only limited quantities of 10% solutions of aluminum chloride or of tannic acid without being materially altered. This limitation in the addition of electrolytes and acidic substances is due more to the characteristics of the emulsifier than to the ester used. Because of the neutral character of the esters, incompatibility with dilute acids, alkalies and electrolytes should not be encountered.

Physical limitation precludes the preparation of the large number of possible combinations of the 12-hydroxystearic acid esters in varying proportions with related fatty alcohols and acids. But the examples cited should suffice to show the adaptability of these esters to formulations involving wax-type components. The desirable qualities of these esters invite further studies of their pharmaceutical and industrial applications. The problem of making synthetic products which approximate or even improve upon the natural waxes is indeed a complex one, but it is believed that a satisfactory approach has been made through the synthesis of these esters of 12-hydroxystearic acid.

SUMMARY

Methods have been given for the isolation of 12-hydroxystearic acid from commercial hydrogenated castor oil, and for the syntheses of a series of twelve of its esters with normal primary alcohols. A number of their physical properties have been evaluated and some of their applications to ointments and similar bases have been suggested. The results indicate the possibilities of utilizing these esters in the formulation of waxes having reproducible properties and whose qualities should prove superior to the natural waxes in respect to stability and freedom from color, odor and rancidity.

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A Phytochemical and Pharmacological Study of the Berries of *Phytolacca americana* Linné (Fam. Phytolaccaceae)

By Laurine D. Jack and Charles H. Rogers†*

The berries of *Phytolacca americana* (*decandra*) have been known in clinical medicine for many years. Phytochemical and pharmacological investigations have consisted of a few incomplete reports. The present work was undertaken with the purpose of making a more complete study.

* Abstracted in part from a thesis submitted by Laurine D. Jack to the Graduate Faculty of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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EXPERIMENTAL

A. PHYTOCHEMICAL INVESTIGATION

Preliminary Study.—Moisture Determination: The moisture content was determined by the volatile immiscible solvent method of the United States

Pharmacopœia XI (4). Moisture was present to the extent of 6.5 per cent.

Ash Determination: The total ash was found to be 4.22 per cent, of which 1.78 per cent was water insoluble and 2.45 per cent was water soluble. The alkalinity of the water insoluble ash was 1.41. The amount of acid insoluble ash was 1.05 per cent.

Extraction with Various Solvents: In order to ascertain the general character of the constituents of Phytolacca Berries 40 Gm. of the drug were extracted in a Soxhlet apparatus with the following solvents: petroleum ether, ethyl ether, chloroform, ethyl alcohol, and water. After the soluble constituents had been completely extracted with each solvent, the latter was removed by evaporation. The residual extracts were dried over sulfuric acid at room temperature until constant weight resulted. Petroleum ether extracted 7.87 per cent; ethyl ether, 0.51 per cent; chloroform, 0.84 per cent; ethyl alcohol, 26.19 per cent; and water, 10.79 per cent.

Test for Alkaloid: Ten grams of the drug were macerated with prolius fluid for several days after which time the fluid was decanted from the drug. It was shaken in a separatory funnel with 2 per cent sulfuric acid. The acid extract was tested for alkaloids with the usual alkaloidal reagents. All tests were negative.

The drug (100 Gm.) was examined by the Stas-Otto method (2) in order to test for alkaloids. The residues which were tested with the alkaloidal reagents yielded negative results.

Large Scale Extraction.—Fifty pounds of the drug (No. 20 powder) were extracted in a Barnstead Extractor with ethyl alcohol until the drug was exhausted. Most of the alcohol was removed from the extract by distillation. The semi-solid residue was incorporated with a sufficient amount of dried marc and was packed in a percolator. It was then extracted with petroleum ether.

Investigation of Petroleum Ether Extract.—The residue from the petroleum ether extract was a green oil. After standing for a few days, minute crystals separated from the oil.

Investigation of Crystals: Microscopically the crystals appeared both as rosettes and as platelets. They were very soluble in petroleum ether and in ethyl alcohol. The melting point range of the purified crystals was 296° C. to 297° C. The specific rotation was +14° at 20° C. Qualitative analysis indicated the absence of nitrogen, sulfur and halogens. The Liebermann-Burchard test for sterols resulted in a permanent red color. A chloroformic solution of the substance decolorized a bromine solution. The molecular weight as determined according to the Rast method was 443.9.

Analysis: Calcd. for $C_{28}H_{46}O_3$: C, 78.07; H, 10.77. Found: C, 77.47; H, 10.62.

The crystals (0.3838 Gm.) were dissolved in 30 cc. of amyl alcohol, 10 cc. of 15 per cent hydrochloric acid were added, and a sufficient amount of ethyl alcohol to form a homogeneous mixture. The solution was heated for 6 hours under a reflux condenser. The alcohol was removed by steam distillation and the product was recovered from the aqueous solution with ethyl ether. The crystals melted at 286.5° C. to 288° C.

Analysis: Calcd. for $C_{27}H_{44}O_3$: C, 77.81; H, 10.65. Found: C, 78.02; H, 10.68.

