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Structural Studies on Glaucarubin from *Simarouba glauca*

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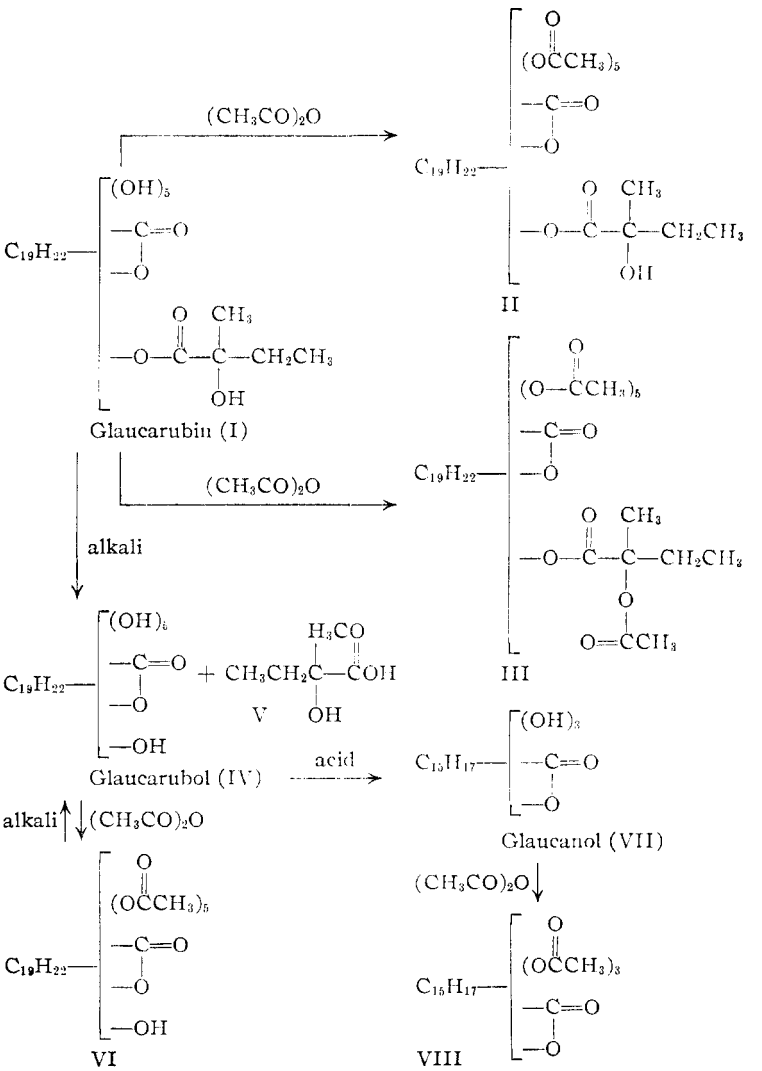
The molecular weight, functional groups and part of the structure of glaucarubin, isolated from *Simarouba glauca*, have been determined. Glaucarubin appears to have the molecular formula $C_{25}H_{36}O_{10}$. The ten oxygen atoms are accounted for by the presence of one lactone, one ester and six hydroxyl groups. Alkaline hydrolysis of glaucarubin cleaved the ester linkage to give an optically active α-methyl-α-hydroxybutyric acid and glaucarubol, $C_{20}H_{28}O_8$, a hexahydroxylactone. Glaucarubol was further degraded by acid hydrolysis to glaucanol, $C_{15}H_{20}O_5$, a trihydroxylactone.

In 1908, Gilling[1] isolated from *Simarouba* bark a crystalline, bitter substance, simaroubin, to which he assigned the formula $C_{22}H_{30}O_9$. Some thirty years later, Glemser and Ott[2] examined the crystalline constituents of the bark of *Simarouba amara*. They isolated simaroubin and confirmed the formula $C_{22}H_{30}O_9$; two other compounds, simaroubidin, $C_{22}H_{32}O_9$, and another of undetermined composition, were also obtained.

Recent studies in these laboratories led to the isolation of a crystalline compound from *Simarouba glauca*, which has been designated glaucarubin. The physical and chemical properties of glaucarubin are different from those reported for simaroubin, simaroubidin, or any previously reported *Simarouba* constituent. We wish to present here work on the chemical characterization and degradation of glaucarubin.

