110. *Structure of Gentianine.*

By T. R. GOVINDACHARI, K. NAGARAJAN, and S. RAJAPPA.

Gentianine isolated from *Enicostemma littorale* is shown to be 4-2′-hydroxyethyl-5-vinylnicotinic lactone by degradative and synthetic experiments.*

FROM *Enicostemma littorale* Bl. (Gentianaceae) we have isolated an alkaloid, $C_{10}H_9O_2N$, m. p. 82—83°. The molecular formulæ and melting points of the alkaloid and its derivatives corresponded closely to those reported for gentianine [1,2] to which structure (Ia) has been assigned. However, the assertion by the Russian authors that gentianine contained a *C*-methyl group and our failure to detect a *C*-methyl group by Kuhn–Roth method † or (more important) in the infrared absorption spectrum, and the non-availability of a sample

* Part of this work was published in *Chem. and Ind.*, 1956, 1017.

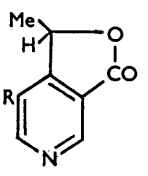
† Duplicate *C*-methyl determinations by Drs. Weiler and Strauss, Oxford.

[1] Proskurnina, *J. Gen. Chem.* (*U.S.S.R.*), 1944, **14**, 1148; *Chem. Abs.*, 1946, **40**, 7213.

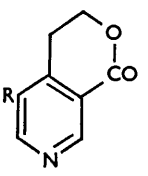
[2] Proskurnina, Shpanov, and Konovalova, *Doklady Akad. Nauk S.S.S.R.*, 1949, **66**, 437; *Chem. Abs.*, 1950, **44**, 159.

for comparison till the completion of the work reported here, made us proceed on the assumption that our alkaloid was isomeric, and not identical, with gentianine.

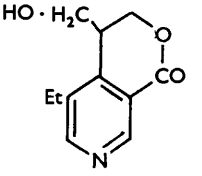
The infrared absorption spectrum of the alkaloid showed bands at 1719 (αβ-unsaturated δ-lactone) and 1634 cm.$^{-1}$ (conjugated double bond) and no bands in the region 1300—1400 cm.$^{-1}$ (*C*-methyl). The alkaloid was optically inactive and could not be resolved. Treatment with alcoholic sodium hydroxide gave a sodium salt from which the alkaloid was recovered by acidification. The alkaloid gave a dihydro-derivative, m. p. 74—76°, on hydrogenation in presence of Adams catalyst (bands at 1716 and 1385 cm.$^{-1}$ and no



(I) a : R = $CH{:}CH_2$
b : R = H
c : R = Et



(II) a : R = $CH{:}CH_2$
b : R = Et



(III)

band at 1635 cm.$^{-1}$). Ozonolysis of the alkaloid yielded formaldehyde. Oxidation with potassium permanganate in acetone solution yielded a lactonic acid, $C_9H_7O_4N$, showing the presence of a vinyl group. Vigorous oxidation yielded pyridine-3 : 4 : 5-tricarboxylic acid, whose identity was confirmed by comparison with a sample synthesised by oxidation of 5-ethyl-4-methylnicotinic acid. Pyridine-3 : 4 : 5-tricarboxylic acid has been prepared [3] by selective decarboxylation of the dipotassium salt of pyridinepentacarboxylic acid, a method both tedious and ambiguous. Hydrolysis of the lactonic acid, $C_9H_7O_4N$, followed by decarboxylation, yielded a basic oil from which a picrate, m. p. 155—160°, was obtained whose analysis was intermediate between that of a vinylpyridine and of a pyridylethanol. Oxidation of the basic oil yielded *iso*nicotinic acid, establishing the alcoholic side chain as in position 4.

These degradations lead to structure (Ia) or (IIa) for the alkaloid, but the infrared evidence favours the latter, a six-membered lactone. Attempts to decarboxylate the acid from the dihydro-derivative of the alkaloid in the hope of obtaining a 5-ethyl-4-hydroxyethylpyridine led to a mixture of bases from which no pure compound could be isolated. A choice between these structures could be made only by synthesis.