A portion of the crystals (0.1 Gm.) were acetylated with a mixture consisting of 10 cc. of acetyl chloride and 5 cc. of pyridine. The crystalline product obtained melted at 303° C. to 304° C. The molecular weight as calculated upon the basis of the acetyl number was found to be 468.7.

Analysis: Calcd. for $C_{30}H_{48}O_4$: C, 76.21; H, 10.24. Found: C, 76.36; H, 10.41.

The acetyl number and the combustion indicated that one acetyl group had been introduced into the molecule. It was concluded that one oxygen atom existed as a free hydroxyl group.

According to the alkoxy determination, no alkoxy groups were present. An attempt was made to reduce the compound catalytically at room temperature, but no reduction occurred. Chromic acid oxidation yielded a residue which apparently was a mixture, for all attempts to crystallize it failed.

Determination of Oil Constants: The oil obtained from the petroleum ether extract was decolorized with charcoal and the following constants were determined: Specific gravity $\frac{25°}{25°}$, 0.89937; index of refraction, $N_D^{20°}$, 1.47046; specific rotation, $[\alpha]_D^{26°}$, +0.28°; acid value, 15.76; saponification value, 189.13; ester number, 173.37; iodine value, 105.22.

Saponification of Oil: The oil was saponified with alcoholic potash. A dilute aqueous solution of the saponified product was prepared and extracted with several portions of ethyl ether in order to remove the unsaponifiable material. From the aqueous solution the free fatty acids were liberated upon the addition of a diluted mineral acid. The resultant aqueous solution was evaporated to dryness.

Detection of Glycerol: The above residue was extracted with absolute alcohol. The residue, which was obtained after evaporation of the alcohol, was dissolved in a small amount of water, a few crystals of potassium bisulfate were added, and the mixture was heated. The odor of acrolein was perceptible which indicated the presence of glycerol.

Isolation of Free Fatty Acids: The combined fatty acids were separated into the saturated and unsaturated fractions according to the lead-alcohol precipitation method of Twitchell (3). About 12 per cent of the fatty acids were saturated.

The methyl esters of the saturated fatty acids were prepared and fractionally distilled *in vacuo* at 4 mm. Hg. Each fraction was saponified and the free fatty acids were liberated upon the addition of a diluted mineral acid. The melting point range

and the mean molecular weight of each fraction was determined. Assuming that no fraction contained more than two adjacent homologs the percentages of the acids were calculated. Accordingly, the mixture of saturated fatty acids consisted of: Palmitic acid, 50.7 per cent; stearic acid, 45.6 per cent; arachidic acid, 3.7 per cent. Qualitative tests for palmitic and stearic acids were positive. The qualitative test for arachidic acid was negative.

The unsaturated fatty acids were methylated and distilled in the same manner as previously described. The iodine value for each fraction was determined. Each fraction was reduced catalytically at room temperature; ethyl alcohol was used as the solvent and platinum black as the catalyst. The residues were saponified and crystals were obtained. The melting points and saponification values of the crystals were determined. The molecular weights were calculated. The values corresponded to those of stearic acid. The *p*-brom phenacyl ester of each crystalline fraction was prepared. Mixed melting points with Eastman *p*-brom phenacyl ester of stearic acid showed no depression. It was concluded that the reduced acid in each case was stearic acid. A portion of the unsaturated acids were brominated. No hexa- or tetrabrom derivatives were prepared so it was believed that the eighteen carbon unsaturated acid was oleic acid.

Unsaponifiable Material: The unsaponifiable material consisted of two crystalline products which were impregnated with an oily substance. The latter was removed inasmuch as it was more soluble in petroleum ether than the crystalline portion. The crystalline products were separated by fractional crystallization from ethyl alcohol and finally from isopropyl ether. According to the Liebermann-Burchard test one of the products was a sterol and the other product was later identified as Hentriacontane.

Investigation of Sterol: The crystals melted at 166° C. Qualitative analysis indicated the absence of nitrogen, sulfur and the halogens. It was believed that the compound was a sterol because the Liebermann-Burchard, Rosenheim and Salkowski tests for sterol were positive. A chloroformic solution of the crystals absorbed bromine.

Analysis: Calcd. for $C_{31}H_{56}O.H_2O$: C, 80.44; H, 12.64.

Found: C, 80.54; H, 12.54.

The crystals were acetylated with acetic anhydride. The melting point of the acetylated product was 175° C. to 175.5° C. The acetyl determination indicated that one acetyl group had been introduced into the molecule. The molecular weight was calculated to be 484.7.

Analysis: Calcd. for $C_{33}H_{58}O_2$: C, 81.40; H, 12.01.
Found: C, 81.13; H, 10.96.

Investigation of Hentriacontane: The melting point of the crystalline product was 67.5° C. Qualitative analysis indicated the absence of nitrogen, sulfur and the halogens. It was insoluble in hot concentrated sulfuric acid. A chloroformic solution did not absorb bromine. The molecular weight was determined by the Rast method and was found to be 432.2. The crystals were optically inactive.

Analysis: Calcd. for $C_{31}H_{64}$: C, 85.24; H, 14.77.
Found: C, 84.24; H, 14.87.