Glaucarubin (I) isolated from *Simarouba glauca* was purified by recrystallization from methanol. The pure compound crystallized as white platelets, bitter to the taste, and melting at 250–255° dec. It is dextrorotatory in pyridine or methanol solution. Glaucarubin is neutral to litmus, insoluble in sodium bicarbonate solution, but readily soluble in 0.1 *N* sodium hydroxide solution. Evidence provided by elementary analyses, potentiometric titrations and an ebullioscopic molecular weight determination indicated that the molecular formula of glaucarubin was $C_{25}H_{36}O_{10}$. The compound exhibited no distinctive ultraviolet absorption. Its infrared spectrum revealed the presence of hydroxyl groups and also of ester, lactone or carbonyl absorption; no carboxyl group absorption was noted. Acetylation of glaucarubin yielded a pentaacetyl II and a hexaacetyl derivative III. Infrared examination showed the latter derivative to be completely acetylated.

Glaucarubin dissolved readily in aqueous alkali with opening of the lactone ring and salt formation; prompt acidification of the solution resulted in the recovery of unchanged starting material. Upon longer standing in alkaline solution, cleavage into two fragments occurred. Acidification of the solution caused the separation of a crystalline precipitate which after purification was found to possess the formula $C_{20}H_{28}O_8$. It has been designated glaucarubol (IV). A hydroxyacid V was isolated by ether extraction of the acidified mother liquor from the precipitation of glaucarubol and was observed to have the composition $C_5H_{10}O_3$. Both degradation products were dextrorotatory.



Glaucarubol exhibited an infrared spectrum characterized by hydroxyl group and ester, lactone or carbonyl group absorption. The compound dissolved in aqueous alkali to yield a sodium salt which in the infrared showed only hydroxyl and

(1) C. Gilling, *Pharm. J.*, **80**, 510 (1908).
(2) O. Glemser and E. Ott, *Ber.*, **70B**, 1513 (1937).

carboxylate ion absorption, thus demonstrating the absence in glaucarubol of carbonyl and ester groups. Acetylation of glaucarubol gave a pentaacetyl derivative VI which still possessed hydroxyl group absorption in its infrared spectrum. Treatment of the pentaacetate with alkali regenerated unchanged glaucarubol. The oxygen content of glaucarubol is thus accounted for by the presence of one lactone and six hydroxyl groups, one of which is hindered and perhaps tertiary.

Lead tetraacetate oxidation of the five-carbon hydroxyacid V gave methyl ethyl ketone, indicating an α-methyl-α-hydroxybutyric acid structure for the degradation product. This was confirmed by the identity of the infrared absorption spectra of the degradation product and its *p*-bromophenacyl ester with those of synthetic racemic α-methyl-α-hydroxybutyric acid[3] and its corresponding derivative.

Acid hydrolysis of glaucarubol led to further degradation and to the isolation of a crystalline product glaucanol, $C_{16}H_{20}O_5$ (VII). Infrared studies of glaucanol and of its sodium salt showed it to contain hydroxyl groups and a lactone. Glaucanol formed a triacetyl derivative VIII which was completely acetylated, as indicated by the absence of hydroxyl group absorption in the infrared spectrum. Accordingly, this degradation product may be characterized as a trihydroxylactone.

The suggested relationships of glaucarubin and its degradation products are shown in formulas I–VIII. It appears likely that in glaucarubin the five-carbon hydroxy acid is attached through an ester linkage to the hindered (tertiary?) hydroxyl group of glaucarubol. In glaucarubin, the presence of the free tertiary hydroxyl group of the hydroxyacid, which is undoubtedly somewhat resistant to acylation, would explain the mixture of penta- and hexaacetyl derivatives which is obtained. After removal of the hydroxyacid by alkali, the resulting glaucarubol then contains a new unreactive hydroxyl group. This group, however, apparently is lost in the subsequent acid hydrolysis, since all three hydroxyl groups of glaucanol are readily acetylated.

Further studies directed toward the elucidation of the structure of glaucarubin are in progress.

