 cc. of petroleum ether containing 25 cc. of benzene. This solution was chromatographed through a column of aluminum oxide 16 x 250 mm. and the column washed with 100 cc. of petroleum ether. The filtrate (2) was temporarily set aside.

The column consisted of 3 color zones. The top zone, 100 mm. long, was pale red in color; the center zone, 80 mm. long, was a pale pink color; and the bottom zone was practically colorless. The column was removed from the tube and divided between zones. Each of the 3 segments was eluted with acetone.

The top segment yielded 0.484 gram of a red oily material from which no crystalline material could be isolated. The center segment also yielded a red oily material, weighing 0.493 gram, from which no crystalline material could be isolated.

The bottom segment yielded 0.265 gram of a white solid material which when crystallized from methanol gave crystals (F) melting at 159 to 163°C. (corrected).

The crystals melting at 159 to 163°C. (F) were combined with (B) and (D) (total weight 0.186 gram), dissolved in 35 cc. of petroleum ether, and chromatographed through a column of aluminum oxide 18 x 100 mm., the column being washed with 40 cc. of petroleum ether. The filtrate yielded 8 mg. of an oily material which was discarded. After removal of the column from the tube, the top 3.5 mm. of the column, containing all of the color was cut away and the remainder of the column divided into 3 equal segments. Each segment was eluted with acetone.

The top segment yielded 0.130 gram of material which, when crystallized from methanol, gave crystals (G) melting at 163 to 165°C. (corrected).

The center segment yielded 27 mg. of material, and the bottom segment yielded 17 mg. of material. No crystalline material could be obtained from these residues by treatment with methanol.

A mixture of crystals (A) and (G) gave a melting point of 163 to 165.2°C. (corrected). These 2 fractions, (A) and (G), weighing 56 mg., were combined. A sample of 14.8 mg. was used to determine the optical rotation and 40 mg. were reserved for a combustion analysis.

The acetate showed no optical activity in chloroform. The blank gave a reading of -3.67° and a solution of 14.8 mg. of the acetate in

2 cc. chloroform gave a reading of −3.66°.

The chloroform solution used for the optical rotation was evaporated to dryness and the residue refluxed with alcoholic sodium hydroxide for 2 hours. The alcoholic solution was then diluted with water and extracted with ether. The ether was removed and the residue, after crystallisation from methanol, melted at 163.2 to 166.2°C. (corrected). A chloroformic solution of the residue decolorized bromine.

A sample of 38.9 mg. of the acetate was analyzed for carbon and hydrogen. The results of the analysis are found below. Calculated from these results the empirical formula for the acetate appears to be $C_{28}H_{46}O_2$ from which the empirical formula for the sterol would be $C_{26}H_{44}O$. However, most naturally occurring phytosterols reported have 27 or more carbon atoms. Since the sterol decolorized bromine, there must be at least one double bond; and since it formed an acetate there must be at least one hydroxyl group. Considering the fact that most naturally occurring sterols have 27 carbon atoms, a sterol acetate having these functional groups would have the molecular formula $C_{29}H_{48}O_2$. For comparison, the composition of such a sterol is given below.

	Carbon %	Hydrogen %	Oxygen % (by difference)
Found	81,23	11.10	7.67
Calc. for $C_{28}H_{46}O_2$	81.09	11.18	7.73
Calc. for $C_{29}H_{48}O_2$	81.22	11.28	7.50

The filtrate (E), after removal of the solvent, left an oily residue containing some solid material. The residue was dissolved in boiling acetone and on cooling, 5 mg. of crystals melting at 40 to 50°C. (corrected) was obtained. The crystals were insoluble in concentrated sulfuric acid and did not decolorize bromine solution, indicating that the material was probably an impure saturated hydrocarbon.

The oily material crystallized from acetone at -7°C. but melted when filtered at 10°C. The oil was soluble in concentrated sulfuric acid, producing a dark reddish brown color. A chloroformic solution of the oil decolorized bromine and potassium permanganate. In view of the fact that it passed through the column of aluminum oxide, it may have been an unsaturated hydrocarbon.

None of the material isolated from the non-saponifiable fraction could be identified.

Examination of the Alcoholic Extract

Isolation of d-Mannitol

The alcoholic extract described on page 29 was washed by decantation with boiling ether until 10 cc. of the ether washings left no residue upon evaporation to dryness. The ether insoluble residue was then washed successively with alcohol at room temperature, and with warm alcohol (45°C.) until the washings were colorless in each case. Finally the residue was taken up in boiling alcohol. On cooling the last solution, fine, white crystals formed. These were filtered off, and the concentration and cooling of the filtrates repeated until no more crystals formed. All the crystal crops were combined and purified by crystallization from 95 per cent alcohol to a constant melting point of 164.6 to 166.2°C. (corrected). The purified material was a white crystalline substance.

The solubility behavior of the compound placed it in group II of the analytical scheme of Kamm(10). On elementary analysis, the compound was found to contain no nitrogen, sulfur, or halogen. Its aqueous solution was neutral to litmus and the addition of hydrochloric acid produced no precipitate. The addition of a solution of silver nitrate to an aqueous solution of another sample did not produce a precipitate. On ignition, the compound left no ash, eliminating the possibility that the compound might be a metallic salt of an organic acid. This experimental data thus indicated a polyhydroxy-alcohol or a sugar.

The compound did not reduce Fehling's solution, but reduced Tollen's reagent when heated on a steam bath.

The melting point of the substance (164.6-166.5°C. corrected) corresponded to that recorded for mannitol. There was no depression of the melting point when the unknown was mixed with a known sample of d-mannitol (Eastman).

Landolt(11) states that the optical rotation of d-mannitol in aqueous solution is laevo. Grün, Husmann, and Kossowitsch(12) found $[\alpha]_D^{25}$ -0.49 for d-mannitol in aqueous solution. Dodge(13) reported $[\alpha]_D^{22}$ -0.39. The optical rotation was determined on the unknown material melting at 164.6 to 166.5°C. with the following result;

$[\alpha]_D^{25}$ -0.26° (32.5 mg. in 2 ml. of water α_D -0.186, λ 1 dm.)

Fischer(14) noted that an aqueous solution containing 5 per cent of d-mannitol and 7.4 per cent of borax had $[\alpha]_D$ $+$ 28.3. Dodge(15) reported $[\alpha]_D^{25}$ $+$ 28.5. Under these same conditions, the unknown gave the following result;

$[\alpha]_D^{22}$ $+$ 27.75 (60.6 mg. in 2 ml. of 7.4 per cent aqueous solution of borax α_D 0.842, λ 1 dm.)

This value compares favorably with those of Fischer and Dodge.

The acetyl derivative was prepared according to the method of Shriner and Fuson(16) using 1 gram of the unknown, 0.5 gram of sodium acetate, and 5 cc. of acetic anhydride. After 4 crystallizations from 95 per cent alcohol, the derivative had a constant melting point of 123 to 123.5°C. (corrected). A mixed melting point with an authentic sample of mannitol hexa-acetate gave no depression. The optical rotation was determined on the hexa-acetate with the following result;

$[\alpha]_D^{25}$ $+$ 21.85 (29.5 mg. in 2 ml. of chloroform α_D 0.43, λ 1 dm.)

The following data have been reported on the hexa-acetate of d-mannitol:

M.P.	$[\alpha]_D$	
120	——	Shriner and Fuson(17)
122	+24.4	Fuson and Rich(18)
126	$[\alpha]^{27}_D$ +18.22	Patterson and Todd(19)
——	+18.8	Iwata(20)
123.5-124.5	$[\alpha]^{27}_D$ +21.3($CHCl_3$)	Dodge(15)

In addition to the hexa-acetate, two tetra-acetates and one di-acetate are known:

Acetate	M.P.	$[\alpha]_D$	
2,3,4,5 tetra	122-125	+ 5.0	Micheel(21)
3,4,5,6 tetra	92	+31.6	v. Vargha(22)
3,4 di	128	+45.3	v. Vargha(22)

From a comparison with the melting points and specific rotations recorded for the hexa-acetate by the various authors, it would appear that the unknown substance was mannitol and that its acetyl derivative was the hexa-acetate. This is the first reported isolation of mannitol from Citrullus Vulgaris Schrader.

Selective Solvent Action

The alcoholic extract, freed of mannitol, was mixed with sufficient sand to give a dry crumbly mixture. This mixture was placed in a muslin bag in a stainless steel soxhlet type extractor, and exhaustively extracted successively with petroleum ether, ether, and 95 per cent alcohol. The solvents were removed by distillation and the residues weighed:

Petroleum ether extract:	28.6 grams
Ether extract:	34.0 grams
Alcohol extract:	94.6 grams

The ether extract: The ether extract (34.0 gms.) was washed with several portions of cold ether over a period of 3 weeks. The ether was removed by distillation, followed by evaporation and finally evaporation *in vacuo*. The residue was a reddish brown mass.

The mass was suspended in about 20 cc. of cold alcohol and filtered. The residue, a reddish amorphous material, was dissolved in hot alcohol, cooled, and the solution filtered. A tan, amorphous precipitate was obtained. After 3 crystallizations from 95 per cent alcohol, 11 mg. of a white amorphous material was obtained which melted at 262.6°C. (corrected). There was a previous softening at 212°C. and a gradual darkening from 213 to 262.6°C. At the melting point the material was quite dark.

An attempt was made to acetylate the material as described on page 47 using 5 cc. of acetic anhydride and 10 cc. of glacial acetic acid. After removal of the ether, the residue weighed 19 mg., but attempts to crystallize the material were unsuccessful.

The petroleum ether extract: The petroleum ether extract (28.6 gms.) was dissolved in chloroform and precipitated by the addition of 3 volumes of 95 per cent alcohol. The precipitate was separated by centrifuging and then washed with alcohol until the washings were colorless, the alcohol being decanted after centrifuging. The precipitate was refluxed with 95 per cent alcohol and filtered. The filtrate was concentrated and on cooling, an amorphous material precipitated which, after 5 crystallizations from 95 per cent alcohol, melted at 65 to 67°C. (corrected) and weighed 11 mg. A chloroformic solution of the material did not decolorize bromine, and upon removal of the chloroform and bromine, the residue did not dissolve in concentrated sulfuric acid. Another sample of the untreated material was not soluble in concentrated sulfuric acid.

