CONCLUSION.

Variations in serum calcium of the rabbit do not depart from normal with the administration of ethylene glycol in toxic amounts or with small doses given over a period of time.

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A STUDY OF THE CONSTITUENTS IN CASCARA SAGRADA EXTRACT I. ISOLATION OF A RHAMNO-GLYCOSIDE OF EMODIN.*[1]

BY HORACE L. SIPPLE, C. G. KING AND GEORGE D. BEAL.[2]

For more than fifty years cascara sagrada extracts have been under investigation in order to determine the chemical nature of the compounds which are responsible for the characteristic cathartic properties. The extracts are usually classified as anthracene, anthraquinone or emodin cathartics, because the characteristic constituents separated have been derivatives of methyl anthraquinones. Dohme and Englehardt (1) reported finding a substance present in cascara which resembled frangulin (m. p. 237°), but their conclusion was questioned by Jowett (2). Beal and Tumminkatti (3) and Daels (4) have shown that the anthraquinone-type substances present are in part free and in part combined in a form which is liberated by hydrolysis. It is now generally accepted that the anthraquinone derivatives are present in part in a glycosidic type of linkage (5), (6). Thorpe and associates have reported finding a glycoside (frangulin) of rhamnose and emodin in Rhamnus frangula (7). Definite identification of the most active substance or substances remains to be accomplished, however. This study of the separation of the chemical constituents of cascara sagrada extract has been made to obtain further information leading toward the identification of the cathartically active constituent or constituents. A glycoside of rhamnose and emodin has been identified as one of the substances present in considerable quantity.

EXPERIMENTAL.

A procedure has been developed for the first stages of a separation of the chemical constituents in an alcoholic extract of the dry cascara bark. The various fractions thus obtained were representative of types of material present, providing a

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basis for continued study of the individual compounds. The chief steps in the procedure may be summarized as follows:

1. A 300-Gm. sample of dry cascara bark was ground to approximately 10 mesh and extracted with 2 liters of 96% ethanol in a modified Soxhlet extractor for fifty hours. Extraction with ethanol rather than water prevents hydrolysis of glycosides (an undesired side reaction that would occur in an aqueous extract due to the action of accompanying enzymes), thus affording greater assurance that any products isolated would represent original constituents of the bark. The substances extracted by ethanol are chiefly water-soluble compounds. Except for a small amount of lipid material, they are precipitated from alcoholic solution upon the addition of solvents such as ethyl acetate, ethyl ether, isopropyl ether, petroleum ether, benzene or chloroform.

2. The alcoholic extract was filtered, evaporated *in vacuo* below 50° C. to approximately one-tenth of the original volume, treated with eight volumes of water and filtered.

3. The filtrate was stirred for two hours with approximately 35 Gm. of ferric hydroxide in suspension to give a clear filtrate which would not cloud when stirred with saturated NaCl solution.

4. The liquid phase from step 3 was treated with 300 cc. of a saturated solution of neutral lead acetate ($(CH_3COO)_2Pb.3H_2O$) and filtered, the small amount of precipitate formed being discarded.

5. A basic lead complex was precipitated at a p_H of approximately 7.4 (phenol red) by the addition of dilute ammonium hydroxide. The lead complex was centrifuged, the supernatant liquid decanted and discarded.

6. The lead complex was decomposed in concentrated sodium sulphate solution, the mixture being made slightly acid with acetic acid. Lead sulphate formed was removed by centrifuging and decanting the liquid phase. Reagents such as phosphoric acid, sulphuric acid and hydrogen sulphide have been avoided because of undesired side reactions which might occur.

7. The decanted liquid was evaporated *in vacuo* to 100 cc., treated with 4 volumes of methanol and filtered. Salts such as sodium sulphate, lead sulphate and some sodium acetate were precipitated. More salts, chiefly sodium acetate, were removed by evaporating the liquid to 50 cc. and filtering.

8. The liquid fraction was treated with one volume of absolute methanol, forming a brown precipitate which was centrifuged and discarded.

9. After evaporation to one-half the original volume, the liquid fraction was treated with one volume of absolute ethanol. A brown precipitate formed slowly. The mixture was allowed to stand in the refrigerator over night, centrifuged and decanted.

10. The liquid fraction, after evaporation to dryness, *in vacuo*, was taken up in 10 cc. of absolute methanol. The small amount of material not soluble in methanol was centrifuged and discarded.

11. The methyl alcohol solution was treated with five volumes of absolute acetone, causing the formation of an orange-red precipitate. The precipitate was separated in the centrifuge, washed with acetone and all liquid phases combined.

The precipitation of this acetone-insoluble material was repeated until a uniform and reproducible product was obtained. This product was then washed once with absolute acetone and dried *in vacuo* over calcium chloride. The amount of this material recovered was approximately 5 Gm. per Kg. of original bark.

12. The liquid phase from step 11 was evaporated *in vacuo* to a volume of 10 cc. and treated with ten volumes of petroleum ether, precipitating a small amount of red, oily material. A very small amount of a yellow, wax-like substance was recovered by decanting and evaporating the petroleum ether liquid phase.

The purified acetone-insoluble material obtained in step 11 did not reduce Benedict's reagent. An ether extract of an aqueous solution of the material gave no coloration with dilute ammonium hydroxide, indicating the absence of free hydroxy-methylanthraquinones (8), (9).