Compound (Ib) was first synthesised from 4-ethylnicotinic acid in order to determine the experimental conditions for synthesis of (Ic). This acid has been prepared [4] by sulphonation of 4-ethylpyridine, fusion with sodium–potassium cyanide, and hydrolysis of the resulting nitrile. An alternate method of wider applicability developed by us is illustrated in the case of 4-ethylnicotinic acid. Ethyl β-oxovalerate was condensed with cyanoacetamide and the resulting dihydroxypyridine converted into 4-ethylnicotinic acid by treatment with phosphorus oxychloride, reduction, and hydrolysis. Its *N*-oxide on treatment with acetic anhydride [5] gave 4-1′-hydroxyethylnicotinic lactone (Ib). Use of vigorous conditions in this reaction led to 4-vinylpyridine and 4-1′-hydroxyethylpyridine acetate. Starting from ethyl α-ethyl-β-oxovalerate, 4 : 5-diethylnicotinic acid was synthesised in an analogous manner and converted into 5-ethyl-4-1′-hydroxyethylnicotinic lactone (Ic), through the *N*-oxide which was treated with acetic anhydride. The picrate, m. p. 148—149°, of this lactone (Ic) showed an infrared absorption band at 1763 cm.$^{-1}$ (αβ-unsaturated γ-lactone) and was different from the picrate, m. p. 140—142°, of the

[3] Weber, *Annalen*, 1887, **241**, 16; see also Rügheimer and Friling, *Annalen*, 1903, **326**, 269.
[4] Wawzonek, Nelson, jun., and Thelen, *J. Amer. Chem. Soc.*, 1952, **74**, 2894.
[5] Boekelheide and Linn, *J. Amer. Chem. Soc.*, 1954, **76**, 1286; Boekelheide and Harrison, *Chem. and Ind.*, 1955, 1423.

dihydro-derivative of the alkaloid, the latter showing an absorption band at 1720 cm.$^{-1}$. This eliminated structure (Ia) for the alkaloid.

Proof for structure (IIa) for the alkaloid was then sought by synthesis of the dihydro-derivative (IIb). 5-Ethyl-4-methylnicotinic acid was obtained starting from ethyl α-ethylacetoacetate. Treatment of the acid with formaldehyde yielded the lactone of 2-(3-carboxy-5-ethyl-4-pyridyl)propane-1 : 3-diol (III), identical with that obtained from the dihydro-derivative of the alkaloid by similar treatment. This conclusively establishes structure (IIa) for the alkaloid. Action of formaldehyde on the sodium salt of the nicotinic acid yielded the dihydro-derivative of the alkaloid.

After completion of this work, a sample of gentianine received through the courtesy of Sojuzchimexport, Moscow, established the identity of our alkaloid with gentianine, for which structure (IIa) must therefore be assigned. Iyer, Pathak, and Bose [6] recently also reported the isolation of gentianine from *Enicostemma littorale*. The alkaloid erythricine,[7] $C_{10}H_9O_2N$, m. p. 78—80°, may also prove to be identical with gentianine.

Experimental

In view of the identity of our alkaloid with gentianine, it is referred to below by this name.

Ultraviolet measurements are for 95% ethanol solutions. Microanalyses are by Mr. S. Selvavinayagam.

