(6) George B. Roth, "The Physiological Assay of Aconite," JOUR. A. PH. A., 2 (1913), 705–712.

(7) L. W. Rowe, "The Bioassay of Aconite," JOUR. A. PH. A., 14 (1925), 968–970.

(8) E. R. Squibb, "Aconite," Ephemeris of Materia Medica, 1 (1883), 123–137.

(9) E. E. Swanson and A. L. Walters, "The Standardization and Stabilization of Aconite Preparations. I.," JOUR. A. PH. A., 12 (1923), 957–963.

(10) E. E. Swanson, "The Standardization and Stabilization of Aconite Preparations. II.," JOUR. A. PH. A., 13 (1924), 1108–1112.

(11) E. E. Swanson and C. C. Hargreaves, "The Standardization and Stabilization of Aconite Preparations. III.," JOUR. A. PH. A., 16 (1927), 296–301.

(12) F. O. Taylor, "Aconite," *Ind. & Eng. Chem.*, 1 (1909), 549–567.

(13) United States Pharmacopœia X, page 387.

PHARMACOLOGICAL LABORATORY,
FOOD, DRUG AND INSECTICIDE ADMINISTRATION,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

A CHEMICAL STUDY OF THE RIND OF CALIFORNIA ORANGES.*,[1]

BY MARION BROOKS MATLACK.

The material used in the following investigation was collected daily from the Wisconsin General Hospital during a period covering about six weeks. The material consisted of the residue left after halved orange had been extracted with a revolving bur. The oranges were all California Valencias. The inner membranous material or "rag" was removed thus leaving only the rind. The rind was then dried over a radiator until brittle and ground in a food chopper. It was then placed in a large galvanized iron percolator and immediately covered with 95 per cent alcohol. Thus it was allowed to stand for some time and then the alcohol was drawn off; 33–36 liters at a time. The alcohol was recovered by distillation using a copper steam jacketed still. The residue remaining was drawn off into a flask and kept until all had been collected. The alcohol was returned to the percolator, and the process repeated until the residue from about two hundred liters of alcoholic percolate had thus been obtained.

The material was a dark brown to black liquid from which some solid matter separated out. This material was then steam distilled to remove volatile oil. The fixed oil separated on top of the aqueous layer in the distillation flask and was colored black by pigment.

The fixed oil which had thus separated out was removed by dissolving it in petroleum ether. After the petroleum ether had been evaporated off there remained a very dark almost black liquid residue.

The aqueous material remaining after removal of both volatile and fatty oil, was evaporated to a thick viscous consistency over a radiator and set aside until desired for use.

In this manner the following materials for further work were obtained:

---

* Part of a thesis submitted for the degree of Doctor of Philosophy, University of Wisconsin, 1928.

[1] Presented before Scientific Section, A. PH. A., Portland meeting, 1928.

1. The volatile oil obtained by steam distillation from the alcoholic extract.
2. Fatty oil and similar material soluble in petroleum ether which had separated from the aqueous liquid after steam distillation of the alcoholic extract in order to separate the volatile oil.
3. The residual alcoholic extract deprived of volatile oil, also of fatty and resinous material, soluble in petroleum ether, resulting after evaporation of the accumulated water.

ISOLATION OF HESPERIDIN.

In the process of extraction of the fat, which floated on top of the aqueous liquid after removal of the volatile oil by steam distillation, a quantity of brownish solid was noticed floating under the petroleum ether layer. This material appeared to be almost crystalline in some cases and in others a black tarry mass. The material which was nearly crystalline was washed with alcohol until light in color. A small quantity was recrystallized from alcohol. This had a melting point of 251° and was soluble in dilute alkali with a light yellow color, and could be precipitated by acids from this solution.

The black resinous mass was treated with boiling alcohol and filtered. There remained on the filter a substance which when dry melted at 250° without further purification. It was also soluble in dilute alkali and was concluded to be the same as the above, *viz.* hesperidin.

The alcoholic filtrate from the impure glucoside was mixed with ether which precipitated a brown solid. Petroleum ether produced still more precipitate. At least part of this brown material appears to belong to that ill-defined group of substances known as resins.

SAPONIFICATION OF THE FAT AND SEPARATION OF THE SATURATED FROM THE UNSATURATED FATTY ACIDS.

The petroleum ether was removed from the fat by distillation. To it were added fifty per cent aldehyde-free alcohol and an excess of potassium hydroxide (as determined by saponification). The saponification of the fat was carried out on a water-bath under a reflux condenser for a period of three hours. The alcohol was removed by distillation and more water added. Next the soaps were extracted with ether until the extract had only a faint yellow color. This ethereal extract was reserved for the examination for unsaponifiable constituents.

