succinic acid anhydride was added. The succinic anhydride dissolved and the product crystallized on standing; yield 3.4 g., m.p. 133–135°.

Anal. Calcd. for $C_9H_{11}NO_3S$: N, 6.6. Found: N, 6.5.

Mucic Acid Mono-α-thenyl Amide.—To 2.88 g. of sirupy mucic acid monolactone[20] 3.4 g. of α-thenylamine was added with cooling, followed by 5 cc. of ethanol. After standing overnight, the product was filtered off, dissolved in water and the acid precipitated with concd. hydrochloric acid. After recrystallization from water, the mucic acid thenyl amide melted at 197–200°.

Anal. Calcd. for $C_{11}H_{15}NO_7S$: C, 43.3; H, 4.9; N, 4.6. Found: C, 43.2; H, 4.7; N, 4.8.

Gluconic Acid α-Thenyl Amide.—To a solution of 3.5 g. of α-thenylamine in 7 cc. of ethanol, 4.7 g. of gluconolactone was added. The reaction mixture warmed up and solidified, then 10 cc. of ethanol was added. After standing overnight the product was filtered off and recrystallized from methanol; yield 5.9 g., m.p. 170–172°.

Anal. Calcd. for $C_{11}H_{17}NO_6S$: N, 4.8. Found: N, 4.8.

Allyl Amide of 2-(D-Glucopentahydroxyamyl)-5-benzimidazolecarboxylic Acid.—By refluxing 19.5 g. of 3-nitro-4-formamidobenzoic acid[21] with 60 cc. of thionyl chloride for 90 min., and then cooling, the acid chloride was obtained crystalline from the reaction mixture. It was filtered off and washed with benzene; yield 15.9 g., m.p. 104–107°. A solution of 8.0 g. of allylamine in 30 cc. of benzene was added dropwise with stirring to 15.9 g. of 3-nitro-4-formamidobenzoyl chloride dissolved in 350 cc. of benzene. A precipitate formed which was filtered off. On concentrating the reaction mixture a second crop was obtained. The material was recrystallized from ethanol, and apparently consisted of a mixture of the 4-formamido- and 4-amino-3-nitrobenzoic acid allyl amide. This material (11.3 g.) was dissolved in 150 cc. of ethanol, heated to boiling and 25 cc. of 2 *N* sodium hydroxide was then added. On cooling, 4-amino-3-nitrobenzoic acid allyl amide crystallized. Recrystallization from ethanol gave 11.0 g., m.p. 200–203°.

Anal. Calcd. for $C_{10}H_{11}N_3O_3$: N, 19.0. Found: N, 19.0.

Hydrogenation of 20.0 g. of 4-amino-3-nitrobenzoic acid allyl amide with Raney nickel in 250 cc. of ethanol, followed by recrystallization of the product from ethyl acetate gave 17.0 g. of 3,4-diaminobenzoic acid allyl amide, m.p. 125–126°. Following the procedure of Link, *et al.*,[22] 4.6 g. of gluconic acid lactone, 5.0 g. of 3,4-diaminobenzoic acid allyl amide, 15 cc. of water, 4 cc. of ethanol and 2.6 cc. of concd. hydrochloric acid were combined. The reaction mixture was warmed gently until a solution was obtained, then heated to 135° for 2 hours. On working up, the allyl amide of 2-(D-glucopentahydroxyamyl)-5-benzimidazolecarboxylic acid, m.p. 230–232°, was obtained.

Anal. Calcd. for $C_{16}H_{21}N_3O_6$: N, 12.0. Found: N, 11.8.

N-Allylglycylgluconamide.—Glycinallyl amide[16] (1.3 g.) prepared according to Sheehan's[23] method was shaken with 1.62 g. of δ-gluconolactone in 5 cc. of ethanol; yield 2.25 g., m.p. 161–163°.

Anal. Calcd. for $C_{11}H_{20}N_2O_7$: N, 9.6. Found: N, 9.5.

D-Glucopentahydroxyhexylmercaptoacetic Acid N-Allyl Amide.—To a mixture of 36 g. of 1-thiosorbitol (80% pure) dissolved in 120 cc. of water and 12.6 g. of KOH in 60 cc. of water, 29.83 g. of α-chloro-N-allylacetamide[16] was added dropwise with stirring under N_2. Stirring was continued until the test for –SH groups (sodium nitroprusside) was negative. The reaction mixture was neutralized with hydrochloric acid and concentrated *in vacuo*. The residue was extracted with methanol and the potassium chloride filtered off. Evaporation of the filtrate to dryness and treatment with acetone gave a crystalline product which was recrystallized three times from ethanol; yield 25.2 g., m.p. 103–105°.