Extraction of Pigment.—The whole berries of *Phytolacca americana* which were obtained from the University of Minnesota Medicinal Plant Gardens were extracted with cold glacial acetic acid. Upon the addition of ethyl ether a red gummy substance was precipitated. It was very soluble in water. Its color reactions with acids, alkalies and lead acetate place it in the same class as the anthocyanins of unboiled beet juice and the Chenopodiaceæ.

B. PHARMACOLOGICAL INVESTIGATION

In carrying out the pharmacological investigation the principal motive was to ascertain the relative toxicity of the berries of *Phytolacca americana*. For this purpose two fluidextracts were prepared—one containing 50 per cent of alcohol by volume and the other containing 25 per cent of alcohol by volume. Also an infusion and a colloidal solution were used. The latter represented a fraction of the alcoholic extract.

Effect on Frogs.—For this purpose the fluidextract containing 50 per cent of alcohol was used and when injected into frogs *via* the ventral lymph sac, a depression was produced. Likewise, a depression occurred in the control frog. One of the frogs which received the fluidextract died, whereas the control which received an injection consisting of a corresponding amount of alcohol survived. It was believed that there might be something in addition to the alcohol which was producing a depressant effect upon the frog.

Effect on Rats.—When intraperitoneal injections of the fluidextract (50 per cent alcohol) were made into rats, a deep degree of narcosis resulted. This also occurred in the control, however the effect was not so pronounced. In the rats which had received the drug it was noted that the reflexes were largely absent, whereas in the control they were present but the reflex time was prolonged. The drug caused a decrease and an irregularity in the respiratory rate. One rat died as a result of respiratory paralysis. It was concluded that the toxic effect of the drug was of the nature of a narcotic or depressant which was more intense than that caused by alcohol.

Effect on Rabbits.—Intraperitoneal injections of the fluidextract (25 per cent alcohol) into rabbits had no effect. The reflex time was normal and there was no indication of any paralysis.

The fluidextract (25 per cent alcohol) was administered orally to rabbits by means of a stomach tube. No abnormal effects were produced.

Several experiments were conducted in which the drug in the form of the fluidextract, infusion or colloidal solution was administered intravenously and the blood pressure was recorded simultaneously. All of these experiments indicated that the drug caused a general depression. The blood pressure fell after each injection of drug but it was not sustained at the lower level. It rose almost as quickly as it fell. However, it never reached its preceding value. Therefore, throughout the course of the experiment there was a gradual decrease in the blood pressure. In general the carotid pulse rate decreased throughout the experiment. Respirations appeared to be normal until the fatal injection was approached. Eight deaths were due to respiratory failure and two deaths were due to cardiac paralysis. For the sake of comparison, two experiments were carried out in a similar manner in which Fluidextract of Phytolacca Root was used. One death was due to respiratory failure and the other was due to cardiac paralysis. These experiments indicated that Phytolacca Root was five to eight times as active as Phytolacca Berries.

It was believed that the action of the drug was primarily upon the medullary centers. The heart rate in some cases was quite irregular and in a few experiments it was believed that extra systoles had occurred. In the majority of the experiments, as fatality was approaching the rate of respiration decreased and the pressure waves were shallow, which indicated either a decreased demand for oxygen or lack of the normal gas exchange. In several of the experiments it was noted that the pulse pressure increased. This was due to a decrease in the diastolic pressure. Such a condition might be indicative of a failing circulation.

Effect on Cats.—Intraperitoneal injections were made into cats. There was no immediate indication of any toxicity; however, a slow death occurred. Goldstein, Jenkins and Thompson (1) had observed under the same conditions with Fluidextract of Phytolacca Root that an ascending paralysis had occurred.

When intravenous injections of the fluidextract (25 per cent alcohol) were made into a cat the same effect was produced as with rabbits.

SUMMARY

From the berries of *Phytolacca americana* the following constituents were isolated:

1. Glycerol as the glyceryl ester of naturally occurring fatty acids.
2. Saturated Fatty Acids.
3. Oleic Acid.
4. A Sterol.
5. A Compound which resembled a Sterol.
6. Hentriacontane.

The pharmacological experiments showed that:

1. The Fluidextract of Phytolacca Berries produced a mild degree of depression.
2. The toxicity of Phytolacca Berries was not as great as that of Phytolacca Root.

From the pharmacological data it can be concluded that Phytolacca Berries have no advantage over Phytolacca Root.

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Tabasco a Substitute for Capsicum*

By Carmel H. Olden and E. V. Lynn†

In a recent number of the Journal, Youngken (1) called attention to the pungency of tabasco peppers, the fruit of *Capsicum anuum* L. var. *conoides* Irish. Preliminary tests by us showed that the material is at least as pungent as capsicum, and we decided to determine by extraction whether it could advantageously be employed as a substitute and whether it actually contains capsaicine. We had available also a quantity of waste from making tabasco sauce and used this for extraction. Only the pulpy part of the fruit is employed in making sauce, the waste being largely seeds and cortical tissue.

EXPERIMENTAL

The material was ground to a coarse powder and extracted according to Tice (2) with ether. It

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