The aqueous soap solution was acidified with dilute sulphuric acid and the resulting mixture extracted with ether in order to obtain the fatty acids. The aqueous solution remaining was reserved for examination for glycerol.

In order to separate the saturated fatty acids from those which were unsaturated, use was made of the lead salt-ether method of Varrentrap. Some of the lead salt separated out as small brown lumps and some as a flocculent white precipitate. The lead soaps were extracted with ether twice and the mixtures filtered. Both the soluble and the insoluble lead soaps thus obtained were treated with dilute hydrochloric acid in order to regenerate the original fatty acids. The liberated acids were dissolved in ether, the solutions dried over anhydrous sodium sulphate and reserved for further examination as described below.

EXAMINATION OF THE UNSATURATED FATTY ACIDS.

The unsaturated fatty acids were examined by the bromination method as given by Lewkowitsch.[1] The fatty acids were dissolved in ether containing a small amount of glacial acetic acid, the solution cooled to 5° and a solution of bromine in acetic acid (1:1) was added until a slight excess of bromine was present. A precipitate settled out before the operation was concluded. The solution and solid were allowed to stand over night at a freezing temperature. The precipitate was filtered off and washed with ether until practically white. The dried substance melted at 182°. After recrystallization from benzene the m. p. rose to 183° C.

The filtrate was nearly freed from ether and poured into two liters of distilled water. A solid separated out which was yellowish brown in color and appeared almost crystalline. This solid was washed with water in order to remove any excess of acetic acid and bromine, allowed to dry, then dissolved in ether and the ethereal solution dried over anhydrous sodium sulphate. The solvent was removed by distillation and the residue extracted with a large volume of petroleum ether. On cooling to about —10° a flocculent precipitate settled out. This was redissolved in boiling petroleum ether and on cooling came out with a light yellow color. To the ethereal solution thereof petroleum ether was added until precipitation just started, the mixture then set outside where the temperature was about —10° C. and allowed to stand over night. Pearly white crystals settled out which had a m. p. of 113–114°.

The petroleum ether solution from which the first lot of crystals had been separated, was evaporated leaving a light brown oily residue.

The bromine content of the two solids obtained was determined by the method of Stepinow.[2] The average of two determinations on the solid melting at 183° was 63.46 p. c. Calculated for linolenic hexabromide 63.3 p. c. The acid present was therefore linolenic acid. The average of the two bromine determinations on the solid melting at 113–114° was 53.15 p. c. Calculated for linolic tetrabromide 53.27 p. c. The acid present is therefore linolic acid. Corrections were made for the chlorine content of the sodium used.

The light brown oily residue mentioned above was debrominated by means of twenty mesh zinc in order to obtain the free acid. On the free acid an iodine number determination was made. The average of two determinations was 95.4. Calculated for oleic acid 90.07. There was therefore probably some isolinolic acid present as has been found to be the case in several instances.

A drop of the above oily brown liquid dissolved in a few cc. of concentrated sulfuric acid gave a deep violet ring when a dilute alcoholic solution of vanillin was superimposed. On shaking, the color spread throughout the liquid.

A positive elaidin test was obtained.

The acid present may be concluded to be oleic acid.

EXAMINATION OF SOLID FATTY ACIDS.

Systematic fractional crystallization was resorted to and nine fractions were collected. Those fractions having the same melting range were combined. This

[1] Lewkowitsch, 6th Edition, I, 581.

[2] *Ber.*, 39 (1906), 4056; Kamm. Qual. Org. Anal., 168.

gave two lots of material one which crystallized readily and gave a good granular precipitate, melting at from 57–58° to a turbid liquid which cleared at 59°; and another which crystallized in flocks and dried to a leathery mass which melted at 66–68°. Its silver salt melted below 100° and the potassium salt was insoluble in cold water. Stephan[1] found evidence of cerotic acid in the residue left after distillation of sweet orange oil.

A silver salt was made of the first substance and the percentage of silver determined.

0.1330 Gm. of the unknown gave 0.0386 Gm. Ag	= 28.8 p. c.
0.0877 Gm. of the unknown gave 0.0252 Gm. Ag	= 28.7 p. c.
Average	= 28.75 p. c.
Silver palmitate contains	29.7 p. c.
Silver stearate contains	27.6 p. c.

Since the per cent of silver in the unknown is almost exactly half way between that of the two salts, it would indicate that the unknown is a mixture of approximately fifty-fifty palmitic and stearic acids. The melting point also corresponds to that given for a 50 per cent mixture of palmitic and stearic by Lewkowitsch.