Isolation and Degradation of Gentianine.—(a) *Extraction of* Enicostemma littorale (with Mr. U. Ramadas Rao). Powdered plant material (whole plant, 2 kg.) was made into a paste with aqueous ammonia (2 l.; *d* 0·9) and water and dried at 30° in the shade, then continuously extracted with hot chloroform for several hours. The extract was shaken repeatedly with N-sulphuric acid until negative to Mayer's reagent. The acid extracts were neutralised with barium carbonate and filtered. The barium sulphate cake was washed thoroughly with water. The aqueous filtrates were rendered acidic with acetic acid, concentrated to a small volume *in vacuo*, basified with concentrated aqueous ammonia, and extracted thoroughly with ether. The ether extracts, after drying (Na_2SO_4) and evaporation, left the crude alkaloid (6—12 g.). Crystallisation from moist ether gave colourless needles of gentianine (4—8 g.), m. p. 82—83°, $[\alpha]_D^{30}$ ±0° (in $CHCl_3$), $\lambda_{max.}$ 220 mμ (log ε 4·38), $\lambda_{infl.}$ 245, 280 mμ (log ε 3·9, 3·2) (Found : C, 68·2, 68·2; H, 4·9, 4·7; N, 8·0; *C*-Me, 0. Calc. for $C_{10}H_9O_2N$: C, 68·6; H, 5·1; N, 8·0%), yielding a hydrochloride, m. p. 169—170° (decomp.), colourless needles from alcohol–ether, hydrobromide, m. p. 178° (decomp.), needles from alcohol–ether, nitrate, needles, m. p. 113° (decomp.), oxalate, m. p. 156° (from alcohol), a (+)-tartrate, m. p. 138° (from alcohol), *picrate*, yellow needles (from water), m. p. 123—124° (Found : C, 48·0; H, 2·7. $C_{16}H_{12}O_9N_4$ requires C, 47·5; H, 3·0%), and methiodide, m. p. 193° (from alcohol–ether) (Found : C, 41·4; H, 3·9. Calc. for $C_{11}H_{12}O_2NI$: C, 41·6; H, 3·8%). (M. p.s reported by Proskurnina *et al.*: [1] gentianine, 79—80°; hydrochloride, 171—172°; hydrobromide, 177—178°; nitrate, 238—240°; oxalate 152—153°; methiodide, 190—191°).

(b) *Sodium gentianinate.* Gentianine (0·1 g.) in ethanol (2 ml.) was treated with sodium hydroxide (0·035 g.) in ethanol. To the solution, ether (10 ml.) was added. The precipitate was washed with ether (20 ml.) to give sodium gentianinate, decomp. >200° (Proskurnina *et al.*[1] reported m. p. 132—134°). An aqueous solution of the above salt was acidified with hydrochloric acid, left overnight, basified with ammonia, and extracted with ether. Evaporation and crystallisation of the residue (80 mg.) from ether gave gentianine, m. p. and mixed m. p. 82°.

(c) *Dihydrogentianine.* Gentianine (0·8 g.) in methanol (25 ml.) was shaken with hydrogen at 55 lb. per sq. in. in the presence of Adams catalyst (50 mg.). Hydrogen uptake ceased after absorption of 1 mol. The solution was filtered and evaporated. The residual oil solidified and crystallised from ether–light petroleum, to give dihydrogentianine (0·6 g.), m. p. 74—76° (Proskurnina *et al.*[1] reported m. p. 75—76°), $\lambda_{max.}$ 270 mμ (log ε 3·4) (Found : C, 67·8, 67·7; H, 6·1, 6·2. Calc. for $C_{10}H_{11}O_2N$: C, 67·8; H, 6·2%), yielding a *picrate*, yellow needles (from water), m. p. 140—142° (Found : C, 47·9; H, 3·4. $C_{16}H_{14}O_9N_4$ requires C, 47·3; H, 3·4%).

(d) *Ozonolysis of gentianine.* The alkaloid (0·5 g.) in dry chloroform (50 ml.) was treated

[6] Iyer, Pathak and Bose, *Naturwiss.*, 1956, **43**, 251.
[7] Feofilaktov and Ban'kovskiĭ, *Farmatsiya*, 1946, **9**, No. 5, 10; *Chem. Abs.*, 1947, **41**, 7676.

with ozone (0·2 g.) at 0° during 6 hr. The residue obtained on evaporation of the solvent at 30° *in vacuo* was refluxed with water (100 ml.) for 1 hr. Acetic acid (1 ml.) and water (100 ml.) were added and the solution was distilled in steam. The distillate was received in water (200 ml.) containing dimedone (1·5 g.), boiled, and filtered hot. The crystals obtained on cooling were recrystallised from dilute alcohol and had m. p. and mixed m. p. with formaldehyde-dimedone, 187°.

(e) *Mild oxidation of gentianine.* The alkaloid (1·45 g.) in acetone (50 ml.) was treated slowly with potassium permanganate (4·4 g.) in acetone (300 ml.) till a pink colour persisted. After 1 hr. the solvent was distilled off and the residue digested with hot water and filtered. The filtrate was evaporated nearly to dryness, acidified with hydrochloric acid, and cooled. The precipitate, recrystallised from hot water, gave the lactone (0·94 g.), m. p. 260—262°, $\lambda_{infl.}$ 265 mμ (log ε 3·1), of 4-2′-hydroxyethylpyridine-3 : 5-dicarboxylic acid (Found : C, 56·0, 55·6; H, 3·4, 3·1. Calc. for $C_9H_7O_4N$: C, 56·0; H, 3·6%). Proskurnina *et al.*[1] reported m. p. 264°.