The melting point and these latter properties indicate that the substance probably is a saturated hydrocarbon.

The Alcohol Extract: The alcohol extract (94.6 Gms.) was extracted with boiling chloroform. Nothing could be crystallized from the chloroform solution and addition of cold 95 per cent alcohol to the solution gave no precipitate.

The residue from the chloroform extraction was extracted with boiling ethyl acetate. No crystalline material could be isolated.

From the entire alcoholic extract of watermelon seeds nothing could be isolated except considerable amounts of mannitol, a few mg. of an unknown, high melting substance, and a few mg. of a substance which appeared to be a hydrocarbon. The residue consisted of a black tarry material.

Summary

1. Attempts to isolate a substance giving a saponin test from watermelon seed, according to the method of Barksdale, were unsuccessful.

2. The ground whole seeds were subjected to selective solvent action successively with petroleum ether, 95 per cent alcohol, and 50 per cent alcohol. Only the first two extracts were investigated.

3. From the physical and chemical properties of the saturated fatty acids, it appeared probable that they consisted chiefly of palmitic and stearic acids with a small amount of arachidic acid.

4. Linoleic acid was identified in the unsaturated fatty acids by conversion to the tetrabromo- and tetrahydroxy- stearic acids. From the physical properties of the mixed bromo- and mixed hydroxy-acids, it appeared that small amounts of oleic acid were present. No acid more highly unsaturated than linoleic acid was found.

5. Glycerol was identified by the odor of acrolein produced on heating with potassium bisulfate.

6. Four unidentified substances were isolated from the non-saponifiable fraction in small amounts. Two were sterols yielding acetates which melted at 123 to 127°C. (corrected) and 167.2 to 168.2°C. (corrected). The latter on saponification, yielded a sterol melting at 163.2 to 165.2°C. (corrected). Of the other two substances, one was probably a saturated hydrocarbon and the other was possibly an unsaturated hydrocarbon.

7. d-Mannitol was isolated from the alcoholic extract. This is the first reported isolation of _d_-mannitol from _Citrullus vulgaris_

Schrader.

8. Two other substances were isolated from the alcoholic extract, both in quantities too small for identification. One was a high melting (262.6°C. decomp., corrected), white, amorphous material. The second, melting at 65 to 67°C. (corrected), was probably a saturated hydrocarbon.

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(________)

Pharmacopoea Hispana, 1st ed., p.___ (Bruntz, L. and Jaloux, M., Plantes Officinales et Plantes á Drogues Medicamenteuses, (1918), p. 210.)

(Lists <u>Citrullus vulgaris</u> Schrader as official in the 1st edition of the Spanish Pharmacopoeia.)

(Committee) 1797

Anguria

Pharmacopoea Hispana, 2nd ed., p. 4

Gives the part used and synonyms of the plant

(Committee) 1803

Citrullus

Pharmacopea Hispana, 3rd ed., p. 19

Gives the part used and synonyms of the plant.

Broteri, F. A. 1804

(Cucurbita citrullus)

Flora Lusitanica, I, p. 312 (Pharmacopea Portuguesa, (1876), p. 266.)

(The Portuguese Pharmacopoeia lists Flora Lusitanica as a reference to Cucurbita citrullus)

Collegio Sanitatis Regio Medico-Chirirgico Hafniensi 1805

Citrullus

Pharmacopoea Danica, 2nd ed., p. 22

Gives the part used and synonyms of the plant.

(Committee) 1817

Citrullus

Pharmacopoea Hispana, 4th ed., p. 28

Gives part used and synonyms of the plant.

Facultate Medica Parisiensi 1818

Semina Dicta Vulgo Frigida

Codex Medicamentarius Sive Pharmacopoea Gallica, 1st ed., p. 283

Lists the four cold seeds.

Shrank, ___. 1824

(Cucurbita pinnatifida)

Syll___ Ratisb___, 2nd ed., v.1, p.190 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 665.)

(The above botanical name is a synonym for Citrullus vulgaris Schrader.)

Frigueiredo, J.J. de 1825

(Cucurbita citrullus)

Flora Pharmaceutica e Alimentar Portugueza, p. 527 (Pharmacopea Portuguesa, 3rd ed., (1876), p. 266.)

(The Portuguese Pharmacopoeia gives Flora Pharmaceutica as a reference to Cucurbita citrullus.)

(Committee) 1826)

Semences froides

Code Pharmaceutique, 2nd ed. p. 446.

Gives formula for mixing the four cold seeds.

Sageret, . 1826

(Citrullus pasteca)

Ann. Sci. Nat. ser. I, *8*, p.____ (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 550)

(The above botanical name is a synonym for *Citrullus vulgaris* Schrader.)

Schrader, H. A. 1827

(Cucumis amarissimus)

Hortus Gottingensis, p.____ (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 664.)

(The above botanical name is a synonym for *Citrullus vulgaris* Schrader.)

Seringe,____. 1828

Cucumis citrullus

DeCandolle's Prodromus, v.3, p. 301.

Gives description, habitat, and synonyms with references.

DeCaisne, J. 1834

(Cucumis dissectus)

Annales du Museum, iii, p. 449 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 664.)

(The above botanical name is a synonym for Citrullus vulgaris Schrader)

Schrader, H.A. 1834

(Citrullus caffer)

Ind___ Sem___ Hort___ Gotting___, p.___ (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 550)

(The above botanical name is a synonym for Citrullus vulgaris Schrader.)

Ecklon, C.F. and Zeyher, K. 1836

(Cucurbita caffra)

Enumeratio Plantarum Africae Australis Extratropicae, p. 279 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 665.)

(The above botanical name is a synonym for Citrullus vulgaris Schrader.)

Schrader, H.A. 1836

(Citrullus vulgaris)

Enumeratio Plantarum Africae Australis Extratropicae, p. 279 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 550).

(Index Kewensis recognises the above botanical name as the standard name for watermelon)

Schrader, H.A. 1836

(Citrullus amarus)

Enumeratio Plantarum Africae Australis Extratropicae, (C. Ecklon and K. Zeyher), p. 279 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 550.)

(The above botanical name is a synonym for Citrullus vulgaris Schrader.)

Fal___, ___., and Dunal, ___, 1836

(Citrullus chodospermus)

Bull. Soc. Agr. Herault, p. 264 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 550)

(The above botanical name is a synonym for Citrullus vulgaris Schrader)

Wood, G. B. and Bache, F. 1836

Cucurbita citrullus

United States Dispensatory, 3rd ed., p.1069; ibid., 4th ed. (1839), p.1139; ibid., 5th ed. (1843), p. 1250; ibid., 6th ed. (1845), p. 1250; ibid., 7th ed. (1847), p. 1250; ibid., 8th ed. (1849), p. 1254; ibid., 9th ed. (1851), p. 1322; ibid., 10th ed. (1854), p. 1323; ibid., 11th ed. (1858), p. 1404; ibid., 12th ed. (1869), p. 1507; ibid., 13th ed. (1871), p. 1584; ibid., 14th ed. (1879), p. 1635; ibid., 15th ed. (1883), p. 1627; ibid., 16th ed. (1892), p. 1776; ibid., 17th ed. (1894), p. 1612; ibid., 18th ed. (1899), p. 1633; ibid., 19th ed. (1907), p. 1468; ibid., 20th ed. (1918), p. 1351; ibid., 21st ed. (1926), p. 1526; ibid., 22nd ed. (1937), p. 1641.

Gives a description of the plant and the seeds, and beginning in 1894 also gives the constituents and uses of the seeds.

(Committee) 1837

Especes Dites Quatre Semences Froides

Codex, Pharmacopee Francaise, 2nd ed., p. 438

Lists the botanical source of each of the four cold seeds.

Schrader, H.A. 1838

(Citrullus cafforum)

Linnaea, 12, p. 413 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 550)

(The above botanical name is a synonym for *Citrullus vulgaris* Schrader)

Spach, E. 1838

Pasteque

Histoire Naturelle des Vegetaux Phanerogames, v.6, p. 214

Gives description, habitat and synonyms of *Citrullus vulgaris* Schrader.

Ecklon, C. F. 1840

Cucumis laciniosus

Nomenclator Botanicus, (E.T. Steudel), 2nd ed., v.1, p. 451

The above botanical name is a synonym for *Citrullus vulgaris* Schrader.

Wood, A. 1847

Cucumis Citrullus Seringe

Class Book of Botany, p. 273

Describes the plant

Hook, J. H. J. 1850

Use of Watermelon Seeds as a diuretic

Charleston Medical Journal, *5*, p. 736

Gives clinical evidence of the diuretic property of the seed.

Stocks, ___, 1851

(Citrullus fistulosis)

Hooker's Kew Journal, iii, 74, t.3 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 560)

(The above botanical name is a synonym for *Citrullus vulgaris* Schrader.)

King, J. and Newton, R. S. 1852

Cucurbita citrullus

Eclectic Dispensatory, 1st ed., p. 149

Gives description, habitat and uses.

DeCandolle, A. 1855

Cucumis citrullus Seringe

Geographie Botanique Raisonee, v.2, p. 908

Gives Habitat and migration of the plant.

Schur, P.J.F. 1866

(Citrullus aquosus)

Enumeratio Plantarum Transilvanae, 2nd ed., p. 221 (Hooker, J.D. and Jackson, B.D., Index Kewensis, (1893), v.1, p. 550.)

The above botanical name is a synonym for *Citrullus vulgaris* Schrader.

Lowe, R. T. 1868

Cucurbitaceae

A Manual Flora of Maderia, p. 291

A description of the plant is given.

Fluckiger, F. A. 1872

Die Frankfurter Liste

Arch. d. Pharm. *201*, p. 433

Lists watermelon seeds in an inventory of a Pharmacy in Frankfort-on-Main, Germany, of the year 1450.

La Sociedad Farmaceutica Mexicana 1874

Sandia O Zandia

Nueva Farmacopea Mexicana, 1st ed., p. 192

Gives synonyms, habitat, and uses.

King, J. 1875

Cucurbita citrullus

American Dispensatory, 10th ed., p. 311

Gives description, habitat and uses.