ISOLATION OF GLYCEROL.

The aqueous liquid from which the fatty acids had been liberated by dilute sulphuric acid was exactly neutralized with sodium carbonate. This solution was evaporated until nearly dry and crystals of sodium sulphate had separated. These were filtered off and the mother liquor evaporated down still further and the residue extracted with alcohol containing a small amount of ether. The alcoholic solution thus obtained was allowed to evaporate over a radiator. A brown viscous residue was left which was dissolved in water. Basic lead acetate was added in excess and the precipitate filtered off. The excess lead was removed from the filtrate by hydrogen sulphide, and the filtered liquid was boiled with animal charcoal and again filtered. This gave a clear light yellow liquid. The water was evaporated off and the light brownish, viscous residue was tested as follows:

1. A small amount was dissolved in a dilute copper sulphate solution and sodium hydroxide added. A clear deep blue solution was obtained.

2. Some of the material was heated with potassium bisulphate and the evolved gases absorbed in water. This solution was found to reduce the reagent named above under 1, Fehling's solution and ammoniacal silver nitrate solution. The gases evolved during heating had the odor of acrolein. The presence of glycerol can, therefore, be concluded as proved.

IDENTIFICATION OF A PHYTOSTEROLIN.

When the fatty acids from the soap had been liberated by dilute sulphuric acid and the organic acids extracted with ether, a brownish colored solid was noticed floating next to the ether layer. After the extraction was complete the aqueous layer was drawn off and the brownish solid separated; NaOH solution was added to the moist solid with the hope of getting it into solution. The brown color dissolved and left a white voluminous solid which partly floated on and partly sank in the solution. The solution was deep brown to black in color. The white precipitate

[1] J. Stephan, *Journ. prakt. Chem.*, 170 (1900), 523.

was filtered off and dried. The brown to black colored solution was acidified with dilute sulphuric acid and a voluminous brown precipitate separated out. This was washed by decantation until free from acid and then filtered off and dried. It appeared like a shiny black resin. It has not been examined further.

The crude white solid was found to have a melting point of 271–273°. It was slightly soluble in boiling alcohol from which it separated on cooling. It was not, however, crystalline. This purified material melted at 271°. A small amount was crystallized from diacetone alcohol from which it came out in granules melting at 271–273°. Crystallization from dilute aqueous pyridine gave microscopic needles melting at 280°. It was found that the material gave a carbohydrate test with α-napthol and concentrated sulphuric acid but did not reduce Fehling's solution. Positive phytosterol color reactions were obtained (Salkowski and Liebermann-Burchard).

A benzoyl and an acetyl derivative were prepared. The former was made by dissolving a small amount of the material in pyridine and warming this solution with benzoyl chloride. After crystallizing once from a mixture of chloroform and alcohol and twice from alcohol it gave a melting point of 198°. The latter derivative was prepared by heating in acetic anhydride for one-half hour at a temperature sufficient to boil the acetic anhydride. After evaporation of the anhydride and two crystallizations from alcohol the crystals melted sharp at 164–164.5°.

One-half gram of the original material was hydrolyzed by the method of Power and Salway.[1] To the material dissolved in amyl alcohol aqueous hydrochloric acid was added and then sufficient ethyl alcohol to produce a homogeneous liquid. The mixture was heated for two hours under a reflux condenser. The amyl alcohol was then removed by steam distillation. There remained in the distilling flask a white solid floating in an aqueous solution. The solid was removed by filtration and crystallized three times from ethyl acetate-alcohol mixture. Large leaf-like, flaky crystals were obtained which melted at 136–136.5°. Their acetyl derivative formed leaf-like crystals, which, when crystallized from ethyl acetate-alcohol mixture, melted at 124.5–125°. The white solid obtained by the above hydrolysis gives both the Salkowski and Liebermann-Burchard reactions for sterols.

The aqueous solution reduces Fehling's solution and gives an osazone which, after two crystallizations from 60 p. c. ethyl alcohol, has a melting point of 204° with very little charring.

The above-mentioned properties agree quite closely with those of synthetic phytosterol-*d*-glucoside prepared by Salway[2] and with the natural phytosterolins found by others *i. e.*, Power & Salway's Ipuranol from *Ipomoea purpurea*.[3]

PLANTS IN WHICH PHYTOSTEROLINS ARE KNOWN TO EXIST.

Reference.		Substance, m. p.	Acetate, m. p.
(1)	*Ipomoea purpurea*	290–295°	160°
(1)	Colocynth (Appears to be identical with material from *Euonymus atropurpureas* and *Caulophyllum thalictroides*)	285–290°	167°
(1)	Bryony root	210–212°	152°

[1] *Chem. Soc. J. Trans.*, 103 (1913), 399.
[2] *Ibid.*, 103 (1913), 1022.
[3] *Ibid.*, 103 (1913), 399.