(f) *Vigorous oxidation of gentianine.* Gentianine (0·5 g.) in 2N-sodium hydroxide (20 ml.) was treated at 100° with potassium permanganate (2 g.) in water (20 ml.) till a pink colour persisted. After a further hour's heating the solution was filtered and the residue washed with water. The filtrate was decolorised with sulphurous acid, evaporated *in vacuo*, acidified, and again evaporated to dryness. The residue, on crystallisation from hot water, gave an acid (150 mg.), m. p. about 250° (decomp.), which left an alkaline residue on combustion. A solution of this in water (50 ml.) was passed through a 4 in. column of Zeo-Karb 315 (Permutit), and the column eluted with water. Evaporation of the eluate and recrystallisation of the residue from water gave pyridine-3 : 4 : 5-tricarboxylic acid, m. p. and mixed m. p. (see below) 262—264° (decomp.) (Found : C, 45·2; H, 2·8. Calc. for $C_8H_5O_6N$: C, 45·5; H, 2·4%).

(g) *Decarboxylation of the lactone-acid.* Various methods of decarboxylation failed. The potassium salt of the lactone-acid [made by evaporating a solution of the acid (0·94 g.) in water (3 ml.) containing potassium hydroxide (0·55 g.)] was heated strongly with soda lime (3 g.). The distillate (200 mg.) yielded a picrate, m. p. 155—160°, unchanged by repeated crystallisation from various solvents (Found : C, 45·2; H, 3·5. Calc. for $C_{13}H_{10}O_7N_4$: C, 46·7; H, 3·0. Calc. for $C_{13}H_{12}O_8N_4$: C, 44·3; H, 3·4%).

(h) *Oxidation of the decarboxylation product.* The above crude base (100 mg.) in water (20 ml.) containing 2N-sodium hydroxide (1 ml.) was oxidised at 100° with potassium permanganate (0·3 g.). The solution was filtered and the residue washed with hot water. The combined filtrates were acidified and evaporated. The residue was extracted with boiling alcohol, the mixture filtered, and the filtrate evaporated. The residue on treatment with aqueous picric acid (50 mg.) gave a picrate, m. p. and mixed m. p. 215° with *iso*nicotinic acid picrate.[8] Admixture with nicotinic acid picrate [8] (m. p. 210°) lowered the m. p. to 160—170°.

4-1′-*Hydroxyethylnicotinic Lactone.*—(a) 3-*Cyano*-4-*ethyl*-2 : 6-*dihydroxypyridine.* Ethyl β-oxovalerate [9] (9 g.), cyanoacetamide (5 g.), piperidine (10 ml.), and methanol (15 ml.) were refluxed for 3 hr. The methanol was distilled off, and the residue diluted with water (50 ml.) and acidified with dilute hydrochloric acid, to yield the *nitrile* (3 g.). Recrystallised from water, this blackened above 250° and effervesced at 260° (Found : C, 58·3; H, 4·8. $C_8H_8O_2N_2$ requires C, 58·6; H, 4·9%).

(b) 4-*Ethylnicotinonitrile.* The above pyridone (9 g.) was heated with phosphorus oxychloride (18 ml.) at 180° for 4 hr. in a sealed tube. The contents were poured on crushed ice. The solution was allowed to come to room temperature and extracted repeatedly with ether. The combined extracts were washed with water and dried (Na_2SO_4), and the solvent was distilled off, yielding the crude chloro-compound (8 g.) which was hydrogenated in methanol (100 ml.) containing potassium acetate (10 g.) and palladium chloride (0·7 g.) at 2 atm., till no more hydrogen was absorbed (½ hr.). The solution was filtered and the residue washed with methanol. The filtrate was evaporated, and the residue treated with water (50 ml.), saturated with sodium hydrogen carbonate, and extracted with ether. Evaporation of the dried (Na_2SO_4) ether extract, and vacuum-distillation of the residual oil gave 4-ethylnicotinonitrile (3·7 g.), b. p. 92°/3—4 mm., yielding a *picrate* (from water), m. p. 153—155° (Found : C, 46·0; H, 3·3. $C_{14}H_{11}O_7N_5$ requires C, 46·5; H, 3·0%). Wawzonek *et al.*[4] reported b. p. 72—74°/2 mm. for the nitrile.

