

The filtrate was distilled in vacuum and the portion boiling at 24°C./27 mm. collected. A total of 55 Gm., representing a yield of 34%, was obtained. This product which was colorless and very volatile had a boiling point of 98°C. when determined by micro methods

This volatile liquid yielded a phenylurethane melting at 232–233°C.; an  $\alpha$ -naphthylurethane melting at 217°C.; and a dinitro-osazone consisting of bright yellowish orange crystals melting at 148°C. No color was observed on the addition of a small amount of this product to anhydrous copper sulfate. Schiff's reagent gave a negative reaction.

Because of its extreme volatility, only an approximate specific gravity was determined between the range of 0.84–0.86 at 15°C. The product rapidly decolorized a solution of bromine in chloroform leading us to suspect the presence of an unsaturated structure. Several iodine determinations were carried out with values ranging from 91 to 139. This variance may be attributed, possibly, to the extreme volatility of the liquid.

## CONCLUSIONS

1. Preliminary studies of the polymerization of pentanone-3, using barium hydroxide as the condensing agent have been made.

2. The principal product formed appeared to be an unsaturated aliphatic alcohol of low molecular weight.

3. The failure to isolate the desired 3-ethyl-octanol-3-one-6 is being investigated.

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# The Phytochemistry of Helonias

## I. Preliminary Examination of the Drug\*

By E. L. Cataline and D. E. Francke†

*Chamaelirium luteum* (L.) A. Gray, is a perennial, dioecious herb of the lily family found growing in low grounds from New England to Georgia and westward. It is known by such synonyms as False Unicorn, Blazing Star, Starwort, Devil's Bit and Helonias, the last named being the title by which the dried rhizome and roots are recognized by the National Formulary. The drug has long been recognized by the Homeopathic Pharmacopœia of the United States, and it was used as a remedy by the American Indians (1, 2, 3).

Moser (3) has reported on the pharmacognosy of the drug, but chemical investigation is apparently limited to the work of Greene and Kruskal<sup>1</sup>. Greene (4, 5) reported

the presence of a saponaceous glycoside, reducing sugar, tannic acid and a small quantity of a white substance which he did not characterize but believed to be a fatty acid. He further showed that the glycoside was hydrolyzed when heated with 2% hydrochloric acid, the products of the hydrolysis being a reducing sugar and a white, resinous substance which he named chamæliretin. Ultimate analyses carried out by Kruskal (6) indicated that the empirical formula of the glycoside may be represented as  $C_{18}H_{32}O_9$  or  $C_{36}H_{62}O_{18}$ .

Helonias has been used as a sialagogue, emetic and uterine tonic. In connection with the latter use it is of interest to note that Pilcher (7) has shown that the drug has no action on the excised uterus of the guinea pig.

## EXPERIMENTAL

### PRELIMINARY EXAMINATION

The moisture content, determined by heating at 100°C. to constant weight, was found to be 10.8%. Using the U. S. P. methods, the total ash was found to be 4.33% and acid-insoluble ash was 1.58%. No

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<sup>1</sup> In a paper published subsequent to the completion of this paper, Marker [*J. Am. Chem. Soc.*, 64 (1942), 1238] showed that diosgenin could be isolated from *Chamaelirium luteum*.

volatile oil was found by the method of Clevenger (8). No alkaloid could be detected by the Stas-OTTO method (9) or by the method for ether soluble alkaloids (10).

#### THE GENERAL METHOD

Two 500-Gm. samples of the drug<sup>1</sup> were subjected to the action of a series of solvents. The percentage of the drug extracted by each solvent is shown in the following table:

Solvent	Per Cent
Petroleum benzin (b. p. 60-75° C.)	2.1
Diethyl ether	1.8
Chloroform	1.1
Alcohol	17.8
Water	14.1
Sulfuric acid (1.25%)	16.0 <sup>a</sup>
Sodium hydroxide (1.25%)	25.3 <sup>a</sup>
Total extractive	78.2

<sup>a</sup> The weight of reagent used was subtracted from the weight of extractive.

#### PETROLEUM BENZIN EXTRACT

The petroleum benzin extract was a dark green, viscous oil. The presence of glycerol was indicated by the production of vapors of acrolein when a small quantity of the oil was heated with potassium bisulfate (9). The Elaidin Reaction (9) was positive indicating the presence of oleic acid. A thin layer of the oil, spread on a glass plate became only slightly more viscous after standing in air for 3 weeks. This was taken to indicate that the more highly unsaturated acids were present only in small amounts, if at all.

The oil was found to possess the following constants:

Specific gravity, 20° C.	0.9456
Refractive index, 25° C.	1.4742
Saponification value	193.8
Iodine value	76.2

*Unsaponifiable Matter.*—The unsaponifiable matter, determined by the modified Kerr-Sober method (11), constituted 10% of the petroleum benzin extract. It was a very viscous, light brown, oily substance which responded to the usual color reactions for sterols (12). An ether solution absorbed bromine. No crystalline material could be isolated from the unsaponifiable matter when it was heated with acetic anhydride or benzoyl chloride. Attempts to fractionate the mixture by means of alcohol were unsuccessful.