(1)	Taraxacum root (Appears to be identical with material from *Cluytia similis*)	297°	161°
(2)	Hops	285–290°	167–168°
(3)	Senna leaves	290°	163°
(4)	*Smilax ornata* Hooper	280–285°	.....
(5)	*Solanum angustifolium*	300°	168–169°
(6)	*Anthemis nobilis* L.	280–283°	159–160°
(7)	*Clematis vitalba* L.	195°	149°
(8)	*Matricaria chamomilla* L.	285°	158–160°
(9)	*Gloriosa superba*	293°	163°
(10)	*Euphorbia pilulifera*	297°	161–162°
(11)	*Phaseolus multiflorus*	275°	162°
(12)	*Dicoma anomala*	260–270°	150°
(13)	*Brauneria (Echinacea) angustifolia*	280–290°	163–164°
(14)	*Ferrula sumbul* before	260–270°	
	after crystallization	290°	159–160°
(15)	*Syzygium jambolina*	275–285°	167–168°
(16)	*Adonis vernalis*	275–285°	167–168°
(17)	*Viburnum prunifolium*	290°	168°
(18)	Cotton plant	218–223°	165–166°
(19)	Synthetic phytosterol-*d*-glucoside, sitosterol-*d*-glucoside)	295–300°	166–167°
	Benzoyl derivative	198°	

REFERENCES.

(1) *Chem. Soc. J. T.*, 103 (1913), 399.
(2) *Ibid.*, 103 (1913), 1267.
(3) *Ibid.*, 103 (1913), 2006.
(4) *Ibid.*, 103 (1913), 201.
(5) *Ibid.*, 105 (1914), 559.
(6) *Ibid.*, 105 (1914), 1829.
(7) *Ibid.*, 105 (1914), 1845.
(8) *Ibid.*, 105 (1914), 2280.
(9) *Ibid.*, 107 (1915), 835.
(10) *Pharm. J.*, 90 (4), 36 (1913), 506.
(11) *Ibid.*, (4) 36 (1913), 550.
(12) *Ibid.*, (4) 36 (1913), 694.
(13) *J. Am. Chem. Soc.*, 37 (1915), 1769.
(14) *Ibid.*, 38 (1916), 432.
(15) *Ibid.*, 38 (1916), 2805.
(16) *Ibid.*, 40 (1918), 436.
(17) *Ibid.*, 42 (1920), 1744.
(18) *Ibid.*, 48 (1926), 2721.
(19) *Chem. Soc. J. T.*, 103 (1913), 1022.

ISOLATION OF TWO PHYTOSTEROLS.

The unsaponifiable material which had been obtained from the soap by extraction with ether was dissolved in petroleum ether. Methyl alcohol was added in large volume with the hope of producing a precipitate but none appeared. Sufficient water was then added to the solution to dilute the methyl alcohol to a concentration of eighty per cent. The solution separated into two layers. A solid crystalline substance settled out in both layers and that from each layer was collected separately. After repeated recrystallization from alcohol-ethyl acetate mixture they were found to melt at 139–139.5° and to give an acetyl derivative which after repeated crystallization from alcohol-ethyl acetate solution melted at 128°. The free alcohol gave the Liebermann-Burchard and Salkowski color reactions for sterols.

The second phytosterol was obtained from the combined filtrates from the crystallization of the first sterol and crystallized in needles instead of the plates quite common to phytosterols. This second phytosterol is more soluble in alcohol than is the first. After repeated crystallization from alcohol it gave a melting point of 150°. From this an acetyl derivative was formed which melted at 113.5°–

114°. These crystals appeared as flat colorless needles. Since phytosterols with the above melting point are not so common as others in plants, it was thought desirable to make a benzoyl derivative. After repeated crystallization this melted at 143°. It crystallizes in colorless leaflets. In the melting points of the sterol and its benzoyl derivative there is complete correspondence with the paraphytosterine of Likiernik[1] obtained from the seed coats of *Phaseolus vulgaris*. He did not prepare the acetyl derivative. The Liebermann-Burchard and Salkowski sterol reactions are both positive with this second phytosterol from orange peel. The specific rotation is —1.3° in chloroform.

EXAMINATION OF RESIDUE LEFT AFTER SEPARATION OF THE PHYTOSTEROLS.