Experimental

Isolation of Glaucarubin.—*Simarouba glauca* (the defatted seeds) (500 g.) was treated with 4 l. of water for 16 hours. The slurry was warmed and then agitated for three hours at 70–75°. After the addition of 150 g. of filter aid (Hyflo Supercel), the hot mixture was filtered, and the material was washed with 500 ml. of hot water. The material was extracted a second time with 3750 ml. of water containing 1.7 ml. of glacial acetic acid.

The extracts (6550 ml.) were allowed to stand overnight at 0–10° and filtered. The solution was concentrated under reduced pressure to 2850 ml. and cooled to 0–10°. The solution was decanted from a small amount of tar and concentrated further to 600 ml. After standing overnight at 0–15° the crude glaucarubin was filtered off, washed with a little cold water and dried. More material was obtained from the aqueous mother liquor by further concentration.

The crude product (wt. 5.88 g., m.p. *ca.* 250°) was twice recrystallized by dissolving in 300 ml. of hot methanol, adding 0.5 g. of decolorizing charcoal and filtering. The filtrate was concentrated at atmospheric pressure to about 40 ml. or until crystallization began. The concentrate was allowed to crystallize at 0–5° for four hours and the product was filtered, washed with cold methanol and dried *in vacuo* yielding 1.9 g. of glaucarubin. Solubility analysis in methanol indicated that this material contained less than 3% impurity.

Further crystallization from methanol yielded white platelets, m.p. 250–255° dec., $[\alpha]^{25}D$ +45° (*c* 1.7 in pyridine), $[\alpha]^{25}D$ +69° (*c* 0.6 in methanol). A molecular weight of 482 was obtained when glaucarubin was treated with 0.05 *N* sodium hydroxide for six hours at 62° and the hydrolysate back-titrated with acid. An equivalent weight of 463 was obtained when the above hydrolysate was acidified to *p*H 3 and allowed to stand overnight before titrating with alkali.

Anal. Calcd. for $C_{25}H_{36}O_{10}$ (496): C, 60.47; H, 7.31. Found: C, 60.60; H, 7.38.

An ebullioscopic determination of the molecular weight in isopropyl alcohol–water azeotrope gave a value of 456.

Acetylation of Glaucarubin.—A solution of 500 mg. of glaucarubin in 2 ml. of pyridine and 2 ml. of acetic anhydride was heated on a steam-bath for 1.5 hours. Removal of the solvents left a 740-mg. residue. The residue was taken up in benzene and chromatographed on 20 g. of acid washed alumina. The column was washed with 50 ml. of 5% ether in benzene solution. Elution with 150 ml. of 35% ether in benzene solution gave 292 mg. of white solid. Two crystallizations from Skellysolve C yielded 195 mg. of white platelet crystals, m.p. 161.5–163.5°, $[\alpha]^{25}D$ +49° (*c* 1.9 in pyridine). The infrared curve showed no hydroxyl group absorption.

Anal. Calcd. for $C_{25}H_{30}O_{10}(CH_3CO)_6$: C, 59.33; H, 6.46; CH_3CO, 34. Found: C, 59.34; H, 6.46; CH_3CO, 34.

Elution of the column with 100 ml. 50% ether in ethyl acetate solution gave 193 mg. of white solid. Crystallization from Skellysolve C yielded 131 mg. of white cubic crystals, m.p. 224–228°, $[\alpha]^{25}D$ +26° (*c* 2.0 in pyridine). Infrared analysis showed some hydroxyl group absorption.

Anal. Calcd. for $C_{25}H_{31}O_{10}(CH_3CO)_5$: C, 59.51; H, 6.47; CH_3CO, 30.46. Found: C, 60.01; H, 6.54; CH_3CO, 30.40.

Alkaline Hydrolysis of Glaucarubin.—A solution of 5.002 g. of glaucarubin in 25 ml. of 1 *N* sodium hydroxide was allowed to stand at room temperature for 18 hours. Acidification with 3 ml. of concentrated hydrochloric acid yielded 3.714 g. (94% yield) of heavy white crystals, m.p. 260–275° dec. Several recrystallizations from methanol yielded white crystals of glaucarubol, m.p. 290–292° dec., $[\alpha]^{25}D$ +38° (*c* 0.8 in pyridine). An equivalent weight of 397 was obtained upon titrating an alkaline solution of glaucarubol. An ebullioscopic molecular weight determination in isopropyl alcohol–water azeotrope gave a value of 403.