Anal. Calcd. for $C_{11}H_{21}NO_6S$: C, 44.7; H, 7.2. Found: C, 44.7; H, 7.2.

Mercuration. General Procedure.—The olefin or thiophene derivative was dissolved in water or methanol, respectively, and an equivalent amount of mercuric acetate as a 20% (w./v.) aqueous or 6% (w./v.) methanolic solution added. In some cases (compounds 18, 25, 27, 28, 31, 32, 33) the mercurated intermediates precipitated and were filtered off. If no precipitate formed after 16 hours, the solution was evaporated to dryness at room temperature. The residue was then treated with methanol, acetone or ethyl acetate to induce crystallization; some products, however, could be obtained only as an amorphous powder.

Combination with 1-Thiosorbitol. General Procedure.—Mercurials with an acidic group were combined with 1-thiosorbitol according to the procedure given above for 3-hydroxymercuri-2-hydroxypropylcarbamylnicotinic acid. Other mercurials: 10 mmoles of substance was dissolved or suspended in 10 cc. of water; 10 mmoles of 1-thiosorbitol dissolved in 5 cc. of water was added. The mercaptomercuri compound was then isolated either by precipitation as an amorphous solid with acetone, or by freeze drying the aqueous solution. Methanol, ethanol or dioxane can also be used as precipitant.

(20) E. Fischer, *Ber.*, **24**, 2136 (1891).

(21) A. Zehra, *ibid.*, **23**, 3625 (1890).

(22) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 293 (1940); *J. Org. Chem.*, **5**, 637 (1940).

(23) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949).

SUMMIT, NEW JERSEY

[CONTRIBUTION FROM THE DEPARTMENT OF PURE CHEMISTRY, UNIVERSITY COLLEGE OF SCIENCE & TECHNOLOGY]

Alkaloids of *Glycosmis pentaphylla* (Retz.) DC. Part I

BY A. CHATTERJEE AND S. GHOSH MAJUMDAR

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From *Glycosmis pentaphylla* (Retz.) DC., three different alkaloids have been isolated: skimmianine, m.p. 175–176°, $C_{14}H_{13}O_4N$; glycosminine, m.p. 225–227°; and glycosine, m.p. 155–156°, $C_{16}H_{14}N_2O$. A complete assignment of the structure for the alkaloid glycosine as 2-benzylidene-1-methyl-4-quinazolone has been possible from studies of its infrared and ultraviolet absorption spectra, from the hydrolysis characteristics of the base and its ozonolysis and from the oxidation experiments of the alkaloid with periodic acid as well as with neutral potassium permanganate in acetone.

Glycosmis pentaphylla (Retz.) DC., commonly known in India as tooth brush plant, belongs to the family *Rutaceae* which is well-known for its varied therapeutically active constituents. Chemical investigation of this plant was first undertaken by Dutta[1] who isolated from this species a neutral compound glycosmin, $C_{22}H_{26}O_{10}$, m.p. 169°, which has been shown to be identical with veratroyl salicin. Recently Chakravarti and Chakravarti[2–4] have shown that *G. pentaphylla* (later identified as *Glycosmis arborea*)[3b] contains two different alka-

(1) S. B. Dutta, *Proc. Acad. Sci., United Province Agra and Oudh, India*, 56 (1935).

(2) R. N. Chakravarti and S. C. Chakravarti, *Proc. Indian Sci. Cong.*, Part III, 79 (1951).

(3) (a) *Ibid.*, 100 (1952); (b) R. N. Chakravarti and S. C. Chakravarti, *J. Proc. Inst. Chemist (India)*, **24**, 96 (1952).

(4) (Mrs.) D. Chakravarti, R. N. Chakravarti and S. C. Chakravarti, *Science and Culture*, **18**, 533 (1953).

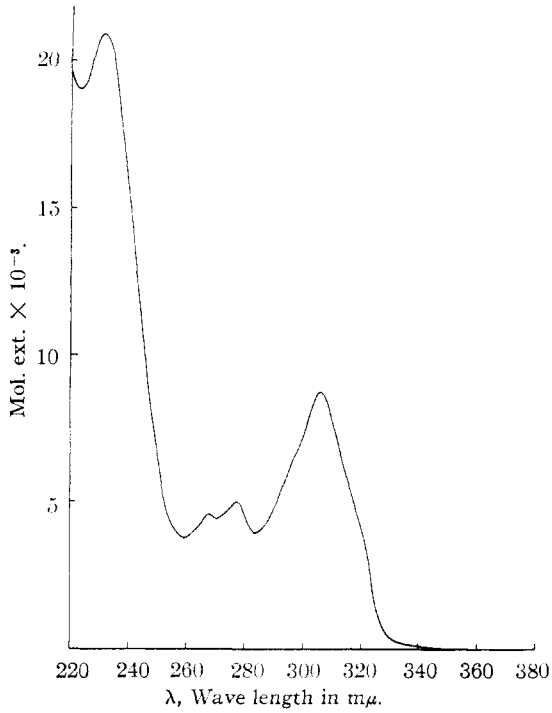
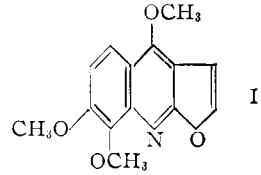


