THE GLUCOSIDES OF CAULOPHYLLUM THALICTROIDES.

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The unexpected difficulties encountered in an attempt to isolate the glucosidal principles from the rhizome and root of *Caulophyllum thalictroides* (Linné), Michaux (Caulophyllum, N. F.), according to the method of Power and Salway, prompted the undertaking of a more extensive examination of the drug with some rather interesting results.

Caulophyllum, commonly known as "Blue Cohosh" and indigenous to North America, has been the subject of considerable chemical study since the first reported finding of an alkaloid by F. F. Mayer in 1863.

A review of the earlier history of Caulophyllum may be found in the report of J. U. Lloyd on "Caulophylline."[1] This review includes the work of F. F. Mayer,[2] A. E. Ebert,[3] and also his own earlier work.[4] In brief, these records show the isolation in pure form of an alkaloid, crystallized only as a hydrochloride; and of a crystalline glucoside.

Power and Salway,[5] continued the work and determined the chemical composition of these and other principles which they found. They isolated and crystallized the alkaloid and found it to be methyl cytisine, $C_{12}H_{16}ON_2$, m. p. 137° $[\alpha]_D$ —221.6; two glucosides were found and named, caulosaponin, $C_{54}H_{89}O_{17}.4H_2O$, m. p. 250–255° and caulophyllosaponin, $C_{66}H_{104}O_{17}$, m. p. 250–260° $[\alpha]_D$ +32.3; also citrullol $C_{28}H_{48}O_5$ or $C_{28}H_{45}O_2(OH)_3$, an enzyme, fixed oil and a small amount of volatile oil.

At the suggestion of Dr. Torald Sollman the writers began the work of isolating the various constituents from the drug with the intention eventually to determine to which of these agents the physiological activity may be assigned. We are also indebted to him for valuable suggestions on the work.

The drug used was obtained in crude form soon after collection, carefully assorted and dried at 43° before grinding, the same lot of drug being used for all work reported in this paper. We hereby express our thanks to Dr. E. E. Stanford for the selection and examination of the crude drug.

A. Isolation of the Glucosides.—The method of Power and Salway for the separation of the glucosides was followed, namely, (1) extraction of the drug with hot alcohol, with subsequent evaporation of the alcoholic solution to extract, (2) steam distillation of the extract for the separation of the volatile oil, (3) removing the glucosides from the aqueous residue with amyl alcohol, with subsequent removal of most of the amyl alcohol by distilling under reduced pressure, (4) precipitating the glucosides from amyl alcohol extract with ether, (5) crystallization of the glucosides from alcohol. The method resulted in the recovery of alcohol-soluble glucoside, which, however, was not crystallizable.

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[1] PROCEEDINGS AMERICAN PHARMACEUTICAL ASSOCIATION, 41, 115 (1893).
[2] JOUR. A. PH. A., 99 (1863).
[3] JOUR. A. PH. A., 203 (1864).
[4] "Drugs and Medicines of North America," 2, 154 (1887).
[5] *J. Am. Chem. Soc.*, 103, 193 (1913).

B.—The disappointing results obtained by this method (*A*) caused us to vary the procedure by taking advantage of the water insoluble quality of the glucoside. The drug was extracted with ether in a modified continuous extraction apparatus. The extract, after distilling the ether, was steam distilled, thereby obtaining a small amount of volatile oil. The residue was then extracted with petroleum ether, giving fixed oil. (A small amount of glucoside was also recovered.) After freeing the extracted drug from ether it was digested with hot alcohol, percolated, and the percolation continued with hot alcohol. Upon distilling the alcohol from the percolate a reddish brown extract was obtained. This was thoroughly mixed with pumice and quartz sand; then water added, after which it was filtered. The deposit on the filter was thoroughly washed with water, dried, dissolved in hot alcohol and filtered through animal charcoal. (Another portion was filtered through "Norit" with no particular advantage.) The alcohol was again removed and the above washing repeated, after which the precipitate was dried, dissolved in alcohol, filtered and evaporated. The dried product was then washed successively with petroleum ether and chloroform. This again resulted in the separation of the alcohol-soluble glucoside. The color was light yellow m. p. 210–220° $[\alpha]_D$ +31.2, but the material was not crystallizable from alcohol, varying hydroalcoholic solutions, alcohol with chloroform, with benzene, with carbon tetrachloride, or with benzine. Alcoholic solutions allowed to evaporate spontaneously produced no crystals.