(Committee) 1876

Melancia

Pharmacopea Portugueza, 3rd ed., p. 266

Gives synonyms, habitat, references, and part used

Dymock, W. 1879

Citrullus vulgaris Schrader

Pharm. J. Trans. *38*, p. 1015

Gives the original four cold seeds and the four cold seeds of Paris and of Bombay.

Sociedad Farmaceutica de Mexicana 1884

Sandia O Zandia

Nueva Farmacopea Mexicana, 2nd ed., p. 96

Gives synonyms, habitat, and use.

(Committee) 1884

Sandia

Farmacopoea Oficial Espanola, 6th ed., p. 91

Gives synonyms, habitat and part used.

Oldberg, O. and Wall, O. A. 1884

Cucumis citrullus

Companion to the United States Pharmacopoeia, p. 387

Gives Origin, habitat, part used, description, constituents, properties, medicinal use, and dose.

Stille, A. and Maisch, J. M. 1884

Cucurbita citrullus

National Dispensatory, 3rd ed., p. 523

Gives a botanical description of the seed.

Popow, ____. 1888

(________________)

Pharm. Zeit. f. Russl. 27, p. 765 (Dragendorf, G., Heilpflanzen, (1888), p. 649.)

(The seeds contain albumin, dextrose, and resin.)

Hehn, V. and Sallybrass, F. S. 1888

Cucumis citrullus

Wanderings of Plants and Animals, p. 239

Suggests a course of migration of *Citrullus vulgaris* Schrader from India.

Nardini, G. 1890

(______________)

Stag. Sperim. Agr. Ital. 18, p. 448 (König, J., Chemie der Menschlichen Narungs-Genussmittel, (1903), p. 1496.)

(Gives an analysis for water, sugars, total nitrogen, protein, crude fiber, and ash of the seed of *Citrullus vulgaris* Schrader.)

King, J. and Lloyd, J. U. 1889

Cucurbita citrullus

American Dispensatory, 16th ed., p. 311

Gives description, habitat and uses.

Hooker, J. D. and Jackson, B. D. 1893

Citrullus vulgaris Schrader

Index Kewensis, v.1, pp. 136,550,664, and 665.

Gives the synonyms, with references, for *Citrullus vulgaris* Schrader.

(____________) 1896

(____________)

Nueva Farmacopoea Mexicana, 3rd ed., p.___ (Bruntz, L. and Jaloux, M., Plantes Officinales et Plants á Drogues Medicamenteuses, (1918), p. 210.)

(Bruntz and Jaloux list *Citrullus vulgaris* Schrader as being official in the 3rd edition of the Mexican Pharmacopoeia.)

Dragendorf, G. 1898

Citrullus vulgaris Schrader

Heilpflanzen, p. 649

Gives synonyms, composition, and habitat.

Wilwitsch, F. 1898

Colocynthis amarissima

Catalogue of the African Plants, v. 3, p. 397

Gives a botanical description of several African varieties.

Weinarowskaja, S. and Naumowa, S. 1902

(Technische Analyse des Öles aus Wassermelonensamen)

J. Russ. Phys.-Chem. Soc. *34*, p. 695 (Chem. Centralblatt, (1903), I, p. 41)

(Physical and chemical constants are given for watermelon seed oil.)

Wijs, J.J.A. 1903

Uber einige Unbekannte und Weniger Bekannte Öle

Zeit. f. Untersuch. der Nahr. u. Genussm., 6, p. 492

Gives physical constants of the oil of the seed.

Peckolt, T. 1904

Heil- und Nutz- Pflanzen Brasiliens

Ber. d. Deutsch. Pharm. Ges. 14, p. 172

In Brazil, the seed kernels are mixed with sugar and water and used as a substitute for almond emulsion.

Sociedad Farmaceutica de Mexico 1904

Sandia

Nueva Farmacopea Mexicana, 4th ed., p. 278

Gives synonyms, habitat, and use of the seed.

Hare, H. A., Caspari, C., and Rusby, H. H. 1909

Cucurbitaceae - The Gourd or Cucumber Family

National Standard Dispensatory, 2nd ed., p. 527

Constituents and properties of the family are similar to those of Bryonia, Colocynth and Elaterium.

Power, F. B. and Rogerson, H. 1910

Chemical Examination of Jalap

J. Am. Chem. Soc., 32, p. 80

Cucurbitol is a member of an homologous series of dihydric alcohols.

Power, F. B. and Salway, A. H. 1910

Chemical Examination of Watermelon Seed

J. Am. Chem. Soc., 32, p. 360.

A description of the procedure used in a detailed chemical analysis of the watermelon seed is given. The seed consisted mainly of fatty acids. During the investigation, "cucurbitol", believed to be a new dihydric alcohol, was isolated.

Heering, W. and Grimme, C. 1911

Untersuchungen uber die Weideverhaltnisse in Deutsch- Sudwestafrika

Arb. Deutsch. Landw. Ges. no. 197, p. 1 (Chem. Centralblatt, (1911), II, p. 1743).

Gives constants on watermelon seed oil and on the mixture of fatty acids obtained upon saponification of the oil.

Power, F. B. and Moore, C. W. 1911

The Constituents of Bryonia Root

Pharm. J. and Pharmacist, 86, p. 626

Cucurbitol is a member of an homologous series of dihydric alcohols of which bryonol, ipurganol, and grindelol are members and have the general formula $C_nH_{2n-8}O_4$.

Mitlacher, W. 1912

<u>Citrullus vulgaris</u> Schrader

Die Offizinellen Pflanzen und Drogen, p. 93

Lists the above plant as official in the third edition of the Portuguese Pharmacopoeia.

Power, F. B. 1912

Constituents of Some Cucurbitaceous Plants

Am. J. Pharm. <u>84</u>, p. 145

Cucurbitaceous plants are characterized by the occurence of acrid or purgative principles and for this reason considerable numbers have been used as medicinal agents.

A summary of phytochemical investigations of cucurbitaceous plants.

Power, F. B. and Salway, A. H. 1913

The Identification of Ipuranol and Some Allied Compounds as Phytosterol Glucosides

J. Chem. Soc. <u>103</u>, pp. 339 and 1022

It was determined that cucurbitol was a phytosterol glycoside.

Piermerts, J. 1917

L'huile de Sole

Bull. Sci. Pharmacol. <u>24</u>, p. 204

Gives physical and chemical constants for watermelon seed oil.

Bruntz, L. and Jaloux, M. 1918

Citrullus vulgaris Schrader

Plantes Officinales et Plants à Drogues Medicamenteuses, p. 210

Lists the National Pharmacopoeias in which the above plant was official prior to 1918.

Sturtevant, E. 1919

Citrullus vulgaris

Notes on Edible Plants, p. 169

Gives description and habitat of the above plant.

Cogniaux, A. and Harms. H. 1924

Citrullus

Das Pflanzenreich (A. Engler), IV, 275, v.2, p. 102

Gives description and synonyms of *Citrullus vulgaris* Schrader.

(________________) 1925

The Seed of Citrullus Vulgaris as a Source of Oil

Bull. Imp. Inst. *23*, p. 149

Gives physical and chemical constants of watermelon seed oil obtained from several different regions of Africa.

Barksdale, I. S. 1926

Studies on the Blood Pressure Lowering Principle in the Seed of the Watermelon

Am. J. Med. Sci., 171, p. 111

A detailed procedure is given for the extraction of a substance from watermelon seed, supposedly a saponin, which tends to lower blood pressure.

The effect of the principle extracted, "Cucurbitin", on the human capillaries, capillaries of the frog, blood pressure of normal dogs and on arterial hypertension in man are briefly discussed.

Nadkarni, K. M. 1927

Citrullus vulgaris

The Indian Materia Medica, p. 205

Gives uses and habitat.

Althausen, T. T. and Kerr, W. T. 1929

Watermelon-Seed Extract in the Treatment of Hypertension

Am. J. Med. Sci. 178, p. 470

Clinical experiments showed cucurbitin to be effective in lowering the blood pressure in the majority of cases.

Beljajew, N. 1929

(Oel der Wassermelonensamen)

Oel- u. Fett- Ind., no.7, p. 38 (Chem. Centralblatt, (1929), II, p. 2532.

(Gives constants of watermelon seed oil.)

Iwanow, S. and Kurotschkina, N. 1929

(Die Kaufmannsche Rhodanzahl und die Bestimmung der Quantitativen Zusammensetzung Einiger Ole Russlands)

Oel- u. Fett- Ind. no.12, p. 45 (Chem. Centralblatt, (1930), I, p. 2333.)

(Gives iodine and thiocyanogen values of watermelon seed oil.)

Cargill, S. L. and Rudy, A. 1931

The Value of Cucurbitin in the Treatment of Arterial Hypertension

Am. J. Med. Sci. <u>181</u>, p. 639

Only a small percentage of patients treated with cucurbitin showed a great reduction in systolic blood pressure and the effects lasted only a short time.

Wehmer, C. 1931

<u>Citrullus vulgaris</u> Schrader

Die Pflanzenstoffe, 2nd ed., v.2, p. 1197

Gives composition of the seed with references.

Jono, Y. 1931

(The Enzyme Content of Dormant and Germanating Seeds)

Acta Schol. Med. Univ. Imp. Kioto, <u>13</u>, p. 211 (Chem. Abstr., <u>25</u>, (1931), p. 3030.)

(Catalase, nuclease, and lipase are present in watermelon seed.)

Gutman, J. 1934

Citrin

Modern Drug Encyclopedia and Therapeutic Guide, 1st ed., p. 67

Describes citrin and its action.

Watanabe, K. 1936

(Citricodehydrase in the Seeds of Watermelons)

J. Agr. Chem. Soc. Japan, 12, p. 745 (Chem. Abstr. 31, (1937), p. 1461.)

(A description of the extraction of citricodehydrase from watermelon seed.)

Damodaran, M. and Sivaramakrishnan, P. M. 1937

New Sources of Urease for Determination of Urea

Biochem. J. 31, p. 104

Watermelon seeds are an excellent source of urease.

Cabbab, A. G. and Soliven, F. A. 1938

The Proximate Physical and Chemical Composition of 26 Species of Citrus and 12 non-Citrus Fruits Grown in the Philippines

Philippine Agriculturist 26, p. 644

Gives an analysis for water, sugars, protein and ash of watermelon seed.

Hylander, C. J. 1939

Watermelon

The World of Plant Life, p. 467

Describes the plant and gives its origin as Africa.