Anal. Calcd. for $C_{20}H_{28}O_8$ (396): C, 60.60; H, 7.12. Found: C, 60.87; H, 7.27.

The acid filtrate from above was continuously extracted with ether for 22 hours. Sublimation of the residue from the ether extract at 50–75° (1 mm.) gave 1.100 g. (94% yield) of fine white needles of a hydroxy acid, m.p. 75–76°, $[\alpha]^{25}D$ +9° (*c* 1.3 in chloroform).

Anal. Calcd. for $C_5H_{10}O_3$ (118): C, 50.81; H, 8.53. Found: C, 51.21; H, 8.46; equiv. wt., 118, and pK_a 4.8.

The *p*-bromophenacyl ester of the hydroxyacid, prepared in the usual way, melted at 85–87°.

Anal. Calcd. for $C_{13}H_{15}O_4Br$: C, 49.54; H, 4.80; Br, 25.36. Found: C, 50.03; H, 4.80; Br, 25.50.

Comparison of the Hydroxyacid with α-Methyl-α-hydroxybutyric Acid.—A sample of racemic α-methyl-α-hydroxybutyric acid, m.p. 69–69.5°, was prepared.[3] A comparison of the infrared absorption curves of the synthetic and natural products showed identity. The *p*-bromophenacyl ester, m.p. 87.5–88.5°, of the synthetic hydroxyacid was prepared by the usual methods. Again, a comparison of the infrared absorption curves of the *p*-bromophenacyl esters of the synthetic and natural hydroxyacids showed identity.

Lead Tetraacetate Oxidation of the Natural Hydroxyacid.—A 100-mg. sample of the hydroxyacid, m.p. 75–76°, from glaucarubin was treated with a slurry of lead tetraacetate crystals in acetic acid. The gaseous oxidation product was swept with nitrogen into a solution of 2,4-dinitrophenyl-

(3) W. G. Young, R. T. Dillon and H. J. Lucas, THIS JOURNAL, **51**, 2531 (1929).

hydrazine while the oxidation mixture was heated to boiling. A 2,4-dinitrophenylhydrazone formed which melted at 109–116° after two crystallizations from ethanol.

Anal. Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.76; H, 4.34; N, 21.96.

A mixed melting point with authentic methyl ethyl ketone 2,4-dinitrophenylhydrazone, m.p. 109–117°, was 109–116°.

The oxidation product from a similar experiment was converted to a semicarbazone melting at 142–143° after sublimation at 130–145° (1 mm.).

Anal. Calcd. for $C_5H_{11}N_3O$: C, 46.49; H, 8.59; N, 32.54. Found: C, 46.67; H, 8.71; N, 32.49.

A mixed melting point with authentic methyl ethyl ketone semicarbazone, m.p. 142–142.5°, was 141–142.5°.

Acetylation of Glaucarubol.—A 500-mg. sample of glaucarubol was dissolved in 2 ml. of pyridine and 4 ml. of acetic anhydride with warming. The clear solution was heated on a steam-bath for 1.5 hours. Removal of the solvents gave 760 mg. of a white solid. This was dissolved in benzene and chromatographed on 20 g. of acid-washed alumina. The column was washed with benzene and ether. Elution with 150 ml. of 35% ethyl acetate in ether gave 528 mg. of oil which was dissolved in 125 ml. of hot Skellysolve C. Cooling gave a precipitate which afforded 465 mg. of long white crystals, m.p. 199–201°, $[\alpha]^{25}D$ +23° (*c* 1.0 in pyridine), on standing for two days.

Anal. Calcd. for $C_{20}H_{23}O_8(CH_3CO)_5$: C, 59.40; H, 6.31; CH_3CO, 35.5. Found: C, 59.71; H, 6.23; CH_3CO, 36.

Hydrolysis of the Acetate of Glaucarubol.—A 100-mg. sample of the acetate of glaucarubol was treated with 3 ml. of 5% alcoholic potassium hydroxide and heated under reflux for 45 minutes, during which time the acetate dissolved. The solution was allowed to stand for one day. The ethanol was removed and the residue dissolved in water. Addition of acid gave 44 mg. of white crystals, m.p. 280–285°. A mixed melting point with glaucarubol was 278–285°. A comparison of the infrared absorption curves of the hydrolysis product and glaucarubol showed identity.