Fig. 1.—Molecular extinction curve of glycosin in alcohol, using Beckmann quartz spectrophotometer, Model DU; length of the cell, 1.0006 cm; concentration of glycosin, 7.46 mg./10³ cc.

loids, arborine,[3,4] m.p. 155–156°, $C_{16}H_{14}N_2O$ (the original formula assigned to arborine[3] was $C_{31}H_{26}N_4O_2$) and arborinine, m.p. 175–176°, $C_{26}H_{24}O_6N_2$.

We have worked on *Glycosmis pentaphylla*, and our findings differ from those of Chakravarti, *et al.*[2,3] We find[5–7] that *Glycosmis pentaphylla* contains three alkaloids, *viz.*, skimmianine (I) (yield 0.03%), m.p. 175–176°, $C_{14}H_{13}O_4N$; glycosminine (yield 0.003%), m.p. 225–227°; and a third which we have called glycosine, m.p. 155–156° (yield 0.25%), $C_{16}H_{14}ON_2$



I

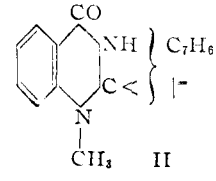
which is probably identical with arborine.[4] The properties and constitution of glycosine have been studied and complete elucidation of its structure has been possible from studies of its infrared absorption and from degradation experiments. The results obtained so far are reported here.

Hydrochloric acid washings of the ethereal extract of the plant liberated on basification a mixture of skimmianine[8] and glycosminine which were separated by fractional crystallization. The former has been compared with an authentic sample of skimmianine $C_{14}H_{13}O_4N$ and their identity has been established by mixed melting points of the bases and their picrates, the latter melting at 198° alone. Both have identical ultraviolet absorption spectra having absorption maxima at 249, 320 and 331 mμ. These findings have been independently confirmed by McKenzie and Price[9] in Australia, who have isolated from same plant (*G. pentaphylla*) skimmianine and one of its isomers, kokusaginine $C_{14}H_{13}O_4N$, m.p. 172–173°, a 4,6,7-trimethoxyfuro-(2,3,2′,3′)-quinoline.

The second alkaloid glycosminine has been obtained in very poor yield (0.003%). Further study of this alkaloid is in progress.

Hydrochloric acid washings of the chloroform extract of *G. pentaphylla* deposited glycosine hydrochloride, m.p. 209–210°, which on basification liberated glycosine, $C_{16}H_{14}N_2O$, m.p. 155–156°; the molecular weight of the base was determined by the Rast method and by its chloroplatinate. The alkaloid retains solvents of crystallization, except ethyl alcohol, tenaciously. Glycosine is a monoacidic base as shown by conductometric titrations. It produces only one series of salts with acids. It is optically inactive and does not produce any coloration with ferric chloride. Glycosine contains no methoxyl, oxide or C-methyl groups but does contain N-methyl and –CONH groups. It is unsaturated. The presence of 2 active hydrogen atoms has also been observed in the base. The ultraviolet absorption spectrum of glycosine (Fig. 1) shows maxima at 231, 268, 277 and 306 mμ. The infrared absorption spectrum of the alkaloid (Fig. 2) shows absorption bands at 3 μ (—NH or OH group or both), at 6.1 μ (aromatic amido group) and at 6.2 μ (—C=C group). The latter has been confirmed by catalytic hydrogenation of glycosine with platinum oxide when dihydroglycosine, $C_{16}H_{16}N_2O$, m.p. 196°, is obtained which has also been prepared from the parent base by its reduction with lithium aluminum hydride in tetrahydrofuran at room temperature (Figs. 1 and 2). On reduction with lithium aluminum hydride in boiling tetrahydrofuran glycosine yields, however, a product different from dihydroglycosine. Further investigation of this reduction product is in progress.

Glycosine has been subjected to alkaline hydrolysis according to Hutchings, *et al.*[10] From the hydrolysates N-methylanthranilic acid, m.p. 175°, and phenylacetic acid, m.p. 70°, have been isolated and identified. A comparison of the rates of alkaline hydrolysis of glycosine, the Hydrangea alkaloids, and other 4-quinazolone alkaloids[10] demonstrates the following 4-quinazolone structure II for the alkaloid glycosine.