Krishnan, P. S. and Krishnaswamy, T. K. 1939

Protein and Other Nitrogenous Constituents of Watermelon Seeds

Biochem. J. 33, p. 1284

A detailed chemical analysis of the nitrogenous constituents of watermelon seeds. They have been shown to be made up of glutelin, globulin, water soluble protein, proteoses and peptones.

Nolte, A. J. and Loesecke, H. W. von 1939

Characteristics and Composition of Watermelon Seed Oil

J. Am. Chem. Soc. 61, p. 889

Gives physical and chemical constants and composition of the oil.

Merck and Company, Inc. 1940

Cucurbitol- Glucoside

Merck Index, 5th ed., p. 175

Gives physical constants of cucurbitol.

II. A STUDY OF THE REACTION OF THEOPHYLLINE WITH BARBITURATES

INTRODUCTION

A study of the reaction of theophylline with barbiturates was undertaken when it was found that a prescription containing sodium phenobarbital and aminophylline in aqueous solution produced a precipitate on standing for a few hours. The melting point of the precipitate indicated that it was neither theophylline nor phenobarbital, nor was it any of the other ingredients of the prescription. A search of the literature revealed that a complex of aminophylline and phenobarbital had been prepared. However, this complex, unlike the substance precipitated above had no definite melting point and was quite water soluble. The interest stimulated by this incompatibility lead to the presence chemical investigation of the product and to a survey of the literature on the reaction products of barbiturates and nitrogenous compounds.

SURVEY OF THE LITERATURE

In 1910, Knoll and Co.(1) described and patented a process for preparing the reaction product of diethyl barbituric acid and codeine or of sodium diethyl barbituric acid and codeine hydrochloride.

In the course of the next 25 years a number of other companies and individuals have extended the reaction to other alkaloids and other nitrogenous compounds with other 5,5-dialkyl and 5-alkyl, 5-aryl barbituric acids, the reactants combining in a definite molecular ratio to form molecular compounds. These compounds were prepared by fusing the reactants or by dissolving the reactants in a suitable organic solvent from which the product crystallized.

Patents have also been granted for molecular compounds prepared by the reaction between hydrochlorides of the alkaloids and the sodium salts of the 5,5 disubstituted barbituric acids. These compounds were prepared by dissolving the reactants in water and allowing the products to crystallise.

According to patents (3,19,21,22,23,24,28), the same molecular compound may be prepared by several different methods. As long as the same ratio of reactants is retained, identical molecular compounds may be prepared by crystallisation from different organic solvents, by fusion of the reactants, and by double decomposition of the salts of the reactants in aqueous solution. On the other hand, more than one molecular compound may be formed from the same reactants(2,6). Compounds may be formed containing one molecule of the alkaloid and one molecule of the barbiturate, one molecule of the alkaloid and two molecules of the barbiturate, or two molecules of the alkaloid and one molecule of the barbiturate, depending upon the ratio of reactants used.

These molecular compounds are quite stable to air and to separation by solvent extraction. However, most of them are easily decomposed into their components by heating with dilute mineral acids(1,20,23).

The patents claim for these compounds a combination and intensification of the physiological action of each component.

The method of preparing the compounds may be grouped into three types, although minor variations such as the concentration of the solutions used, complete removal of the reaction solvent before crystallization, etc. exist for each specific compound.

Method I — Fusion

The barbiturate and the alkaloid are mixed intimately in the proper molecular ratio. The mixture is heated in an oil bath to the fusion point and held there until completely melted. The melt is poured into a mortar and after solidification, is ground and recrystallized from a suitable solvent.

Method II — Crystallization from an Organic Solvent

The barbiturate and the alkaloid are dissolved, either together or separately, in a suitable solvent in the proper molecular ratio and heated. On cooling, the product precipitates and is crystallized from fresh solvent.

Method III — Crystallization from Water

The sodium salt of the barbiturate and the alkaloid hydrochloride are dissolved in water in the proper molecular ratio. On standing for a time, or on heating and cooling the product precipitates and is crystallized from fresh solvent.

The reactions which have been carried out are tabulated below.

TABLE I

Barbiturate	Mols	Alkaloid or Nitrogen Compound	Mols	Method	M.P. of Product	Reference
β-bromallyl isopropyl	1	1-phenyl, 2,3-dimethyl, 4-isopropyl, 5-pyrazolon	1	I,II(benzene)	128	3
Cyclohexenyl ethyl	1	6,8 dimethoxy quinoline	1	III	*	4
Diallyl	1	Allyl morphine base	1	II(Methanol)	**	5
Diallyl	1	Cinchonine	1	II(Benzene)	217	6
Diallyl	1	Codeine	1	II(dil. Alcohol)	**	7
Diallyl	1	Diacetyl morphine	1	II(Methanol)	186	8
Diallyl	1	Dihydrocodeine	1	II(Dil. Alcohol)	95	9
Diallyl	1	Dihydromorphine	1	II(Dil. Alcohol)	135	10
Diallyl	1	Ethylhydrocupreine	1	II(Alcohol)	135	11
Diallyl	**	Ethyl quinine carboxylate	**	**	79	12
Diallyl	1	Hydroquinine	1	I	**	9
Diallyl	1	Morphine	1	**	258	13
Diallyl	1	Quinine	1	**	128	14
Diallyl	1	Sparteine	1	**	139-140	15
Diallyl	2	Sparteine	1	**	140	15
Di-n-butyl	1	Pyramidon	2	II(Alcohol)	94-96	16

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*Not given in original article

**Original not available and information not available in abstract reference.

TABLE I (continued)

Barbiturate	Mols	Alkaloid or Nitrogen Compound	Mols	Method	M.P. of Product	Reference
Diethyl	1	α-aminopyridine	2	II(Dil.Alcohol) III	82	17
Diethyl	1	α-bromisovaleryl urea	1	I	130-132	2
Diethyl	1	α-bromisolvaleryl urea	2	I	117	2
Diethyl	2	α-bromisolvaleryl urea	1	I	164-168	2
Diethyl	1	Bromodiethyl acetyl urea	1	I	96	2
Diethyl	2	Bromodiethyl acetyl urea	1	I	98	2
Diethyl	1	Codeine	1	II(Dil.Alcohol)	85	1
Diethyl	1	2-ethoxy,5-acetamide pyridine	2	I	112	17
Diethyl	1	Ethyl p-amino benzoate	2	**	119	18
Sodium Diethyl	1	Ethylhydrocupreine sulfate	1	II(Alcohol)	161-162	19
Diethyl	1	Hydroquinine	1	I, II(Acetone, Chloroform),III	110	19
Diethyl	1	4-hydroxy, 5-nitro pyridine	1	II(Water)	245	17
Diethyl	1	N-methyl, α-pyridone	1	II(Benzene)	120	17
Diethyl	1	Pyramidon	1	II(Acetone)	115	21
Diethyl	1	Pyramidon	2	II(Alcohol)	95-97	20
Diethyl	1	1-phenyl,2,3-dimethyl,4-isopropyl,5-pyrazolon	1	II(80% Methanol)	138-139	3

TABLE I (continued)

Barbiturate	Mols	Alkaloid or Nitrogen Compound	Mols	Method	M.P. of Product	Reference
Diethyl	1	Procaine	2	**	140	18
Diethyl	1	Quinine	1	I,II (Acetone, Chloroform, Alcohol), III	136	22,23,24
Diethyl	1	Sparteine	1	**	138	18
Dipropyl	**	Codeine	**	I	89.5	25
Dipropyl	1	Ethylhydrocupreine	1	II(Alcohol)	127-128	19
Dipropyl	1	Papaverine	1	I	120	25
Dipropyl	1	Propylhydrocupreine	1	II(Alcohol)	103	19
Dipropyl	1	Quinine	1	II(Acetone, Chloroform, Alcohol)	127-128	22,23,26
Ethyl allyl	1	Quinine	1	II(Alcohol)	90	27
Ethyl-n-butyl	1	Pyramidon	2	II(Alcohol)	88-90	16
Ethyl-sec.-butyl	1	Pyramidon	2	II(Alcohol)	84-86	16
Ethyl isoamyl	1	Pyramidon	1	II(Alcohol)	90-94	16
Ethyl isopropyl	1	Sparteine	1	**	140	15
Isopropyl allyl	1	Pyramidon	1	I,II (Acetone, Alcohol)	93	21,28
Isopropyl allyl	1	1-phenyl,2,3-dimethyl,4-isopropyl,5-pyrazolon	1	I,II (Benzene)	128	3
Isopropyl allyl	2	Sparteine	1	**	122	15
Phenyl ethyl	1	α-bromisovaleryl urea	1	I	131	2

TABLE I (continued)

Barbiturate	Mols	Alkaloid or Nitrogen Compound	Mols	Method	M.P. of Product	Reference
Phenyl ethyl	1	α-bromisovaleryl urea	2	I	119	2
Phenyl ethyl	1	Bromodiethyl acetyl urea	1	I	125-128	2
Phenyl ethyl	2	Bromodiethyl acetyl urea	1	I	148-150	2
Phenyl ethyl	1	6,8 diethoxy quinoline	1	III	133	4
Phenyl ethyl	1	Ethyl-p-amino benzoate	2	I	**	18
Phenyl ethyl	1	Hydroquinine	1	II(Alcohol)	165	29
Phenyl ethyl	1	N-methyl pyridone	1	III	123	17
Phenyl ethyl	1	Papaverine	1	II(Alcohol)	145-146	17
Phenyl ethyl	1	1-phenyl, 2,3-dimethyl, 4-isopropyl, 5-pyrazolon	1	II(Acetone)	161	3,21
Phenyl ethyl	1	Procaine	2	**	155	18
Phenyl ethyl	1	Pyramidon	1	I	132	30
Phenyl ethyl	1	Quinine	1	II(Alcohol)	182-183	29
Phenyl ethyl	2	Sarcosine anhyd.	1	I	127	30
Phenyl ethyl	1	Sparteine	1	**	151	15
Phenyl ethyl	2	Sparteine	1	**	157	15

A patent was granted to Grüter(31) in 1938 for a molecular compound containing phenobarbital and aminophylline. This compound was prepared by causing one molecule of theophylline to react with one molecule or slightly more of ethylenediamine and varying proportions up to 0.2 molecule of phenobarbital either in the presence or absence of solvent. In contrast to the molecular compounds of barbiturates just discussed, this compound was easily soluble in cold water and had no definite melting point.