Aqueous Hydrochloric Acid Hydrolysis of Glaucarubol.—A 1.000-g. sample of finely ground glaucarubol was treated with 100 ml. of 0.1 *N* hydrochloric acid and the resultant slurry heated on a steam-bath. The clear solution obtained in 30 minutes was heated for an additional 30 minutes. The resultant solution was lyophilized and the residue taken up in chloroform and chromatographed on 25 g. of acid-washed alumina. The column was washed with 80 ml. of chloroform. Elution with 110 ml. of acetone gave 568 mg. of white glassy solid. A solution of the white solid in 10 ml. of ethylene dichloride, when allowed to stand overnight, gave 277 mg. of square platelet crystals. Two crystallizations from ethylene dichloride gave 229 mg. of white platelet crystals of pure glaucanol, m.p. 229–233°, $[\alpha]^{25}D$ +147° (*c* 0.4 in methanol) and $[\alpha]^{25}D$ −65° (*c* 1.0 in 0.1 *N* sodium hydroxide).

Anal. Calcd. for $C_{16}H_{20}O_5$ (292): C, 65.73; H, 6.89. Found: C, 65.54; H, 6.54; equiv. wt., 291.

Acetylation of Glaucanol.—A solution of 44 mg. of glaucanol in 1 ml. of pyridine and 1 ml. of acetic anhydride was heated on a steam-bath for 1.5 hours. The solvents were removed and the residue taken up in benzene and chromatographed on 5 g. of acid-washed alumina. Elution with 150 ml. of benzene gave 48 mg. of a white solid. Two crystallizations from Skellysolve C gave 44 mg. of white crystals, m.p. 210–211°.

Anal. Calcd. for $C_{16}H_{17}O_5(CH_3CO)_3$ (418): C, 63.16; H, 6.26; CH_3CO, 31. Found: C, 63.53; H, 5.96; CH_3CO, 32; sapon. equiv., 104.

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Garrya Alkaloids. III. The Skeletal Structure of the Garrya Alkaloids

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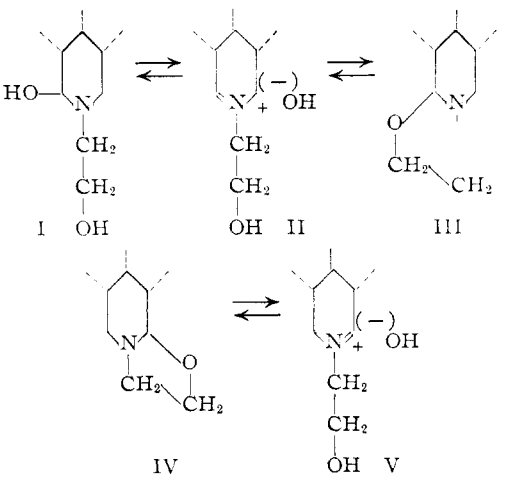
On the basis of oxidation as well as dehydrogenation experiments the diterpenoid structure VI related to phyllocladene is proposed for dihydroveatchine.

Introduction

In our previous communications on the Garrya alkaloids, garryine and veatchine,[1,2] we have shown that these two bases can be represented by the partial structures I, II and III for garryine and IV and V for veatchine.

In the present communication we propose the structure VI for dihydroveatchine and structures VII and VIII for veatchine and garryine in their anhydrous form. The considerations which led us to adopt these structures were in part reported in a preliminary note[3] and were as follows: Selenium dehydrogenation at 340° gave a good yield of 1-methyl-7-ethylphenanthrene and a compound $C_{15}H_{15}N$ which was recognized as an azaphenanthrene by its ultraviolet absorption. It was at first considered to be a phenanthridine, but it is



(1) K. Wiesner, S. K. Figdor, M. F. Bartlett and D. R. Henderson, *Can. Jour. Chem.*, **30**, 608 (1952).

(2) K. Wiesner, W. I. Taylor, S. K. Figdor, M. F. Bartlett, J. R. Armstrong and J. A. Edwards, *Ber.*, **86**, 800 (1953).

(3) K. Wiesner, R. Armstrong, M. F. Bartlett and J. A. Edwards, *Chemistry and Industry*, 132 (1954).