*Liquid and Solid Fatty Acids.*—Because of the relatively small quantity of the oil available, no attempt was made to fractionate the liquid and solid fatty acids quantitatively. A quantity of the oil was saponified, the unsaponifiable matter removed by shaking with ether, and the liquid and solid fatty acids separated by the lead salt-ether method (11). The percentages of the liquid and solid fatty acids were approximately 60% and 30%, respectively.

The liquid fraction was oxidized with potassium

permanganate at 0° C. (13), the reaction mixture filtered and the hydroxy acids precipitated by acidification. The major portion of the precipitate consisted of 9,10-dihydroxystearic acid, m. p. 132-133° C. after recrystallization from alcohol. When it was mixed with an authentic sample of 9,10-dihydroxystearic acid prepared from oleic acid by the same process there was no depression of the melting point.

The remainder of the precipitate was recrystallized from water and then several times from alcohol, m. p. 174-175° C. This acid is undoubtedly the "high-melting" tetrahydroxystearic acid (11, 13). No hexahydroxystearic acid could be isolated by this method. Attempts to brominate the liquid fatty acid mixture yielded virtually no ether-insoluble material. It appears, therefore, that the oil contains very little, if any, linolenic acid (13).

Fractionation of the solid fatty acids using magnesium acetate yielded inconclusive results. One-tenth the quantity of magnesium acetate required to precipitate the solid fatty acids completely was added to a hot alcohol solution of the acids. After the mixture had cooled, the precipitate was filtered off, air dried, suspended in ether and decomposed with dilute hydrochloric acid. The ether layer was separated, dried, the solvent removed, and the residue recrystallized from alcohol. After neutralization of the filtrate with ammonium hydroxide the process was repeated. In some instances no precipitate was obtained and another addition of magnesium acetate was necessary. By this process five fractions were obtained.

The residue from the first precipitation was gummy and no crystalline material could be isolated from it. The second fraction was crystalline and melted at 70.5° C. When mixed with an authentic sample of stearic acid there was no depression of the melting point. The remaining fractions did not exhibit sharp melting points. Fractions 3 and 4 melted at 55-57° C. and were undoubtedly mixtures. Fraction 5 melted over a much wider range.

#### OTHER EXTRACTS

*Diethyl Ether Extract.*—The diethyl ether extract consisted of a brown, semisolid, resinous substance. It was completely soluble in potassium hydroxide solution but only partially soluble in alcohol. No crystalline material could be isolated from it upon extraction with a number of solvents.

No color developed when an aqueous extract of the resinous material was treated with ferric chloride T. S. indicating the absence of gallic acid.

*Chloroform Extract.*—The chloroform extract was similar in appearance and behavior to the diethyl ether extract.

*Alcohol Extract.*—The alcohol extract was obtained as a black, sticky semisolid. A foam was produced when a portion was shaken with water. The aqueous layer did not yield a color with ferric chloride T. S.

<sup>1</sup> Purchased from the S. B. Penick Company. Labeled "Granulated."

Work upon this fraction, which contains the saponaceous glycoside, will be reported in detail later.

*Water Extract.*—The water extract was a brown, granular solid. An aqueous solution of the substance foamed when shaken. The presence of reducing substances was indicated by the production of cuprous oxide upon boiling with Fehling's solution. The ferric chloride test was negative. A deep blue color was produced when a solution of iodine in potassium iodide was added to an aqueous solution of the extract.

## SUMMARY

A preliminary chemical investigation of the rhizome and roots of *Helonias (Chamaelirium luteum* A. Gray) is reported. No volatile oil or alkaloid could be detected. The nondrying, fixed oil was shown to contain oleic, linoleic and stearic acids. The presence of other saturated fatty acids and sterols was indicated. No tannic or gallic acid could be detected.

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## Comparative Stability of Vitamin A in Cod-Liver Oil and in Oleovitamin A and D\*

By Arthur D. Holmes and Madeleine G. Pigott

When it was foreseen that the supply of cod-liver oil would not be adequate for the country's needs, the Revision Committee of the United States Pharmacopœia provided for a substitute product which would possess the same vitamin A and vitamin D value as official cod-liver oil and designated it oleovitamin A and D. The official specifications (1) for this product provide that natural vitamin A and vitamin D may be dissolved in edible vegetable oils and the mixture shall have a U. S. P. vitamin potency of not less than 850 but no more than 1100 vitamin A, and not less than 85 and no more than 110 vitamin D units per gram. Obviously oleovitamin A and D does not contain any iodine (2), arsenic (3) or other substances which are

found in cod-liver oil and its therapeutic value resides wholly in its vitamin content.

In the commercial distribution of oleovitamin A and D, this material, like other pharmaceutical products, is likely to remain in the hands of the wholesaler or upon the druggist's shelf for indefinite periods. Therefore a question naturally arises as to the relative permanency of the fat-soluble vitamins as they naturally occur in cod-liver oil and in solution in vegetable oils. Earlier studies in this laboratory have shown that the permanency (4) of vitamin A in cod-liver oil is influenced by the exposure of the oil to light. In other words, the rate of destruction of vitamin A in cod-liver oil packed in Flint glass containers depends upon both the intensity of the light or direct sunshine and the length of time that the oil is exposed to it. Accordingly, it seemed of inter-

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