C. Isolation of the Alkaloid.—The aqueous filtrate from the washing of the original extract B was evaporated to a syrupy consistence, sodium bicarbonate added and the mass extracted with chloroform for the separation of the alkaloid. (It was found that the dilute aqueous solution did not yield the alkaloid to chloroform even when made alkaline and saturated with sodium chloride.) The alkaloid was purified in the usual manner, using a minimum of dilute acid to shake out the chloroformic solution. The hydrochloride was formed in the final chloroformic solution by passing dry hydrochloric acid into it. This yielded a colorless flocculent precipitate, but upon the addition of alcohol (95%) and warming to dissolve the salt, followed by ether to a slight cloudiness, the alkaloidal hydrochloride was obtained in very fine acicular crystals, m. p. 120°, without decomposition, $[\alpha]_D$ −126.

The base methyl-cytisine reported by Power and Salway had m. p. 137° $[\alpha]_D$ −221.6; the hydrochloride decomposing at 250–255°. They state in a footnote that the water of crystallization in this salt could not be directly determined, since the substance slowly loses hydrogen chloride on dehydration.

D. Enzyme Action. (1) Crystallizable Glucoside Sparingly Soluble in Alcohol.—Our inability to isolate the crystalline glucoside or glucosides directly, led to further work taking into account the presence of enzyme.

A portion of the drug was moistened with water and kept in a closely covered vessel so that at no time did it become dry. It was kept at room temperature for one week (no decomposition was apparent by odor or growth of moulds), then dried, extracted with hot alcohol as before, the alcohol reduced by distillation, sand and pumice added, after which it was washed with water, dried, extracted from the sand pumice mixture with hot alcohol and filtered. The alcoholic solution was still highly colored, but a sparingly-alcohol-soluble glucoside crystallized

very readily from it. After re-crystallizing twice from alcohol, it was obtained in colorless silky needles m. p. 243–246° $[\alpha]_D$ +45. By evaporating the alcoholic liquor from which the above glucoside was crystallized, repeating the washing with water and crystallizing from alcohol, additional glucoside as above was obtained, with a total yield of 15 Gm. from 8 pounds of drug.

This crystalline glucoside is soluble in alkali hydroxides or carbonates, from which it may be recovered without change by precipitating with dilute hydrochloric acid, washing with water and crystallizing from alcohol. The optical activity and melting point remain the same, which is taken as evidence that it is unchanged. Hydrolysis of the glucoside by acids is very slow and in aqueous suspension requires boiling.

(*2*) *Alcohol-Soluble Glucosidal Material.*—The alcoholic solution from which the crystalline glucoside was separated yielded a non-crystalline product, readily soluble in alcohol, insoluble in chloroform, ether, benzine or benzene, is light yellow in color, m. p. 230–240° $[\alpha]_D$ +37. This product gives the characteristic glucosidal reaction and is not crystallizable from alcohol or hydroalcoholic solutions. Dialysis of the sodium salt was tried with negative result. This product is probably contaminated with the crystallizable glucoside D(1). The yield was 1.9 Gm. from 8 pounds. of drug.