II

Further evidence as to the structure of glycosine has been obtained by periodic acid oxidation of the base. With this reagent glycosine liberates a base,

(5) A. Chatterjee and S. Ghosh Majumdar, *Science and Culture*, **17**, 306 (1952).
(6) *Ibid.*, **18**, 505 (1953).
(7) *Ibid.*, **18**, 604 (1953).
(8) Y. Asahina and M. Inubase, *Ber.*, **63**, 2052 (1930).
(9) A. McKenzie and J. R. Price, *Australian J. Sci. Research Ser. A*, **5**, 579 (1952).
(10) B. L. Hutchings, *et al.*, *J. Org. Chem.*, **17**, 19 (1952).

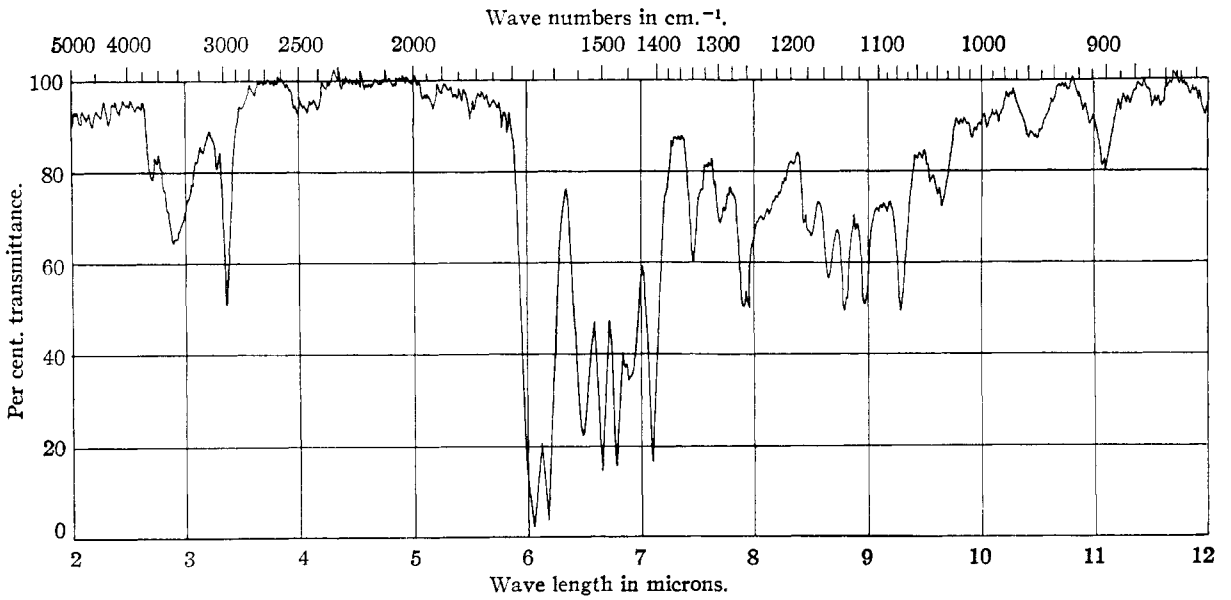
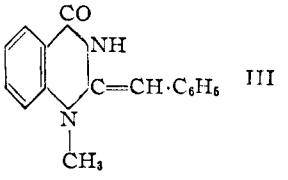


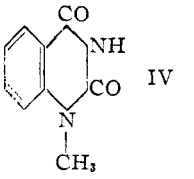
Fig. 2.—Infrared spectrum of glycosin in chloroform taken in Baird double beam spectrophotometer (self recording).

m.p. 255–256° (characterization of which is in progress) and benzaldehyde, isolated and identified as its 2,4-dinitrophenylhydrazone. The isolation of benzaldehyde from the alkaloid in conjunction with the above results clearly indicates glycosine to be 2-benzylidene-1-methyl-4-quinazolone (III).