[8] Johnson, *J.*, 1947, 1626.
[9] Anderson, Halverstadt, Miller, and Roblin, jun., *J. Amer. Chem. Soc.*, 1945, **67**, 2197.

(c) 4-*Ethylnicotinic acid* 1-*oxide*. 4-Ethylnicotinic acid (0·85 g.; from 4-ethylnicotinonitrile [4]) in acetic acid (6 ml.) containing 30% hydrogen peroxide (2 ml.) was heated at 70° for 3 hr. More hydrogen peroxide (2 ml.) was added and the mixture left at 70° for a further 8 hr. The solution was evaporated *in vacuo*, with repeated additions of water to remove last traces of hydrogen peroxide and acetic acid. The residue, on two crystallisations from water, gave the N-*oxide* (0·85 g.), m. p. 187—188° (Found: C, 57·4; H, 5·6; N, 7·9. $C_8H_9O_3N$ requires C, 57·5; H, 5·4; N, 8·4%).

(d) 4-1′-*Hydroxyethylnicotinic lactone*. (i) The above *N*-oxide (0·77 g.) in dioxan (10 ml.) containing acetic anhydride (2 ml.) was heated under reflux for 4 hr. The solvents were removed *in vacuo*; the residue was treated with excess of saturated sodium hydrogen carbonate solution and extracted with ether. The dried (Na_2SO_4) ether extract on evaporation gave an oil (0·45 g.) which, on treatment with picric acid (0·9 g.) in ether (50 ml.), gave a resin. This resin was washed several times with ether and then crystallised from alcohol, to give yellow plates of a picrate, m. p. 130—133°, raised by two recrystallisations from the same solvent to 148—151° (Found: C, 45·5; H, 3·1. Calc. for $C_{15}H_{14}O_9N_4$: C, 45·7; H, 3·6%). The m. p. was undepressed on admixture with the picrate derived from 4-1′-hydroxyethylpyridine acetate, prepared by treatment of 4-ethylpyridine 1-oxide with acetic anhydride.

The ether washings from the above experiment, on spontaneous evaporation, gave the 4-1′-*hydroxyethylnicotinic lactone picrate*, m. p. 153° after two crystallisations from alcohol (Found: C, 44·5; H, 2·7; N, 14·3. $C_{14}H_{10}O_9N_4$ requires C, 44·4; H, 2·6; N, 14·8%). Passage of a benzene solution (50 ml.) of this picrate (200 mg.) through alumina gave the free *base* (from ether–light petroleum), m. p. 87—88°, $\lambda_{max.}$ 255 mμ (log ε 3·18) (Found: C, 65·0; H, 4·4. $C_8H_7O_2N$ requires C, 64·4; H, 4·7%).

(ii) 4-Ethylnicotinic acid 1-oxide (0·5 g.) was warmed with acetic anhydride (2 ml.) till dissolved and left overnight at 30°. Addition of water, saturation with sodium hydrogen carbonate, and extraction with ether gave an oil (0·21 g.), yielding a single picrate (200 mg.), m. p. 153—154°, identical with the lactone picrate described above.

5-*Ethyl*-4-1′-*hydroxyethylnicotinic Lactone*.—(a) 3-*Cyano*-4 : 5-*diethyl*-2 : 6-*dihydroxypyridine*. This could not be obtained by refluxing ethyl α-ethyl-β-oxovalerate with piperidine and cyanoacetamide in methanol for 3 hr. Guareschi's procedure [10] was, however, successful. Ethyl α-ethyl-β-oxovalerate [9] (22 g.) was shaken with aqueous ammonia (40 ml.; *d* 0·9) for 6 days, and the aqueous layer containing the amide was separated and treated with ethyl cyanoacetate (16 ml.). The homogeneous solution obtained by shaking was left at 30° for 4 days. The ammonium salt of the nitrile was filtered off, suspended in water, and acidified. The precipitate, on recrystallisation from water, gave the *nitrile* (7 g.), m. p. 186—187° (decomp.) (Found: C, 62·2; H, 6·4. $C_{10}H_{12}O_2N_2$ requires C, 62·5; H, 6·3%).