Redemann and Niemann(32) prepared 71 compounds of nitrobarbituric acid with various simple primary, secondary, and tertiary amines, simple heterocyclic bases, alkanolamines, alkaloids, amino acids, and proteinogenic amines. Nitrobarbituric acid acts as a strong monobasic acid(33). For this reason the compounds formed were salts and not molecular compounds. No salt was reported with theophylline or theobromine and nitrobarbituric acid but a salt of caffeine, a homologue of theophylline, was prepared.

REFERENCES*

(1) German Patent 239,313, Sept. 18, 1911

(2) British Patent 447,245, May 14, 1936

(3) U. S. Patent 2,014,866, Sept. 17, 1935

(4) British Patent 306,905, May 26, 1930

(5) Swiss Patent 77,197

(6) Swiss Patent 77,194

(7) Swiss Patent 77,199

(8) Swiss Patent 77,198

(9) Swiss Patent 77,201

(10) Swiss Patent 77,200

(11) Swiss Patent 77,195

(12) Swiss Patent 77,196

(13) Swiss Patent 76,997, March 1, 1918

(14) Swiss Patent 76,996, March 1, 1918

(15) German Patent 586,245, Oct. 19, 1933

(16) U. S. Patent 1,478,463, Dec. 25, 1923

(17) British Patent 416,273, Sept. 13, 1934

(18) Lusignani, G., Ann. Chim. Farm., p. 64, Dec. 1939

(19) German Patent 291,421, Mar. 20, 1916

(20) U. S. Patent 1,459,347, June 19, 1923

(21) U. S. Patent 1,798,556, Mar. 21, 1931

(22) German Patent 249,908, July 8, 1912

(23) U. S. Patent 1,185,637, June 16, 1916

(24) Swiss Patent 62,732, Feb. 4, 1913

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* The original German and Swiss Patents and reference(18) were not available. Information from these references was taken from abstract material (see Bibliography).

(25) German Patent 631,097, June 12, 1936

(26) Swiss Patent 62,733, Feb. 4, 1913

(27) Swiss Patent 77,196

(28) U. S. Patent 1,494,127, May 13, 1924

(29) German Patent 247,188, April 29, 1912

(30) Pfeiffer, P. and Seydel, R., Z. Physiol. Chem. <u>178</u>, p. 1, (1928)

(31) U. S. Patent 2,017,279, Oct. 15, 1935

(32) Redemann, C. E. and Niemann, C., J. Am. Chem. Soc., <u>62</u>, p. 590 (1940)

(33) Trübsach, P. A., Z. physik. Chem. <u>16</u>, p. 718, (1895).

EXPERIMENTAL

Isolation of the Material from the Prescription

The following prescription was received in a Madison Pharmacy.

Phenobarbital soluble		gr. xvi
Aminophylline (Theophylline with Ethylenediamine)		gr. 90
Aq. Peppermint	qs	fl.oz. viii

Immediately after it was compounded, the prescription was a clear, colorless solution. A few hours later, the prescription was returned to the pharmacy containing a white crystalline precipitate. In an investigation of the precipitate, the prescription was compounded, dissolving both solids separately and mixing the solutions. The solution remained clear for about 20 minutes at which time a slight cloudiness was observed. After about 30 minutes, the solution was quite cloudy and contained some crystalline precipitate. In an hour's time, the solution was almost clear again and contained a larger amount of a white crystalline precipitate. The precipitate was removed by filtration on a suction filter and dried. The melting point was found to be 249 to 251°C. (corrected).* The melting point of phenobarbital is 174 to 177°C., and of theophylline 269 to 272°C. The unknown was then recrystallized from hot water to a constant melting point, 250.7 to 251.7°C. (corrected). A mixed melting point of the unknown with theophylline showed a depression, giving a melting point of 244.6 to 250.7°C. (corrected). That the unknown

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* The thermometer used was standardized against a Bureau of Standards set of Anschütz thermometers.

could be recrystallized to a constant melting point and that it showed a depression of the melting point when mixed with theophylline is evidence to indicate that some reaction had occurred in the prescription.

Composition and Properties

In order to determine which substances were involved in the reaction, one gram of theophylline was dissolved in 240 cc. of water and then 0.2 grams of sodium phenobarbital added and dissolved. On standing, the solution exhibited the same phenomena as the prescription and at approximately the same time intervals. The precipitate was removed by filtration and recrystallized from hot water to a constant melting point of 250.7 to 251.7°C. (corrected). A mixed melting point of this material with theophylline showed a depression (244.1 to 250.2°C., corrected). A mixed melting point of the product from the prescription with the product from the above reaction showed no depression. The formation of the unknown substance in the absence of ethylenediamine eliminates the latter as one of the materials involved in the reaction.

In order to determine if the sodium ion played any part in the reaction, 1.0 gram of theophylline was dissolved in 50 cc. of 95 per cent alcohol with the aid of heat. When all of the theophylline was dissolved, 0.2 gram of phenobarbital was dissolved in the theophylline solution. On cooling, a white crystalline precipitate formed which was removed by filtration and recrystallized from hot alcohol to a constant melting point of 250.7 to 251.7°C. (corrected). A mixed melting point with theophylline gave a depression (243.1 to

250.2°C., corrected), and a mixed melting point with the product from the prescription gave no depression.

The solubility of the unknown substance in a variety of solvents at 25°C. was determined in a qualitative manner. The results are recorded below:

Water -- slightly soluble, soluble in boiling water.

Alcohol -- slightly soluble, soluble in boiling alcohol.

Ether -- Very slightly soluble.

Acetone -- very slightly soluble.

Carbon tetrachloride -- practically insoluble.

Chloroform -- practically insoluble.

Benzene -- very slightly soluble.

Glycerine -- practically insoluble.

Ethyl acetate -- slightly soluble.

Anhydrous dioxane -- soluble.

Five per cent sodium hydroxide solution -- soluble.

Five per cent hydrochloric acid solution -- slightly soluble.

A sample of the material prepared in alcohol solution was dried overnight at 145°C. <u>in vacuo</u> and analysed for carbon, hydrogen, and nitrogen. The analytical results indicate an atomic ratio of $C_{3.83}H_{4.23}N_{1.41}O$, corresponding to a compound of the empirical formula of $C_{26}H_{30}N_{10}O_7$. From a comparison with the figures for theophylline and phenobarbital, it is evident that the unknown is neither of the starting materials. The composition, however, compares favorably with the theoretical composition of a molecular compound of 2 molecules of theophylline and 1 molecule of phenobarbital ($C_{26}H_{28}N_{10}O_7$).

Table II

		Per cent Carbon*	Per cent Hydrogen*	Per cent Nitrogen**	Per cent Oxygen (by difference)
Unknown	(1)	52.8	4.98	23.27	18.95
	(2)	52.8	5.04	23.18	18.98
	Average	52.8	5.01	23.23	18.96
2 Theophylline-1 phenobarbital		52.8	4.73	23.6	18.87
Theophylline		46.66	4.47	31.11	17.76
Phenobarbital		62.09	5.17	12.05	20.69

*Macro
**Macro Kjeldahl

The composition of the product was further verified by the following experiments carried out on a quantitative basis. The reactants were mixed in a ratio of 1 mole to 1 mole by dissolving 1.000 gram of phenobarbital in 50 cc. of a hot alcoholic solution of 0.775 gram of theophylline. The solution was allowed to stand 15 hours, stirring once after 2 hours. Crystals began to form within 15 minutes. If the compounds reacted 1 mole to 1 mole, there should have been formed theoretically 1.775 grams of the product. If they reacted in the ratio of 2 molecules of theophylline to 1 molecule of phenobarbital, the theoretical yield would be 1.275 grams of product with 0.5 gram of unreacted phenobarbital.

After 15 hours the crystals were removed by filtration and the mother liquid repeatedly concentrated and the precipitate removed until no more product crystallized from the solution. The solution was then evaporated to dryness and the residue extracted with boiling ether to dissolve any excess phenobarbital. There remained a small amount of ether insoluble material. The ether solution was evaporated to dryness leaving a residue of phenobarbital. The weight and melting point was determined on each crystal crop and each residue. The weight of product and of recovered phenobarbital compares favorably with that required for a ratio of 2 molecules of theophylline to 1 molecule of phenobarbital as may be seen from Table III.

A second solution was prepared as above, mixing the reactants in a ratio of 1 molecule of phenobarbital to 2 molecules of theophylline by dissolving 1.000 gram of phenobarbital in 50 cc. of a hot alcoholic solution of 1.550 grams of theophylline. If the reactants reacted in the molecular ratio of 1:1, the theoretical yield would be 1.775 grams of product with 0.775 gram of unreacted theophylline. If they reacted in a molecular ratio of 1 molecule of phenobarbital to 2 molecules of theophylline, the theoretical yield of product would be 2.550 grams. The weight of product recovered compares favorably with that required for a ratio of 2 molecules of theophylline to 1 molecule of phenobarbital as may be seen in Table III.

In each experiment the fractions melting above 240°C. were combined and recrystallized to a constant melting point of 250.7 to 251.7°C. (corrected). A mixed melting point of this material with the analysed material gave no depression. Fractions IV and V in experiments I and II respectively were recrystallized from dilute alcohol to a constant melting point of 174 to 176°C. (corrected). A mixed melting point of this material with phenobarbital gave no depression. The yield of product in each experiment and the recovery of phenobarbital are in accord with the theoretical that would be obtained by the formation of a molecular compound of 1 molecule of phenobarbital and 2 molecules of theophylline.

These experiments also show that 2 molecules of theophylline react with 1 molecule of phenobarbital regardless of the proportion of the reactants.

