[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY]

Saponins and Sapogenins. XX. Bethogenin and Trillogenin, New Sapogenins from *Trillium Erectum*

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During the course of the isolation of diosgenin from commercial powdered beth root (*Trillium erectum*) by the procedure of Marker, Turner and Ulshafer[2] we have obtained a second sapogenin in an amount at least as great as the amount of diosgenin. This compound, which we have named "bethogenin," has the molecular formula $C_{27}H_{40}O_4$. It gives a yellow color with tetranitromethane and is precipitated by digitonin, suggesting that it is an unsaturated steroid sapogenin. Reaction with acetic anhydride in pyridine gave only a monoacetate, while benzoyl chloride and pyridine gave a monobenzoate. Two oxygen atoms tentatively may be assumed to be the inert oxygen atoms in the side chain characteristic of steroid sapogenins. This makes it still necessary to account for one more oxygen atom.

Attempts to dehydrate bethogenin failed, indicating that this oxygen atom is not a tertiary hydroxyl group. This was confirmed by a Zerewitinow determination which showed only one active hydrogen. On catalytic reduction in neutral alcohol solution with hydrogen and palladium catalyst, two moles of hydrogen were absorbed per mole of bethogenin. While isolation of a pure hydrogenated bethogenin has not yet been successful, acetylation of the reduction product gave a readily crystallized compound which proved to be a diacetate. From this it appears that bethogenin contains a carbonyl group.

In order to confirm this view, attempts were made to prepare a carbonyl derivative. It was found that bethogenin reacted with hydroxylamine in pyridine to give a new compound but analyses indicated that this was a dioxime. At present such behavior is difficult to explain. One possibility is that bethogenin is a diketone with one very readily enolizable carbonyl group so that it gives the reactions both of a carbonyl group and of a double bond and hydroxyl group. However, all of the usual tests for an enol group gave negative results. Another possibility is that the second molecule of hydroxylamine reacted with the side chain. Indication that the side chain of bethogenin behaves differently from that of other steroid sapogenins was obtained when it was found that bethogenin could not be converted into a pseudosapogenin.[3] Heating with acetic anhydride at 200° gave only uncrystallizable gums.

A third sapogenin has been isolated in small amount which appears to be unique among the known steroid sapogenins in that the two characteristic inert oxygen atoms are lacking. This compound has the empirical formula $C_{27}H_{48}O_4$ and yields a tetraacetate, $C_{35}H_{56}O_8$. Nothing further has been done with this compound because of the small amount of material that has been available. One is inclined to speculate that the customary side chain has been reduced with the formation of an open chain and two free hydroxyl groups. For this substance we suggest the name "trillogenin."

A very small amount of a fourth sapogenin, which appears to be identical with chlorogenin, also has been isolated.

The authors are indebted to Dr. L. F. Fieser for his interest in this problem and for permitting one of us (F. C. C.) to spend some time in this Laboratory working on the problem.

Experimental

Diosgenin and Trillin.—Ten pounds of powdered beth root[4] was extracted and hydrolyzed according to the procedure of Marker, Turner and Ulshafer.[2] The crude diosgenin was filtered from the second hydrolysis mixture and weighed only 15 g. It could not be purified readily by crystallization but on extraction in a Soxhlet apparatus with 60–70° ligroin, the diosgenin was removed and then readily purified by crystallization from acetone.

The unextracted residue amounting to 2.7 g. was purified by crystallization from glacial acetic acid and melted at 269.5°–271° when the capillary tube was placed in a preheated bath: $[\alpha]^{26}D$ −103.4°. $[\alpha]^{26}Hg$ −127.2° in dioxane. This substance is the "trillin" of Marker and Krueger[5] as shown by analysis, conversion to the acetate, and further hydrolysis to diosgenin. Our analysis checks better for the anhydrous compound than for the hemihydrate of Marker and Krueger.

Anal. Calcd. for $C_{33}H_{52}O_8$: C, 68.7; H, 9.1. Found: C, 69.3; H, 9.0.

(1) Research Assistant on funds from the Rockefeller Foundation.

(2) Marker, Turner and Ulshafer, THIS JOURNAL, **62**, 2542 (1940).

(3) Marker and co-workers, *ibid.*, **62**, 518, 648, 898 (1940).

(4) Purchased from S. B. Penick and Company and described in their price list as "*Trillium erectum* and species."

(5) Marker and Krueger, THIS JOURNAL, **62**, 2548 (1940).

The **acetate**, m. p. 204–205°, has a rotation of $[\alpha]^{30}D$ −71.4°; $[\alpha]^{30}Hg$ −80.2° in dioxane.

Chlorogenin.—The aqueous alcoholic acid filtrate and washings from the crude diosgenin after standing for several days deposited about 0.2 g. of material which, after several recrystallizations from methyl alcohol, melted at 262–272° and did not depress the melting point of chlorogenin, m. p. 270–274°, but depressed the melting point of gitogenin and of trillin. Conversion to the acetate did not give enough product to purify to a melting point above 130°.