A. On concentrating the solution left after the phytosterols had been removed some material separated out and was filtered off. Two attempts were made to hydrolyze this material, once with alcoholic KOH and once with sodium ethylate. When this solution was diluted with water, the alcohol evaporated off and the solution extracted with ether, a small amount of material was obtained. (Only a trace of acid could be liberated from the remaining aqueous solution.) The material from the above solution was crystallized from various solvents including alcohol and petroleum ether and a small amount of substance was obtained which gave an indefinite melting point of 75–77°. In the hope of better purification the material was acetylated and again recrystallized several times. After drying a melting point of 62–63° was obtained. The original substance crystallized from alcohol in fine colorless needles and had the appearance of a wax alcohol. Ceryl alcohol has a melting point of 79° and ceryl acetate melts at 65°. It is thought, therefore, that the unknown substance might be impure ceryl alcohol.

B. The material left after *A* had been filtered off was then examined. Repeated attempts were made to isolate some substance which might be identified but nothing could be separated which could be defined. Saponification was tried twice, once with strong alcoholic KOH and once with sodium ethylate. The material thus treated was extracted with ether and the ether solution dried over anhydrous sodium sulphate. On evaporation of the ether solution a black, almost solid mass was obtained. This mass was fused with phthallic anhydride in the hope of separating an alcohol and a hydrocarbon. No alcohol was obtained and only a trace of acid recovered. The residue from the fusion came out where a hydrocarbon would be expected but all attempts to isolate one have thus far been unsuccessful. It is thought that this material may be oxidized carotinoid pigment.

SUMMARY AND CONCLUSIONS.

1. An examination has been made of the fats, sterols and related compounds of the sweet orange.

2. The following fatty acids have been characterized: oleic, linolic, linolenic, stearic and palmitic.

3. Two phytosterols have been identified one of which crystallizes in flakes and appears to be nearly pure sitosterol, the other one crystallizes in needles and would seem to be identical with the paraphytosterol of Likiernik.

[1] *Z. physiol. Chem.*, 15 (1891), 430.

4. A phytosterol glucoside (phytosterolin) has been separated whose characteristics indicate it to be identical with the synthetic sitosterol-*d*-glucoside of Salway.

5. Glycerol has been identified.

6. Indications have been found of the presence of ceryl alcohol, a small amount of resin and carotinoid coloring matter.

A PROPOSED PHYSIOLOGICAL STANDARD FOR PITUITARIUM, U. S. P.*

BY WM. T. MCCLOSKY AND J. C. MUNCH.[1]

PHARMACOLOGICAL LABORATORY, FOOD, DRUG & INSECTICIDE ADMINISTRATION, DEPT. OF AGRICULTURE, WASHINGTON, D. C.

Glandular products are finding their way into therapeutics and some of them have found their way into the U. S. Pharmacopœia. "Pituitarium is the cleaned, dried and powdered posterior lobes obtained from the pituitary body of domesticated animals which are used for food by man. It is a yellowish or grayish amorphous powder, having a characteristic odor, and is only partially soluble in water" (10). Its official preparation is **Liquor Pituitarii.** No mention is made in the Pharmacopœia as to the physiological strength of Pituitarium. It is known that many manufacturers use Pituitarium to make Liquor Pituitarii. Confronted with this, how can the manufacturer know how much powder to use to make Liquor Pituitarii of the strength mentioned in the Pharmacopœia? Variations in manufacturing methods may cause differences in the physiological potency of Liquor Pituitarii, irrespective of the raw material used. By standardizing the potency of the Pituitarium powder only one of the causes of variation is removed, even though it may be the greatest. It is still necessary for the manufacturer to assay each lot of finished pituitary solution.

The potency of desiccated posterior pituitary powders is most important in considering the recommendation of the Second International Conference on Biological Assays held in Geneva in 1925 (5), that a dried posterior powder be adopted in every Pharmacopœia to be used as the basis for the preparation of all commercial pituitary solutions. This suggestion was not adopted by the Third Conference held at Geneva in 1928 (6), because there was some doubt whether such a powder would retain the diuretic action as well as the pressor and oxytoxic potency. Reports by Mackensie (3), Smith and McClosky (8) and Kestranek, Molitor and Pick (2) all indicate that the diuretic action of the posterior pituitary gland is quantitatively preserved in preparing a desiccated powder by the U. S. P. X method.

A number of samples of commercial posterior powders were obtained directly from the manufacturers, representing their current output, and a study of their activities made. Extracts were made to contain the activity of 1 mg. powder per cc. and 10 mg. powder per cc., and were prepared as follows: A desired amount

---

* Scientific Section, A. PH. A., Portland meeting, 1928.

[1] Resigned Jan. 1, 1928.