A 0.1-Gm. sample of the orange-red material treated with 1% HCl in a boiling water-bath for 30 minutes gave a positive test with Benedict's reagent, and an

ether extract of the hydrolyzed material treated with dilute ammonium hydroxide gave the pink coloration characteristic of a free hydroxy-methylanthraquinone.

A 2-Gm. sample dissolved in a mixture of 70 cc. of ethanol, 30 cc. of water and 6 cc. of HCl (concentrated), and heated on a boiling water-bath for eight hours gave water-soluble and water-insoluble products of hydrolysis. After dilution with three volumes of water and evaporation *in vacuo* below 50° C. in a stream of CO_2 to a volume of about 75 cc., the liquid was filtered in order to remove the water-insoluble product.

The product removed by filtration was recrystallized from glacial acetic acid and, after drying over NaOH *in vacuo*, had a melting point of 250° C. With pure emodin it gave a mixed melting point of 249° C. The pure emodin had a melting point of 251° C. It was concluded that this product of the hydrolysis was emodin.

The water-soluble fraction, after neutralization with silver carbonate and decolorization with charcoal, was evaporated *in vacuo* below 50° C. to a volume of approximately 20 cc. The specific rotation recorded was approximately +5.0.

The optical rotation of the unhydrolyzed glycoside from step 11 could not be determined readily because of the deep red color of solutions of the substance.

The phenylosazone of the water-soluble product of hydrolysis gave a melting point of 181° C., and mixed with rhamnose phenylosazone gave a melting point of 179° C. Rhamnose (Merck) gave a phenylosazone melting at 180° C.

A portion of the water-soluble fraction distilled with HCl (sp. gr. 1.06) gave a distillate that colored aniline acetate paper yellow, a test for methyl furfural (5). It was concluded that the hydrolysis product soluble in water was rhamnose, and that the compound hydrolyzed was a glycoside of rhamnose and emodin.

The recrystallized glycosidic substance (step 11) was inactive as a cathartic in doses of 120 mg. (extract from 3-Gm. bark) when given to mice. The guinea pig dose was above 300 mg. of the dry material. Pure emodin fed to mice in 5-, 10- and 20-mg. doses was also apparently inactive, although possibly slightly active at the 20-mg. level.

The minimum cathartic dose for mice of a concentrated aqueous extract of cascara sagrada was found to be equivalent to the extract from 50-mg. bark. The minimum dose per 250-Gm. guinea pig was equivalent to the extract from 300 mg. of bark.

The alcoholic extract of cascara sagrada fed to mice was active in doses equivalent to the extract from 50 mg. of bark. According to Munch (11) the approximate minimum human dosage is equivalent to the extract from 1 Gm. of bark.

SUMMARY.

A procedure has been developed for the first stages of a separation of the chemical constituents in an alcoholic extract of cascara sagrada.

A glycoside of rhamnose and emodin has been identified as one of the substances present in this alcoholic extract.

Biological tests have been made of the cathartic properties of (*a*) the rhamnose-emodin glycoside, (*b*) pure emodin, (*c*) an aqueous extract of cascara sagrada and (*d*) an alcoholic extract of cascara sagrada.

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STUDIES IN PERCOLATION.*

BY MILTON WRUBLE.

DETANNATED TINCTURE OF CINCHONA.

Tinctura Cinchonæ Detannata was official in the N. F. 1886, 1896 and 1906. The tannins were removed from the tincture as the name implies. As early as 1866, Walker (1) briefly described a method by which he prepared a fluidextract of cinchona of a beautiful red color which held the cinchotannates in solution, by percolating with diluted alcohol. Meumann, in 1886 (2), discussed the method for preparing detannated tinctures by means of ferric hydroxide. His method could be applied to any drug but was outlined for cinchona. The ferric hydroxide magma was added to the bark and intimately mixed. The mixture was transferred to a percolator and percolation allowed to proceed.

A year later, Meumann published a second paper (3) in which he recommended 2 parts of ferrous sulphate to 3 parts of bark. Remington (4), in 1887, furnished a formula for the detannated tincture according to which the U. S. P. fluidextract was treated with ferric hydroxide. In 1888, Tiarks (5) reported on the method generally employed for preparing detannated fluidextracts. He mentions the use of albumin, gelatin, lime and ferric hydroxide. Keutmann (6), in 1903, described a method for detannating cinchona by percolating a mixture of the bark with calcium hydroxide after first forming a magma of the two with water and adding ammonium carbonate. It is doubtful whether he obtained a completely detannated tincture since he reports a cherry-red color for the preparation made in this manner, whereas a completely detannated tincture is yellowish in color. However, the red color may have been due to cinchona red (not to cinchotannic acid) which is reported to be *soluble* in ammonia water.

Experimental Part.—Cinchona bark, No. 20 powder was used. When assayed according to the U. S. P. X, it yielded 4.70%, 4.32% and 4.26% of total alkaloids in as many assays. The tannin content was determined by the hide powder method (7). Three determinations yielded 4.18%, 4.17% and 4.46%, respectively.

* Scientific Section, A. PH. A., Madison meeting, 1933.