Table III

	Experiment I		Experiment II	
Fraction	Weight	M.P.	Weight	M.P.
I	0.967	245-250	2.056	246-250
II	0.167	248-250	0.244	248-250
III	0.029*	163-220	0.021	247-248
IV	0.393**	168-172	0.072*	246-248
V	----	----	0.030**	169-171
Product Obtained	1.163 (91.6%)		2.393 (93.5%)	
Rec. Phenobarbital	0.393 (79.0%)		0.030	

*Ether insoluble residue
**Ether soluble residue

The evidence of these two experiments together with the elemental analysis and the constant melting point indicate that phenobarbital and theophylline react to form a product containing 2 molecules of theophylline for each molecule of phenobarbital. Since the product contains 2 molecules of theophylline for each molecule of phenobarbital and since phenobarbital is a weak monobasic acid ($K_{ion.} = 3.89 \times 10^{-8}$), the indications are that a molecular compound has been formed rather than a salt. This product, which will hereafter be referred to as "theophylline - phenobarbital", differs from that patented by Gruter(1) in the ratio of reactants, absence of ethylenediamine, solubility, and melting point.

When an alkaline solution of theophylline-phenobarbital was acidulated with either concentrated or dilute hydrochloric acid, the molecular compound precipitated provided there was not sufficient water present to hold it in solution. It was then of interest to determine what effect the addition of acid would have on the original prescription. The prescription was prepared as before and acidified with concentrated hydrochloric acid immediately after mixing. Theophylline-phenobarbital precipitated instantaneously.

Treatment with Sodium Hydroxide

Since the molecular compound was soluble in sodium hydroxide, it was of interest to determine if a stable sodium salt of the molecular compound was obtained. Assuming that each component of the molecular compound was free to form a sodium salt, 1.000 gram of theophylline-phenobarbital was dissolved in sufficient 1.000 normal sodium hydroxide to furnish 3 molecules of sodium hydroxide for each molecule of theophylline-phenobarbital. To this solution was added 25 cc. of 95 per cent alcohol. The white precipitate which formed was removed by filtration and dried to constant weight at 105°C. The precipitate weighed 0.554 grams. It was soluble in water, but a concentrated aqueous solution when acidified with concentrated hydrochloric acid did not yield a precipitate of either phenobarbital or the molecular compound. This latter test indicated that no sodium phenobarbital was present in the precipitate. The precipitate was therefore probably sodium theophyllinate. In the course of 2 hours, 2 additional crops of precipitate weighing 0.032 and 0.054 gram were filtered from the aqueous alcohol solution. Each of the last 2 precipitates gave the same tests as the first.

The 3 portions of the material believed to be sodium theophyllinate and totaling 0.650 gram were combined and recrystallized twice from dilute alcohol. A portion of the recrystallized material was dried at 110°C. for 2 hours and assayed for sodium. The results are listed below:

Sample 1.	10.40 per cent
Sample 2.	9.88 per cent
Sample 3.	10.15 per cent
Theoretical for sodium theophyllinate	11.40 per cent

These low and indecisive results are possibly due to varying amounts of water of crystallization which are held quite tenaciously(2). Fifty milligrams of the sodium salt lost only 2 milligrams after drying at 125°C. <u>in vacuo</u> overnight.

A second portion of the recrystallized material believed to be sodium theophyllinate was dissolved in water and acidified with concentrated hydrochloric acid. The solution was evaporated and dried at 105°C. for 30 minutes to remove the excess hydrochloric acid and to decompose the theophylline hydrochloride to theophylline. The residue was then extracted with boiling alcohol to dissolve the theophylline from the sodium chloride. On cooling the alcoholic solution, a white crystalline precipitate formed which, when filtered and dried, melted at 269 to 271°C. (corrected). A mixed melting point with theophylline gave no depression.

The aqueous alcohol filtrate from the sodium theophyllinate was acidified with concentrated hydrochloric acid and evaporated to dryness. The residue which weighed 0.479 gram was extracted with boiling ether and the ether solution evaporated on a steam bath. The ether extract weighed 0.262 gram and melted at 168 to 171°C. (corrected). After 2 crystallizations from dilute alcohol, the melting point was 174 to 176°C. (corrected). A mixed melting point with phenobarbital showed no depression.

The ether insoluble portion of the residue weighed 0.218 gram and melted at 249 to 250°C. (corrected). On recrystallization from alcohol, the substance melted at 250.7 to 251.7°C. (corrected). A mixed melting point with theophylline-phenobarbital showed no depression. The presence of the theophylline-phenobarbital in the residue is explained by the fact that not all of the sodium theophyllinate was precipitated by the alcohol, and as has been pointed out before, in acid medium the molecular compound forms.

The theoretical and actual yields of the products obtained from the treatment of 1.00 gram of theophylline-phenobarbital with 1.000 normal sodium hydroxide are tabulated below.

	Actual	Theoretical (based on 2 theoph.& 1 phenobarb.)
Sodium theophyllinate	0.650 gram	0.680 gram
Phenobarbital	0.262	0.392
Theophylline-Phenobarbital	0.218	----
Total	1.130	1.072

A comparison of the yields gives additional evidence of a combination in the ratio of 2 molecules of theophylline to 1 molecule of phenobarbital. The fact that the actual total recovery is greater than the theoretical is believed due to the formation of hydrated sodium theophyllinate.

Treatment with 10 per cent Hydrochloric Acid

A 0.205 gram sample of theophylline-phenobarbital and 15 cc. of 10 per cent hydrochloric acid were placed in a 50 cc. round-bottomed flask and heated on a steam bath under a reflux condenser. During the heating, complete solution was effected. After heating for 1 hour, the solution was removed from the steam bath and allowed to cool spontaneously during 45 minutes. A white crystalline precipitate formed. The crystals, when filtered off and dried, weighed 0.055 gram and melted at 174 to 176°C. (corrected). A mixed melting point with phenobarbital showed no depression. The solution was concentrated to one-third its volume over a flame and upon cooling, a second crop of crystals was obtained. The crystals when filtered off and dried, weighed 0.016 gram and melted at 173 to 175°C. (corrected). A mixed melting with phenobarbital showed no depression.

The solution was then evaporated to dryness on a steam bath and the residue dried at 105°C. for 30 minutes to remove the hydrochloric acid and to decompose the theophylline hydrochloride to theophylline. The residue, which weighed 0.135 gram melted at 266 to 269°C. (corrected). One crystallization from alcohol gave a product melting at 269 to 272°C. (corrected). A mixed melting point with theophylline showed no depression.

The theoretical and actual yields of the products obtained from the treatment of 0.205 gram of theophylline-phenobarbital with 10 per cent hydrochloric acid are tabulated below.

	Actual	Theoretical (based on 2 theoph.& 1 phenobarb.)
Phenobarbital	0.071	0.080
Theophylline	0.125	0.125
Total	0.196	0.205

A comparison of these yields gives further evidence that the molecular compound contains 2 molecules of theophylline and 1 molecule of phenobarbital.

On standing in cold 10 per cent hydrochloric acid, theophylline-phenobarbital gave no evidence of decomposition.

Treatment with Silver Nitrate

Since theophylline forms an insoluble silver salt and phenobarbital forms no silver salt at all, it was of interest to determine what effect silver nitrate would have on the molecular compound.

A sample of 1.00 gram of theophylline-phenobarbital was dissolved in hot water and an aqueous solution of silver nitrate added. A white gelatinous precipitate formed which appeared to be composed of two types of particles. The solution was filtered with suction and the precipitate washed free of silver nitrate. Considerable difficulty was experienced in washing the precipitate because of its gelatinous character. The precipitate, believed to be a mixture of phenobarbital and of silver theophyllinate, was dried, powdered, and extracted completely with ether to remove any phenobarbital. The residue, presumably silver theophyllinate, after drying overnight at 110°C. weighed 0.951 gram.

A portion of the silver salt was assayed for silver. Two samples of silver theophyllinate were ignited to free silver and the per cent of silver determined with the following results:

	Per cent Silver
Sample 1.	37.39
Sample 2.	37.45
Average	37.42
Theoretical for Silver Theophyllinate	37.46

The results compare favorably with the theoretical per cent for silver theophyllinate.

A second portion of the silver theophyllinate was suspended in alcohol and treated with hydrochloric acid. The alcoholic solution was warmed on a steam bath effecting solution of theophylline hydrochloride and coagulating silver chloride. The precipitate of silver chloride was filtered off and the solution concentrated to about one-third its volume, filtered and cooled. The white needles which crystallized out were removed by filtration, dried and found to melt at 267 to 271°C. (corrected). One crystallization from alcohol gave a product melting at 269 to 272°C. (corrected). A mixed melting point with theophylline showed no depression.

The filtrate from the silver theophyllinate and the washings were combined, acidified with hydrochloric acid, and extracted 3 times with ether. These extractions were combined with the ether extractions of the dried silver theophyllinate, evaporated, and the residue dried at 110°C. for 30 minutes. The residue, which had a slight yellow color, weighed 0.302 gram, and after 2 crystallizations from dilute alcohol melted at 173 to 175°C. (corrected). A mixed melting point with phenobarbital showed no depression.

The theoretical yields and actual yields of the products obtained from the treatment of theophylline-phenobarbital with silver nitrate are tabulated below:

	Actual	Theoretical (based on 2 theoph.& 1 phenobarb.)
Silver Theophyllinate	0.951	0.973
Phenobarbital	0.302	0.392
Total	1.253	1.365

The low yield of phenobarbital is believed due to oxidation of some of the phenobarbital by nitric acid. The nitric acid was formed on acidification of the filtrate from the silver theophyllinate and was not washed out of the ether. This would also account for the color of the crude phenobarbital residue.

Although phenobarbital is quite soluble in most of the organic solvents listed on page 103 and theophylline is not, theophylline-phenobarbital did not decompose into its components when treated with these solvents.

Experiments with Other Barbiturates and Theophylline

Since it has been reported in the literature that the same nitrogenous compound, for example, quinine, will form molecular compounds with different 5,5-disubstituted barbituric acids, it was of interest to determine if molecular compounds could be formed with theophylline and 5,5-disubstituted barbituric acids other than phenobarbital.

Preparation of 1,3 Dimethyl Phenobarbital

Thirteen grams of phenobarbital was dissolved in 2 equivalents (5.6 cc.) of normal sodium hydroxide in a 3-necked flask, fitted with a mechanical stirrer and a dropping funnel, and immersed in an ice-salt bath. A 10 per cent excess over 2 equivalents (11.7 cc.) of freshly distilled dimethyl sulfate was added dropwise with constant stirring. After addition of the dimethyl sulfate was complete, stirring was continued for 3 hours during which crude dimethyl phenobarbital was precipitated. The precipitate was filtered off, washed with water until the washings were neutral to litmus, and dried. The crude yield was 14.6 grams (99.2 per cent). Stuckey(3) obtained a 70 per cent yield using exactly 2 equivalents of dimethyl sulfate. Possibly the 10 per cent excess of dimethyl sulfate produced this better yield.