Bethogenin.—When the above alcoholic filtrates were poured into a large volume of water, a black tar precipitated. This was dissolved in 500 cc. of alcohol, just sufficient water added to prevent miscibility with 60–70° ligroin and the solution exhaustively extracted in a continuous liquid extractor with 60–70° ligroin. The ligroin extract on concentration left a yellow semi-solid residue weighing 20 g. which on repeated crystallization from methyl alcohol gave long white needles with a maximum melting point of 182–185°. Purification takes place more readily from a diluted rather than from a hot saturated solution. If the bethogenin was prepared by hydrolysis of the acetate with methyl alcoholic potassium hydroxide solution or by recrystallization from alcohol containing potassium hydroxide, a product was obtained melting at 193–194°. When this was recrystallized from pure methyl alcohol, the melting point dropped to 184–186° and on a second recrystallization to 163–182°. A sample which melted at 180–184° when first prepared melted at 160–173° after standing two weeks. The first two analyses given below are on material which had been purified by crystallization from methyl alcohol alone, while the second two are on samples that had been crystallized from alkaline alcohol. Bethogenin as obtained by crystallization from methyl alcohol contains solvent of crystallization. All samples were dried for analysis at 110° and 20 mm. to constant weight.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.67; H, 9.40; one OH, 3.97; mol. wt., 428.6. Found: C, 75.60, 75.06, 75.55, 75.60; H, 9.70, 9.87, 9.50, 9.96; OH (Zerewitinow), 3.84; mol. wt. (Rast), 442; $[\alpha]^{24}D$ −98.4° in dioxane.

Bethogenin gives a yellow color with tetranitromethane and is precipitated from alcoholic solution with digitonin. The ferric chloride test for enols, the Legal test, the Rosenheim test and the Zimmermann-Jaffee reaction were all negative. In the Lieberman test, a red color was formed which slowly darkened to a deep green.

The **acetate** was prepared by adding 12 cc. of acetic anhydride to 1.97 g. of bethogenin dissolved in 20 cc. of pyridine and allowing the solution to stand for one hour. The product crystallized and was filtered, washed with water and crystallized twice from a mixture of four parts ethyl alcohol and one part benzene. It melted at 230–232° and gave a yellow color with tetranitromethane.

Anal. Calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 8.99. Found: C, 73.50, 74.00, 74.62; H, 9.70, 9.89, 9.00; $[\alpha]^{24}D$ −94.4° in dioxane.

When 1 g. of bethogenin in 6 cc. of acetic anhydride and 6 cc. of pyridine was heated in a sealed tube at 100° for twenty hours,[6] a brownish colored solution resulted from which only the monoacetate could be isolated.

(6) Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 823 (1937).

A solution of 0.24 g. of bethogenin in 10 cc. of dry pyridine was treated with 3 cc. of benzoyl chloride and allowed to stand overnight. After removing the pyridine under reduced pressure, water was added and the precipitate washed with hot water to remove benzoic acid. The residue was crystallized from methyl alcohol and gave 0.15 g. of the **benzoate** as needles, m. p. 190–191. Three more crystallizations from 1:4 benzene–methyl alcohol mixture raised the melting point to 212–215°.

Anal. Calcd. for $C_{34}H_{44}O_5$: C, 76.65; H, 8.33. Found: C, 76.97; H, 9.10; $[\alpha]^{24}D$ −65.1° in dioxane.

Tetrahydrobethogenin Diacetate.—A mixture of 1.5 g. of bethogenin, 0.3 g. of palladium oxide and 200 cc. of ethyl alcohol was shaken overnight under hydrogen at a pressure of 30 pounds per square inch. The mixture was filtered to remove the palladium and the filtrate evaporated to dryness. This product could not be crystallized readily from any of the common organic solvents. Crystallization did take place from 75% aqueous methyl alcohol but constant melting material could not be obtained even after thirteen crystallizations.

Acetylation of 0.86 g. of the crude product with 5 cc. of acetic anhydride in 7 cc. of pyridine gave 0.77 g., m. p. 126–129°. After fourteen crystallizations from ligroin (60–70°) the melting point was constant at 141–144°. It gave a negative test for unsaturation with tetranitromethane.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.05; H, 9.37. Found: C, 71.87, 72.30; H, 9.25, 9.66; $[\alpha]^{24}D$ −156° in dioxane.

A smaller sample of bethogenin in a semi-micro apparatus showed absorption of four atoms of hydrogen per mole.

Reaction of Bethogenin with Hydroxylamine.—A solution of 0.5 g. of bethogenin and 0.4 g. of hydroxylamine hydrochloride in 4 cc. of pyridine and 4 cc. of absolute alcohol was heated on the steam-bath for one hour and the flask allowed to stand overnight. The precipitate was filtered, washed with 95% alcohol and crystallized from absolute alcohol to a constant melting point of 241–243°.

Anal. Calcd. for $C_{27}H_{42}O_4N_2$: C, 70.72; H, 9.22; N, 6.11. Found: C, 70.42, 70.40; H, 9.54, 9.96; N, 5.94, 5.92.

Attempts to hydrolyze the reaction product to the original compound were unsuccessful.