III

In determining the position of the substituent in a quinazolone nucleus, the possible effect of functional groups adjacent to the quinazolone ring on the rate of hydrolysis should be taken into consideration. It has been shown by Hutchings, *et al.*,[10] that substitution in the 2-position definitely stabilizes the quinazolone nucleus to alkaline cleavage. According to these workers the 2,3-substituted model was approximately 10 times as stable as the 3-substituted 4-quinazolone compounds. Thus the hydrolysis characteristics of different 4-quinazolones studied by Hutchings, *et al.*, suggest that glycosine might be a 3-substituted 4-quinazolone compound. Since the presence of a —CONH group in glycosine has already been established by its infrared absorption spectrum, the benzylidene group has been located in position 2 of the quinazolone nucleus. This appears to be the only logical place for glycosine to accommodate the C_7H_6 residue (II) and the double bond which eventually becomes exocyclic. The ready hydrolysis of glycosine with alkali which should not be expected according to structure III and also the observation of Hutchings, *et al.*,[10] is due to the presence of the exocyclic double bond in position 2 associated with the benzylidene group. This is supported by the fact that dihydroglycosine where the extranuclear double bond has been reduced is quite stable toward aqueous and alcoholic alkalies even at reflux temperatures. More definite proof of this extranuclear double bond in glycosine has been afforded by ozonolysis of the base at −75° as also by its oxidation with neutral potassium permanganate in acetone. On ozonolysis at −75° glycosine liberates benzaldehyde, isolated as its 2,4-dinitrophenylhydrazone. The alkaloid III when oxidized by neutral potassium permanganate (following essentially the method of Robinson and Boyd-Barret[11]) produces 1-methyl-2-keto-1,2-dihydro-4-quinazolone (IV), m.p. 260°. This compound showed no depression in melting point on admixture with the synthetic product prepared from N-methylanthranilamide and ethyl chloroformate on refluxing.



IV

Thus all the evidence establishes III as the structure of glycosine.

Experimental

Isolation of Skimmianine and Glycosminine.—Powdered, sun dried leaves (1.0 kg.) of *Glycosmis pentaphylla* were extracted in a Soxhlet with ether (5 liters) for 48 hours. The deep green ethereal extract was concentrated to 300 ml. and then digested with 2 *N* hydrochloric acid (400 ml. in four portions). The brown acid extract was cooled in ice-water and basified with sodium carbonate. The separated base was taken in ether (300 ml. in four portions). The ethereal extract was washed with water, dried over anhydrous sodium sulfate and concentrated to 15 ml. A turbidity appeared which was just dissolved in 90% ethanol. Colorless rhombohedral prisms of skimmianine (0.3 g.) crystallized on standing. On further concentration the mother liquor deposited long needle shaped crystals of glycosminine (0.03 g.), m.p. 225–227°. Skimmianine melted at 175–176° and it showed no depression in m.p. when mixed with an authentic sample of skimmianine prepared from *Chloroxylon swietenia*.[12]

(11) R. Robinson and H. S. Boyd-Barret, *J. Chem. Soc.*, 319 (1932).
(12) A. Chatterjee and P. K. Bose, *J. Ind. Chem. Soc.*, **23**, 1 (1946).

Anal. Calcd. for $C_{14}H_{13}O_4N$ (skimmianine): C, 64.86; H, 5.02; N, 5.40; OCH_3, 35.96; mol. wt., 259. Found: C, 65.02; H, 4.89; N, 5.29; OCH_3, 35.78; mol. wt., 265, 256 (by chloroplatinate and the Rast method).

Skimmianine Picrate.—Skimmianine picrate was prepared by addition of an ethereal solution of the base to picric acid (in ether). The yellow precipitate crystallized from 90% ethanol in shining yellow needles. It melted at 198° dec. when mixed with skimmianine picrate which alone melted at 198° dec.

Anal. Calcd. for $C_{14}H_{13}O_4N \cdot C_6H_3O_7N_3$: N, 11.48. Found: N, 11.62.

Skimmianine chloroplatinate was prepared by adding an aqueous solution of platinic chloride (5%) to a faintly acidic solution (HCl) of skimmianine. Upon crystallization from water containing a little hydrochloric acid, orange plates separated which did not melt but decomposed above 205°.

Anal. Calcd. for $(C_{14}H_{13}O_4N)_2H_2PtCl_6$: Pt, 21.01. Found: Pt, 20.9.

Isolation of Glycosine.—The air dried powdered leaves of *Glycosmis pentaphylla*, which were left after extraction with ether (1.0 kg.), were extracted in a Soxhlet with chloroform (5 liters) for 48 hours. The deep green extract was concentrated to 300 ml. and then digested with 2 *N* hydrochloric acid (500 ml. in five portions). The brown acid aqueous solution was cooled in ice-water and basified with ammonium hydroxide. The glycosine which separated was extracted with chloroform (400 ml. in four portions). The chloroform extract was washed with water, dried over anhydrous sodium sulfate and concentrated to 15 ml. The thick oily residue was treated with 20 ml. of 2 *N* hydrochloric acid whereby a crystalline solid separated which was purified by several crystallizations from 90% ethanol till the m.p. 209–210° did not change.

The pure hydrochloride was dissolved in 100 ml. of 2 *N* hydrochloric acid, cooled in ice and basified with ammonium hydroxide. Precipitated glycosine was taken up in chloroform (100 ml. in three portions), washed with water, dried over anhydrous sodium sulfate and concentrated to 5 ml. This solution was diluted with ethyl acetate (1–2 ml.) and kept overnight. Colorless rhombohedral prisms of glycosine (2.5 g.) were obtained having a m.p. 155–156°. On repeated crystallization from 90% ethanol, ethyl acetate, benzene and chloroform the m.p. was found to remain unchanged. A sample regenerated from the picrate showed the same m.p.