Preliminary Treatment of Commercial Samples

As indicated in Table IV, most of the 5,5-disubstituted barbituric acids were commercial products. These samples consisted of the 5,5-disubstituted barbituric acids or salts of 5,5-disubstituted barbituric acids in powder, capsule, or tablet form. The barbituric acid powders required no treatment. Capsules of barbituric acids were emptied and the powder extracted with ether and any diluent filtered off. Capsules of soluble salts of barbituric acids were emptied, the powder treated with warm water, and the solution filtered to remove diluents. The filtrate was acidified with hydrochloric acid and extracted with ether. Tablets were crushed and the powder treated as that of capsules. If the salts were water insoluble, the powder was suspended in water, acidified with hydrochloric acid, extracted with ether and any diluent filtered off.

Disubstituted barbituric acids prepared in this way had melting points within 5°C. of those reported for the respective compounds.

Reaction Conditions

The following experiments were carried out on a quantitative basis. A weighed amount of the 5,5-disubstituted barbituric acid was dissolved in 80 cc. of a hot alcoholic solution containing enough theophylline to give a molar ratio of 2 theophylline to 1 disubstituted barbituric acid. On cooling the solution, crystals separated and were removed by filtration. The mother liquor was repeatedly concentrated and the precipitate removed until no more crystals separated from solution. The remaining solution was evaporated to dryness. If the barbiturate were easily soluble in ether, the residue was extracted with boiling ether to remove the barbiturate. If the barbiturate were insoluble or slightly soluble in ether, the residue was extracted with cold 10 per cent hydrochloric acid to remove the theophylline. In either case, the extract was evaporated to dryness to recover the barbiturate or theophylline. The weight and melting point were determined on each crystal crop and each residue.

Since it had been found that phenobarbital would react with theophylline in a ratio of 2 molecules of theophylline to 1 molecule of phenobarbital, regardless of the proportions of the reactants, some of the experiments (indicated on Table IV by *) were carried out in a molar ratio of 1:1 in order to conserve materials.

In Table IV, it can be seen from the percentage recovery of the reactants and the melting points of the residues that no reactions took place. Recrystallization of the fractions gave products melting at the melting point of theophylline (269-271°C., corrected) or at the melting point of the respective barbiturate. Mixed melting points with the proper constituent gave no depression.

TABLE IV

5,5-Disubstituted Barbituric Acid	M.P.	Wt.	Wt. of Theo.	Recovered Theophylline			Recovered Barbiturate		
				Wt.	%	M.P. Range**	Wt.	%	M.P. Range
Diethyl(a,b) (Barbital)	188-191	1.0	1.946	1.728	89.0	267-269	0.972	97.2	180-186
Ethyl isopropyl*(b) (Ipral)	197-198	0.5	0.402	0.345	86.0	268-272	0.476	95.0	195-197
Ethyl n-butyl*(b) (Neonal)	127-128	0.5	0.424	0.376	88.8	266-271	0.490	98.0	120-124
Ethyl 1-methyl butyl(b) (Pentobarbital)	127-130	1.0	1.592	1.376	86.5	265-272	0.950	95.0	119-124
Ethyl isoamyl(b) (Amytal)	153-156	1.0	1.592	1.388	87.3	267-272	0.865	86.5	150-153
Ethyl hexyl*(b) (Ortal)	126	0.5	0.378	0.341	91.0	265-271	0.495	99.0	118-123
Ethyl (1-methyl 1-butenyl)*(b) (Delvinal)	160-162	0.5	0.357	0.331	92.5	267-272	0.470	94.0	159-160
[illegible]	[illegible]	[illegible]	[illegible]	0.344	[illegible]	265-269	0.452	90.5	131-134

TABLE IV (continued)

5,5-Disubstituted Barbituric Acid	M.P.	Wt.	Wt. of Theo.	Recovered Theophylline Wt.	%	M.P. Range**	Recovered Barbiturate Wt.	%	M.P. Range
1,3-Dimethyl phenyl ethyl*(a)	88	0.5	0.346	0.302	87.0	260-266	0.440	88.0	80-84
Phenyl allyl*(d)	154	0.5	0.380	0.295	78.0	260-271	0.480	96.0	145-150
Diphenyl*(d)	290-292	0.5	0.319	0.280	87.6	260-267	0.466	93.5	289-293
Diphenyl hydantoin*(b,e)	295-298	0.5	0.352	0.301	85.5	261-269	0.478	95.5	289-296

Fusion Experiments

Since no products were obtained from theophylline and barbiturates other than phenobarbital by reaction in alcohol, and since some of the patents reported products obtained by fusing the reactants, five of the 5,5-disubstituted barbiturates (diethyl, phenyl methyl, diphenyl, isopropyl bromallyl, and N-methyl phenyl ethyl) were subjected to the following fusion treatments with theophylline. Because of its close relationship to phenobarbital, the failure to obtain a product from phenyl methyl barbituric acid was surprising and it was included in these experiments.

Sufficient theophylline was added to 0.5 gram of the barbiturate in a test tube to give a molar ratio of 1:1 and the mixture heated in a Wood metal bath until liquified. The mixture was stirred, allowed to cool, and dissolved out with boiling alcohol. The crystallization was then continued as described under Reaction Conditions on page 115. In no case were new products obtained by this treatment and better than 85 per cent of the reactants was recovered.

Reaction of Barbituric Acid with Theophylline

Barbituric acid, the parent compound of the barbiturates listed in Table IV, reacted with theophylline in alcohol to yield a product melting at 220.5 to 221.5°C. (corrected) with decomposition. The material was analyzed for carbon, hydrogen, and nitrogen. The analytical results indicate an atomic ratio of $C_{2.3}H_{2.5}N_{1.22}O$, corresponding to a compound of the empirical formula $C_{11}H_{12}N_6O_5$. From a comparison with the percentage composition of barbituric acid and theophylline, it is evident that the unknown was neither of the starting materials. The data, however, compare favorably with the theoretical composition of a compound containing 1 molecule of barbituric acid and 1 molecule of theophylline ($C_{11}H_{12}N_6O_5$). However, since barbituric acid is a comparatively strong acid ($K_{ion} = 1.05\ 10^{-4}$), this compound is probably a salt.

Table V

	Per cent Carbon*	Per cent Hydrogen*	Per cent Nitrogen**	Per cent O_2 (by diff.)
Unknown	42.63	3.86	28.80	24.71
1 Theophylline- 1 Barbituric Acid	42.85	3.89	27.17	26.09
Theophylline	46.66	4.47	31.11	17.76
Barbituric Acid	37.49	3.12	21.88	37.51

*Macro
**Macro Kjeldahl

Experiments with Other Nitrogenous Compounds and Phenobarbital

These experiments were carried out using caffeine, theobromine, and diphenyl guanidine under the conditions described for "Experiments with other Barbiturates and Theophylline" on page 115. With caffeine and theobromine there was no reaction as indicated by better than 90 per cent recovery of the reactants in each case. These experiments were repeated with the same results. Neither could a product be obtained by fusing the reactants according to the conditions stated for Fusion Experiments, p. 119.

Diphenyl guanidine reacted with phenobarbital to yield a product melting at 195.5-196°C. (corrected). The material was analyzed for carbon, hydrogen, and nitrogen. The analytical results indicate an atomic ratio of $C_{8.33}H_8N_{1.67}O$, corresponding to a compound of the empirical formula $C_{25}H_{24}N_5O_3$. From a comparison with the percentage composition of diphenyl guanidine and phenobarbital, it is evident that the unknown was neither of the starting materials. The composition, however, compares favorably with the theoretical composition of a molecular compound of 1 molecule of diphenyl guanidine and 1 molecule of phenobarbital ($C_{25}H_{25}N_5O_3$). However, since diphenyl guanidine is a strong base(4) this compound is probably a salt.

Table VI

	Per cent Carbon*	Per cent Hydrogen*	Per cent Nitrogen**	Per Cent O_2 (by diff.)
Unknown	67.75	5.45	15.96	10.84
1 Diphenyl guanidine 1 Phenobarbital	67.74	5.64	16.15	10.47
Diphenyl guanidine	73.96	6.14	19.90	0.00
Phenobarbital	62.08	5.17	12.06	20.69

*Macro
**Macro Kjeldahl

Suggested Application of the Reaction

In view of the fact that nineteen barbiturates substituted in the 5 position with varying combinations of radicals failed to produce a reaction product, the following procedure is suggested as an identity test for phenobarbital, and as a means of distinguishing theophylline from caffeine and theobromine. However, the possibility of other barbiturates forming molecular compounds with theophylline should be further exhaustively investigated.

Identity Test: Dissolve 0.5 grams of phenobarbital in 25 cc. of a hot alcoholic solution of 0.775 gram of theophylline. Cool the solution and remove the crystals by filtration. After one crystallization from alcohol, the crystals melt between 247 and 252°C.

Summary

1. Sodium phenobarbital reacts with aminophylline in aqueous solution to form a product melting at 250.7-251.7°C. (corrected).

2. The same product can be formed by allowing phenobarbital and theophylline to react in alcoholic solution.

3. Analytical results and the amount of product obtained from known weights of reactants indicate that the product contains 2 molecules of theophylline and 1 molecule of phenobarbital.

4. The product always contains 2 molecules of theophylline and 1 molecule of phenobarbital regardless of the proportion of the reactants.

5. Since there are 2 molecules of theophylline for each molecule of phenobarbital, and since phenobarbital is a weak monobasic acid, it is believed that the product is a molecular compound rather than a salt.

6. The molecular compound is decomposed by sodium hydroxide solution into sodium phenobarbital and sodium theophyllinate, and is regenerated by acidifying the alkaline solution.

7. The molecular compound is decomposed by heating with 10 per cent hydrochloric acid, into phenobarbital and theophylline hydrochloride, but cold hydrochloric acid has no effect.

8. Silver nitrate decomposes the molecular compound into phenobarbital and silver theophyllinate.

9. None of nineteen other 5,5-disubstituted barbituric acids reacted with theophylline under the same conditions or by fusion with theophylline.