(*3*) *The Constants of the Glucosides* which Power and Salway report, together with those reported above under B (D, 1 and 2) are:

	M. P.	$[\alpha]_D$.	Alcohol.
Caulosaponin (cryst.)	250–255°	...	sparingly soluble
Glucoside D (1) (cryst.)	243–246°	45	sparingly soluble
Caulophyllosaponin (cryst.)	250–260°	32.3	very soluble
Glucoside D (2) (non-cryst.)	230–240°	37	very soluble
Glucoside B (non-cryst.)	210–220°	31.2	very soluble

(*4*) *To investigate further the action of the enzyme* in bringing about the change to crystal form of glucoside, it was assumed the enzyme is present in quantity such that it could produce the change in additional non-crystalline glucoside which might be added to it, and such is the case, as is shown by the following test. One Gm. of the non-crystalline glucoside B was thoroughly mixed with 5 Gm. of drug, then moistened and kept in this condition for about a week. The mixture was then dried, extracted with hot alcohol, reduced to extract and washed with water etc., as described in B, after which it was dried, dissolved in hot alcohol and from this was obtained 0.15 Gm. of sparingly alcohol-soluble, but crystalline, glucoside. No attempt was made to recover more of it since this amount indicates the greater portion came from the non-crystalline product B. The same experiment using the non-crystalline material D(2) resulted only in a non-crystalline product. This suggests that glucosides D(1) and D(2) are products of the ferment-hydrolysis of glucoside B. No attempt has been made as yet to subject B to other hydrolytic agents.

E. Acidic Character of the Glucosides.—The behavior of these glucosides toward alkalies indicates that they are acidic. It does not seem likely that this acid character is due to a phenol since they are freely soluble in sodium carbonate. Sodium bicarbonate, however, reacts very slightly with them in cold aqueous solution.

To show the relative acidity of the three forms of glucosides referred to in B, D(1 and 2), one-tenth Gm. of each was dissolved in warm neutral 70% alcohol and the acidity determined with $N/50$ sodium hydroxide, phenolphthalein indicator. They required respectively 6.25 cc., 6.00 cc. and 6.30 cc. In each case the alkali was increased to 10 cc., the excess determined with $N/50$ sulphuric acid and the acid required equalled the excess alkali added. The increased amount of alkali required for B, and D(2), both of which were not crystallizable, may be due to alcohol soluble saponifiable resins.

F. Extractability of Caulophyllum by Alcoholic Menstrua.—It is possible that a considerable proportion of the Caulophyllum under the ordinary condition of handling would be subject to enzyme action, in which case the comparatively alcohol-insoluble glucoside results.

The menstruum prescribed in the National Formulary for the preparation of the fluidextract consists of alcohol, three volumes, and water, one volume. The fluidextract made from the drug where enzyme action has occurred therefore represents only a small proportion of the glucoside in the drug. If the fluidextract is to be used instead of the isolated active constituents, it would seem advisable to add sodium carbonate to the menstruum to render the glucoside soluble. This does not affect the solubility of the alkaloid, as the base is readily soluble in either water or alcohol.

SUMMARY.

1. Analysis of Caulophyllum by the method (*A*) of Power and Salway yielded the alkaloid methylcytisine and a non-crystalline glucosidal material which differed from that described by them.

2. A modification of the above method (*B*) resulted in a similar non-crystallizable product.

3. By moistening the drug to allow enzyme action previous to extraction, a crystalline glucoside D(1), also a non-crystallizable glucosidal product D(2) was obtained.

4. Power and Salway obtained two crystalline glucosides Caulosaponin and Caulophyllosaponin. The crystalline glucoside obtained after fermentation D(1) by its slight solubility in alcohol and its melting point, resembles caulosaponin, but is optically active, while they do not report on the optical activity of caulosaponin.

The caulophyllosaponin reported by them is optically active, very soluble in alcohol and if present in the fermented drug referred to, it would appear in the glucosidal material reported in D(2), which is optically active but did not crystallize from alcohol or hydroalcoholic solutions.

5. The change in the glucoside to crystal form apparently is due to enzyme action since a portion of the non-crystalline product B when mixed with drug and moistened yielded the crystal form. In other words, enzyme action in the drug affects the character of the glucoside obtained therefrom, namely,

In the absence of enzyme action, only non-crystalline glucoside obtained.

Enzyme action results in crystalline and non-crystalline glucosidal products, which differ also by the degree of their solubility in alcohol.

The pharmacologic actions of the isolated constituents are under investigagation and we hope to report on these later.