Glycosine is easily soluble in chloroform, ethyl acetate, benzene and ethanol and is sparingly soluble in ether.

Anal. Calcd. for $C_{16}H_{14}ON_2$: C, 76.80; H, 5.60; N, 11.20; NMe, 6.00; active H, 0.8. Found: C, 76.19; H, 5.51; N, 11.30; NMe, 6.10; active H, 0.84 (by Zerewitinoff method).

It gives the following color reactions: concentrated sulfuric acid, colorless; Erdmann's reagent, nil; Fröhde's reagent, solution slowly turns pink; concentrated sulfuric acid and potassium dichromate, dissolve the alkaloid with a light pink solution; vanillin and concentrated hydrochloric acid, solution slowly turns purple.

Glycosine Picrate.—Glycosine picrate was prepared by adding an aqueous solution of glycosine hydrochloride to an aqueous solution of the picric acid. The yellow precipitate crystallized from 90% ethanol in fine yellow needles, m.p. 171–172° dec.

Anal. Calcd. for $C_{16}H_{14}N_2O \cdot C_6H_3O_7N_3$: C, 55.11; H, 3.55; N, 14.61. Found: C, 54.81; H, 3.20; N, 15.20. Found for arborine: C, 55.6; H, 3.6; N, 15.0.

Glycosine Hydrochloride.—Glycosine hydrochloride was formed by adding 2 *N* hydrochloric acid to a concentrated solution of glycosine in chloroform. Colorless crystals separated in light flakes, m.p. 209–210° dec. (when crystallized from 90% ethanol).

Anal. Calcd. for $C_{16}H_{14}ON_2 \cdot HCl$: Cl, 13.02. Found: Cl, 12.56.

Glycosine Chloroplatinate.—Glycosine chloroplatinate obtained by adding an aqueous solution of platinic chloride (5%) to a faintly acidic solution (HCl) of glycosine. Upon crystallization from water containing a little hydrochloric acid glistening orange colored plates of glycosine chloroplatinate were obtained which decomposed above 160°.

Anal. Calcd. for $(C_{16}H_{14}N_2O)_2 \cdot H_2PtCl_6$: Pt, 21.42. Found: Pt, 21.6, 21.5.

Catalytic Hydrogenation of Glycosine.—Glycosine (0.5 g.) dissolved in ethanol (100 ml.) was treated with hydrogen and Adams platinum oxide catalyst (0.1 g.) which had been previously saturated with hydrogen. The absorption of hydrogen (49.2 ml. at 28°, 760 mm.) corresponds to two atoms of hydrogen per molecule. Ethanol solution was filtered off from the catalyst and concentrated when dihydroglycosine, $C_{16}H_{16}N_2O$, separated out in colorless needles, m.p. 196°.

Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.19; H, 6.35; N, 11.11. Found: C, 76.32; H, 6.31; N, 11.23.

Reduction of Glycosine with Lithium Aluminum Hydride.—Glycosine (0.5 g.) dissolved in tetrahydrofuran (25 ml.) was added dropwise on a sludge of lithium aluminum hydride (1.0 g.) in the same solvent cooled in ice when a brisk evolution of gas was noticed. The mixture was kept stirred for 4 hours and then decomposed with water (5 ml.) under cooling. Aluminum hydroxide which separated was filtered off and the filtrate on evaporation deposited colorless needles (0.4 g.) of dihydroglycosine, m.p. 195–196°. On recrystallization from ethyl acetate, the m.p. did not change.

Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.19; H, 6.35; N, 11.11. Found: C, 76.32; H, 6.31; N, 11.23.

The compound did not depress the melting point of dihydroglycosine when mixed.

Hydrolysis of Glycosine with 0.1 *N* Alkali.—Glycosine hydrochloride (0.5 g.) was dissolved in 100 ml. of 0.1 *N* sodium hydroxide and heated in a steam-bath for 4 hours. The reaction product was cooled in ice-water and thoroughly shaken with benzene (80 ml.) which removed unreacted glycosine (0.05 g.).

The brown aqueous alkaline solution was acidified with acetic acid in the cold when a turbidity appeared and it was extracted with ether. The ethereal extract was washed with water and dried over anhydrous sodium sulfate. Dry ether solution on evaporation left a solid residue which on distillation *in vacuo* at 0.01 mm. gave two different fractions.