Attempts to Prepare a Pseudobethogenin.—Three attempts were made to convert bethogenin into a pseudobethogenin. In the first bethogenin was heated with acetic anhydride at 200°, in the second bethogenin acetate was heated with acetic anhydride at 165° and in the third run bethogenin acetate and acetic anhydride were heated at 200°. Only unchanged acetate or resinous products could be isolated.

Trillogenin.—After standing for several months, the methyl alcohol solution which had been extracted with ligroin deposited a few tenths of a gram of precipitate which, after several crystallizations from methyl alcohol, melted at 206–210°; $[\alpha]^{24}D$ −41.6; $[\alpha]^{24}Hg$ −54.3 in dioxane. The substance does not give a color with tetranitromethane and gives marked depressions in melting point when mixed with diosgenin or tigogenin.

Anal. Calcd. for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08. Found: C, 73.74, 74.17; H, 11.05, 11.08.

The compound was recovered unchanged after several hours of refluxing with alcoholic hydrochloric acid, proving that it is not a prosapogenin. When refluxed with acetic anhydride and sodium acetate, an **acetate** was obtained which, after several crystallizations from aqueous methyl alcohol, melted at 102–103°; $[\alpha]^{24}D$ 0°; $[\alpha]^{24}_{Hg}$ −3.5° in dioxane.

Anal. Calcd. for $C_{27}H_{44}O_4(CH_3CO)_4$: C, 69.51; H, 9.33; acetyl, 28.48. Found: C, 69.97; H, 9.28; acetyl, 29.86, 29.52.

Summary

Bethogenin, a new sapogenin having the empirical formula $C_{27}H_{40}O_4$, has been isolated from the hydrolysis products of extracts of powdered beth root (*Trillium erectum*). It appears to be an unsaturated steroid sapogenin with one hydroxyl group and one carbonyl group.

Another new sapogenin, trillogenin, having the empirical formula $C_{27}H_{48}O_4$, has been obtained in very small amount. This compound is saturated and is unique among the known steroid sapogenins because it lacks the two characteristic inert oxygen atoms, all four oxygen atoms being accounted for by hydroxyl groups.

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Halogen Compounds Derived from 4-Methyl-2,5-diphenylfuran

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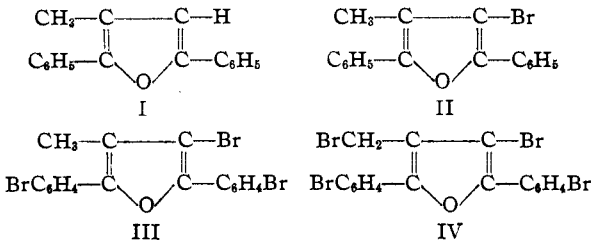
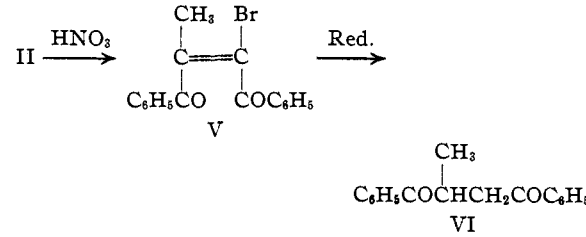
The bromination of this furan was of interest in connection with orientation[2] and the synthesis of certain brominated *cis* unsaturated diketones which could not easily be made otherwise.

The bromination of methyldiphenylfuran (I) proceeded stepwise with the formation successively of the mono,[3] tri and tetrabromo derivatives (II, III and IV).



Zinc and acetic acid reduced only the tetrabromo derivative and eliminated but one bromine to give the tribromo compound. The last bromine introduced therefore must have been aliphatic and located on the methyl group; and the three bromines of the tribromo compound must be aromatic. The tribromo compound on oxidation first with nitric acid and then with potassium permanganate gave more than one molecule of *p*-bromobenzoic acid, showing that two of the halogens occupied the two phenyl para positions. The remaining bromine atom, evidently the first introduced, must, therefore, be in the furan β-position (*cf.* II). This was confirmed by nitric acid oxidation of the monobromo derivative (II) to the unsaturated bromo 1,4-diketone[3] (V) and reduction of this to the saturated diketone (VI) with loss of the bromine. The structures of the three bromination products therefore are as represented in formulas II, III and IV.



Each of the three brominated furans (II, III and IV) could be oxidized by the nitric–acetic acid reagent to the corresponding unsaturated 1,4-diketones (V, VII and IX) which are presumed to be *cis* from the mode of formation. The first of these oxidation products (V) already has been reported.[3] The other two (VII and IX) could be converted into the saturated diketone (VIII) by reduction with zinc and acetic acid and into the furan (X) by reduction under dehydrating conditions.

The *cis*-di-[bromobenzoyl]-propylene (XI) has become available through oxidation of the corresponding furan (X). This new unsaturated diketone in turn was reduced to the saturated diketone (VIII) and to the furan (X).

Unfortunately, it has not yet been possible to

(1) Present location, National Aniline Division, Allied Chemical and Dye Corp., Buffalo.

(2) Lutz and Kibler, THIS JOURNAL, **62**, 1520 (1940).

(3) Lutz and Stuart, *ibid.*, **59**, 2316 (1937).