10. Barbituric acid reacts with theophylline in a molar ratio of 1:1 to produce a compound melting at 220.5 to 221.5°C. (corrected). However, because barbituric acid is a comparatively strong acid, this compound is believed to be a salt.

11. Phenobarbital did not react with caffeine or theobromine but did react with diphenyl guanidine in a molar ratio of 1:1 to produce a compound melting at 195.5 to 196°C. (corrected). However, because diphenyl guanidine is a strong base, this compound is believed to be a salt.

12. Because of the apparent specificity of the reaction of theophylline with phenobarbital forming a molecular compound, it is suggested that the preparation of "theophylline-phenobarbital" could be used as an identity test for phenobarbital and as a means of distinguishing theophylline from caffeine and theobromine.

REFERENCES

(1) U.S. Patent 2,017,279, Oct. 15, 1935

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(3) Stuckey, R.E., Quart. J. Pharm. Pharmacol. 14, p. 217, (1941).

(4) Merck and Co., Inc., Merck Index, 5th ed., p. 204, (1940).

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Knoll and Co. Sept. 18, 1911

(Verfahren zur Darstellung einer Verbindung von Codein mit Diethylbarbitursaure)

German Patent 239313 (P. Friedlaender, Fortschritte der Teerfarbenfabrikation, 10, p. 1223, (1913)).

(Codeine and diethyl barbituric acid will react to form a compound in alcoholic solution or when triturated in aqueous solution. The same compound will be formed by reaction between their salts in aqueous solution. The compound is decomposed into its components by heating with phosphoric acid.)

F. Bayer and Co. April 29, 1912

(Verfahren zur Darstellung von Verbindungen aus arylalkylbarbitursauren und Chinaalkaloiden)

German Patent 247,188 (P. Friedlaender, Fortschritte der Teerfarbenfabrikation, 11, p. 987, (1915)).

(Arylalkyl- or salts of arylalkyl- barbituric acids react with quinine alkaloids to form new compounds.)

E. Merck July 8, 1912

(Verfahren zur Darstellung von Verbindungen aus Chinin und Dialkylbarbitursauren)

German Patent 249,908 (P. Friedlaender, Fortschritte der Teerfarbenfabrikation, 11, p. 988, (1915))

(Dialkyl- or salts of dialkyl- barbituric acids react with quinine, or its hydrochloride, to form new compounds.)

E. Merck Feb. 4, 1913

(Manufacture of a Compound from Quinine and Diethylbarbituric Acid)

Swiss Patent 62,732 (Chem. Abstr., 8, p. 2226, (1914))

(A new compound is formed by bringing together quinine salts and salts of diethyl barbituric acid in water.)

E. Merck Feb. 4, 1913

(Manufacture of a compound of Quinine and Dipropylbarbituric acid)

Swiss Patent 62,733 (Chem. Abstr. 8, p. 2226, (1914))

(A new compound is formed by bringing together dipropylbarbituric acid and quinine)

E. Merck March 20, 1916

(Verfahren zur Darstellung von Verbindungen aus Chininderivaten und Dialkylbarbitursauren)

German Patent 291,421 (P. Friedlaender, Fortschritte der Teerfarbenfabrikation, 12, p. 754, (1917))

(Compounds of dialkylbarbituric acids and quinine derivatives may be prepared by bringing the reactants together in a suitable organic solvent, by fusing the reactants, or by reaction of the salts of each in water.)

G. Diehl June 6, 1916

Compounds of Quinine and Dialkyl Barbituric Acids

U. S. Patent 1,185,637

Compounds of dialkylbarbituric acids and quinine may be prepared by allowing the reactants to react in a suitable organic solvent, by fusing the reactants, or by reaction of the salts of each in water.

Soc. Anon. pour L'ind. Chim. a Bale March 1, 1918

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 76,996 (Chem. Abstr. 12, 2111, (1918))

Dial and quinine when brought together in molecular proportions form a therapeutically active compound.

Soc. Anon. pour L'ind. Chim. a Bale March 1, 1918

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 76,997 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and Morphine)

Soc. Anon. pour L'ind. Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,193 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and ethyl quinine carboxylate.)

Soc. Anon. pour L'ind. Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,194 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and cinchonine)

Soc. Anon pour L'ind Chim. A Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,195 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and ethyl hydrocupreine)

Soc. Anon. pour L'ind. Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,196 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of ethyl allyl barbituric acid and quinine)

Soc. Anon. pour L'ind Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,197 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and allyl morphine base)

Soc. Anon. pour L'ind Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,198 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and diacetyl morphine)

Soc. Anon. pour L'ind Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,199 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and codeine)

Soc. Anon. pour L'ind Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,200 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and dihydromorphine)

Soc. Anon. pour L'ind Chim. a Bale

(Therapeutically Active Derivatives of Diallylbarbituric Acid)

Swiss Patent 77,201 (Chem. Abstr. 12, p. 2111, (1918))

(Compound of Dial and dihydrocodeine and of Dial and hydroquinine)

E. Starkenstein June 19, 1923

Pharmaceutical Product

U. S. Patent 1,459,347

Preparation of a compound of barbital and pyramidon in alcoholic solution.

E. H. Volwiler Dec. 25, 1923

Hypnotic and Analgesic Compound

U. S. Patent 1,478,463

Compounds of dialkylbarbituric acids with pyramidon

E. Preiswerk May 13, 1924

Compound of Isopropylallylbarbituric Acid and Process for Making Same

U.S. Patent 1,494,127

Compound of isopropylallylbarbituric acid and Pyramidon.

Pfeiffer, P. 1925

Uber eine Verbindung aus Veronal und Pyramidon

Zeit. Physiol. Chem. 146, p. 98

Preparation of a eutectic of pyramidon and veronal, veramon, containing one molecule of pyramidon and one molecule of veronal.

Rheinboldt, H. and Kircheisen, M. 1925

Uber Veronal-Pyramidon und das "Veramon"

Arch. d. Pharm. <u>263</u>, p. 513

Veramon consists of a mixture of the compound $C_{21}H_{29}O_4N_5$, (Veronal and pyramidon in equimolecular proportion), with Pyramidon, colored yellow by oxidation products.

Pfeiffer, P. and Angern, O. 1926

Uber eine Verbindung von Veronal mit Pyramidon

Zeit. Physiol. Chem. <u>154</u>, p. 276

Compounds of pyramidon or antipyrine with veronal or sarcosine anhydride are explained by a linkage of the imide grouping of substances of the veronal type to the acid amide grouping of the substance of the pyramidon type.

Mareeuw, W.H.P. 1927

Veramon is een Scheikundige Verbinding en geen Mengsel

Pharm. Weekb. <u>64</u>, p. 753 (Chem. Abstr. <u>21</u>, p. 3102, (1927))

(Veramon is a Chemical compound and not a eutectic mixture)

Pfeiffer, P. and Seydel, R. 1928

Molekulverbindungen der Veronalreihe

Zeit. Physiol. Chem. <u>176</u>, p. 1.

The compounds obtained by uniting one molecule of veronal with one molcule of pyramidon, antipyrine or sarcosine anhydride are due to the saturation of secondary valences between the CO of the pyramidon component and both NH groups of the veronal. If an amide H of the veronal is substituted by alkyl, the product is no longer capable of forming a molecular complex.

I. G. Farbenindustrie Aktiengesellschaft May 26, 1930

Process for the Manufacture of New Therapeutic Agents

British Patent 306,905

Compound of cyclobarbital and 6,8 dimethoxyquinoline

Preiswerk, E. March 21, 1931

Process for Making Compounds of Dialkyl Barbituric Acids and 1-phenyl, 2,3-dimethyl, 4-dimethylamine, 5-pyrazolone

U.S. Patent 1,798,556

When dissolved in a common solvent the two reactants yield yellow crystals which are soluble in organic solvents.

Hoffman La Roche Inc. Oct. 19, 1933

(Compounds of Sparteine with Barbituric Acid

German Patent 586,245 (Chem. Abstr. 28, p. 578, (1934))

(Crystalline compounds are obtained by combining one molecule of sparteine with 1 or 2 molecules of a 5,5-disubstituted barbituric acid by fusing the reactants, by heating the reactants in a solvent, or by double decomposition in a solution between salts of the reactants.)

Chemische Fabrik von Heyden, A.G. Sept. 13, 1934

Manufacture of Derivatives of Oxy- and Amino- Pyridine Compounds

British Patent 416,273

Preparation of compounds of barbital and phenobarbital with oxy- and amino- pyridines.

Schnider, O. Sept. 17, 1935

Barbituric Acid Compounds of 1-phenyl, 2,3-dimethyl, 4-isopropyl-5-pyrazolone

U. S. Patent 2,014,866

Preparation of molecular compounds of dialkylbarbituric acids with 1-phenyl-2,3-dimethyl-4-isopropyl-5-pyrazolone.

Gruter, P.R. Oct. 15, 1935

Process for Making Readily Soluble Complex Theophylline Compounds

U. S. Patent 2,017,279

Phenobarbital, 0.2 mol proportion or less is caused to react with 1 mol proportion of aminophylline until a product is formed which is soluble in water and suitable for therapeutic use as an antispasmotic.

Taeschner, F. and others May 14, 1936

Improvement in and Relating to Processes for the Production of Barbituric Acid Compounds

British Patent 447,245

Preparation of molecular compounds of barbital and phenobarbital with urea derivatives.

Rosenberg, H. June 12, 1936

(Barbituric Acid Derivatives)

German Patent 631,097 (Chem. Abstr. <u>30</u>, p. 6136, (1936))

(Preparation of molecular compounds of dipropylbarbituric acid with papaverine and with codeine by fusion.

Insignani, G. 1939

(Molecular Association of Barbiturates and Local Anesthetics)

Ann. Chim. Farm. p. 64, (Dec.), (Chem. Abstr. 34, p. 2382, (1940)

(Barbitaland phenobarbital form water soluble molecular associations with procaine and ethyl-p-aminobenzoate.)

Redemann, C.E. and Niemann, C. 1940

The Diluturates (5-Nitrobarbiturates) of Some Physiologically Important Bases

J. Am. Chem. Soc., 62, p. 590

Formation of salts of 5-nitrobarbituric acid with amines.

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