(a) B.p. 65–70°, m.p. 70° after crystallization from dilute alcohol: This compound has been characterized as phenylacetic acid by mixed melting point with synthetic sample.

(b) B.p. 80°: It solidified as stout needles (0.1 g.), and was purified by crystallization from the mixture of petroleum ether and ethanol as long needle shaped crystals. It has been identified as N-methylanthranilic acid, m.p. 175°.

Periodate Oxidation of the Alkaloid.—Glycosine hydrochloride (100 mg.) was dissolved in 50 ml. of water and 50 ml. of 0.1 *N* periodic acid was added. After 4 hours the mixture was steam distilled. Benzaldehyde distilled over and it was extracted out from the distillate (100 ml.) with ether which was washed with sodium bisulfite to remove iodine. The ether extract on evaporation left benzaldehyde which with 2,4-dinitrophenylhydrazine gave orange red crystals of 2,4-dinitrophenylhydrazone, m.p. 235°. It showed no depression in m.p. when mixed with freshly prepared 2,4-dinitrophenylhydrazone (m.p. 235°) of benzaldehyde. From the non-volatile portion of the periodic acid oxidation products left after steam distillation, a base, m.p. 255–256°, has been isolated, characterization of which is in progress.

Ozonolysis of Glycosine.—The alkaloid glycosine (0.3 g.) dissolved in ethyl acetate (30 ml.) and acetic acid (4 ml.) was ozonized at −75° until the solution became blue. The ozonide was cleaved reductively by adding magnesium powder (0.5 g.) and 10 ml. of 1:1 acetic acid and keeping it overnight. Next morning the mixture was diluted with 50 ml. of water and extracted with chloroform (50 ml.). The aqueous layer was removed and the organic layer was washed twice with HCl (2 *N*) to remove basic material. The chloroform layer was washed with water, dried over anhydrous Na_2SO_4 and distilled in an atmosphere of nitrogen. The residual oil with 2,4-dinitrophenylhydrazine formed orange red crystals of 2,4-dinitrophenylhydrazone, m.p. 235°. It showed no depression in m.p. when mixed with 2,4-dinitrophenylhydrazone of benzaldehyde.

Isolation of 1-Methyl-2-keto-1,2-dihydro-4-quinazolone from Glycosine by $KMnO_4$ Oxidation.—To an ice-cooled acetone solution (25 ml.) of glycosine (0.5 g.) finely powdered potassium permanganate (1.0 g.) was added with constant stirring. The stirring was continued for 3 hours and the reaction product was left for another 3 hours at room temperature. The excess of permanganate was decomposed with methyl alcohol and the separated brown mass

was filtered and repeatedly washed with hot methanol (15 ml.). The filtrate together with the washings was freed from the solvent. The viscid residue was taken up in ether (15 ml.), washed with sodium bicarbonate solution and dried over anhydrous sodium sulfate. Dry ether solution upon concentration deposited colorless needles of 1-methyl-2-keto-1,2-dihydro-4-quinazolone (0.18 g.), m.p. 255–258°. On several crystallizations from ethyl acetate, ethyl alcohol and benzene shining needles of constant m.p. 259–260° were obtained. It showed no depression in m.p. on admixture with the synthetic product of 1-methyl-2-keto-1,2-dihydro-4-quinazolone, m.p. 260°.

Anal. Calcd. for $C_9H_8N_2O_2$: C, 61.36; H, 4.55; N, 15.91. Found: C, 61.48; H, 4.61; N, 15.98.

Synthesis of 1-Methyl-2-keto-1,2-dihydro-4-quinazolone.—One gram of N-methylanthranilamide[13] prepared from isatoic anhydride was heated with freshly distilled ethyl chloroformate (2.0 g.) on a water-bath for 2 hours and then refluxed for 3 hours when 1-methyl-2-keto-1,2-dihydro-4-quinazolone separated out. It crystallized from alcohol in shining colorless needles (1.2 g.), m.p. 259–260°.

Anal. Calcd. for $C_9H_8O_2$: C, 61.36; H, 4.55; N, 15.91. Found: C, 61.52; H, 4.68; N, 15.79.

(13) Asima Chatterjee and Subhendu Ghosh Majumdar, THIS JOURNAL, **75**, 4365 (1953).

CALCUTTA, WEST BENGAL, INDIA

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Rauwolfia Serpentina Alkaloids. I. Structure of Reserpine

BY NORBERT NEUSS, HAROLD E. BOAZ AND J. W. FORBES

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Hydrolysis of reserpine, a sedative principle of *Rauwolfia Serpentina* Benth., yielded 3,4,5-trimethoxybenzoic acid and reserpic acid. Reduction of reserpine afforded 3,4,5-trimethoxybenzyl alcohol and reserpic alcohol. Empirical formulas are proposed for reserpine and its degradation products. From the study of ultraviolet and infrared spectra of various indole derivatives, a possible structure of reserpine has been suggested.

Rauwolfia Serpentina Benth. is a genus of the *Apocyanaceous* family and has been used for centuries in India as a sedative. Its value as a hypotensive agent was discovered in the early thirties by Chopra, Gupta and Mukherjee[1] and since that time has been discussed by several pharmacologists.[2] The chemical investigation of the plant was first undertaken by Siddiqui and Siddiqui[3] and pursued later by several other investigators.[4] A renewed interest in the alkaloids of *Rauwolfia* was created by the isolation of a sedative principle of the plant, a crystalline alkaloid, named reserpine by Mueller, Schlittler and Bein.[5]

In our recent publication[6] it was shown that reserpine gives, upon hydrolysis with dilute alkali, 3,4,5-trimethoxybenzoic acid and an amino acid which we called reserpic acid. By reduction of reserpine, using lithium aluminum hydride, 3,4,5-trimethoxybenzyl alcohol and an amino alcohol, named reserpic alcohol, were obtained. These two reactions led to the establishment of empirical formulas of reserpine and its degradation products.[7] Based on data, both from ultraviolet and infrared spectra, it was proposed that reserpine has possibly a yohimbine-like nucleus with one methoxyl group at the 6-position of the indole moiety, corresponding to the 11-position of yohimbane.[8] The proof of that assumption came recently from the work of L. Dorfmann, *et al.*,[8] whose degradation studies, carried out on methyl reserpate, led to the isolation of yobyrine, the corresponding hydroxyyobyrine (probably a 7-hydroxy derivative) and 4-methoxyoxalylanthranilic acid. By preparation of a γ-lactone, the same authors established the relative position of the carbomethoxy group and hydroxy group in the ring E. Furthermore, the isolation of 5-hydroxyisophthalic acid from alkali fusion of methyl reserpate indicated the probable position of the second methoxyl group in ring E of reserpine.[9]

As starting material for isolation of reserpine, the oleoresin fraction[10] was used. By chromatography of this fraction on acid-washed alumina, using benzene–chloroform mixtures, a new alkaloid, tentatively called "*Rauwolfia* Alkaloid A" was isolated.[11] After changing to chloroform alone, reserpine was obtained. Repeated recrystallization of the alkaloid yielded long colorless prisms which gave satisfactory analytical results for a $C_{33}H_{40}N_2O_9$ compound. Preparation of a maleate salt and its analysis substantiated the empirical formula of reserpine. The molecular weight determination from X-ray data was also in excellent agreement with the above formulation.

Hydrolysis of reserpine by hot dilute sodium hy-

(1) R. N. Chopra, J. C. Gupta and B. Mukherjee, *Indian J. Med. Res.*, **21**, 261 (1933); **29**, 763 (1941).

(2) Raymond-Hamet, *Bull. Acad. Med.*, **115**, 452 (1936); *Compt. rend. Acad. Sci.*, **223**, 927 (1946); H. J. Bein, *Experientia*, **9**, 107 (1953), and the references cited therein.

(3) S. S. Siddiqui and R. H. Siddiqui, *J. Ind. Chem. Soc.*, **8**, 667 (1931).

(4) *Cf.* A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **36**, 1143 (1953), and references cited therein.

(5) J. M. Mueller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 338 (1952).

(6) N. Neuss, H. E. Boaz and J. W. Forbes, THIS JOURNAL, **75**, 4870 (1953).

(7) Independently, in this country and in Switzerland, A. Furlenmeier, R. Lucas, H. B. MacPhillamy, J. M. Mueller and E. Schlittler assigned the same empirical formulas to reserpine and reserpic acid, *Experientia*, **9**, 331 (1953). The identical formula for reserpine was also proposed by C. Djerassi, M. Gorman, A. L. Nussbaum and J. Reynoso, THIS JOURNAL, **75**, 5446 (1953). A different formula was suggested by M. W. Klohs, *et al.*, *ibid.*, **75**, 4867 (1953).

(8) L. Dorfmann, C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F. St. André, *Experientia*, **9**, 368 (1953).

(9) An earlier announcement of these results by Dr. Schlittler (Symposium on hypotensive drugs, Boston, Massachusetts, September 14–15, 1953) prompted us to discontinue our work on structure elucidation of reserpine.

(10) A. Dutt, J. C. Gupta, S. Ghosh and B. S. Kahali, *Indian J. Pharm.*, **9**, 54 (1947); *J. Am. Pharm. Assoc.*, **36**, 416 (1947).

(11) Data concerning this and other alkaloids from *Rauwolfia serpentina* will be published later; N. Neuss, *et al.*, THIS JOURNAL, in press.