(b) 4 : 5-*Diethylnicotinonitrile*. The preceding compound (10 g.) was heated with phosphorus oxychloride (20 ml.) and worked up as usual, to give the chloro-compound (8 g.) which was hydrogenated in the presence of palladium chloride (0·7 g.) in methanol (100 ml.) containing potassium acetate (8 g.), to give the nitrile (3·2 g.), b. p. 110°/5 mm. The *picrate*, on crystallisation from water, had m. p. 144—145·5° (Found: C, 49·6; H, 3·7. $C_{16}H_{15}O_7N_5$ requires C, 49·4; H, 3·9%).

(c) 4 : 5-*Diethylnicotinic acid*. The preceding nitrile (3·2 g.) was heated at 140° for 6 hr. with 75% sulphuric acid (32 ml.). The mixture was poured on crushed ice, and the solution treated with calcium hydroxide to pH 5—6 and filtered. The residue was repeatedly washed with boiling water. The combined filtrates were evaporated to dryness *in vacuo*, extracted with boiling alcohol, and filtered. The filtrate was evaporated to dryness, and the residual amino-acid sulphate passed in aqueous solution through De-Acidite E (Permutit). Evaporation of the eluate and recrystallisation from alcohol–ether gave 4 : 5-*diethylnicotinic acid* (2·5 g.), m. p. 115—116° (Found: C, 66·6; H, 7·1. $C_{10}H_{13}O_2N$ requires C, 67·0; H, 7·3%).

(d) 4 : 5-*Diethylnicotinic acid* 1-*oxide*. On treatment with hydrogen peroxide in acetic acid and crystallisation from water, the above acid (2·3 g.) gave the N-*oxide* (1·3 g.), m. p. 190—192° (Found: C, 61·8; H, 6·6. $C_{10}H_{13}O_3N$ requires C, 61·6; H, 6·7%).

(e) 5-*Ethyl*-4-1′-*hydroxyethylnicotinic lactone*. The oxide (0·5 g.) was treated with acetic anhydride (2 ml.) with occasional warming and shaking. The dark red solution was left overnight and worked up as above. The red oil (180 mg.) thus obtained, on treatment with ethereal picric acid (500 mg.) and crystallisation from methanol and then twice from water, gave the

[10] Guareschi, *Ber., Ref.*, 1896, **29**, 655; Ruzicka and Fornasir, *Helv. Chim. Acta*, 1919, **2**, 338.

lactone picrate (80 mg.), m. p. 148—149° (Found : C, 47·8; H, 3·5. $C_{16}H_{14}O_9N_4$ requires C, 47·3; H, 3·5%).

2-(3-*Carboxy*-5-*ethyl*-4-*pyridyl*)*propane*-1 : 3-*diol Lactone*.—(a) 5-*Ethyl*-4-*methylnicotinonitrile*. 3-Cyano-5-ethyl-2 : 6-dihydroxy-4-methylpyridine [10] (5 g.), when heated with phosphorus oxychloride (10 ml.) at 160° for 4½ hr., gave solid 2 : 6-dichloro-3-cyano-5-ethyl-4-methylpyridine (4·5 g.). Hydrogenation in presence of palladium chloride (0·4 g.) in methanol (50 ml.) containing potassium acetate (5·5 g.) yielded the nitrile (2·5 g.), b. p. 120°/7 mm., characterised as the *picrate*, m. p. 157—158° (from water) (Found : C, 48·3; H, 3·8. $C_{15}H_{13}O_7N_5$ requires C, 48·0; H, 3·5%).

(b) 5-*Ethyl*-4-*methylnicotinic acid*. The preceding nitrile (2 g.) was hydrolysed with 75% sulphuric acid (20 ml.) and worked up as above, giving the *acid* (1·5 g.), crystallising from alcohol–ether in flakes, m. p. 163—165° (Found : C, 65·6; H, 6·4. $C_9H_{11}O_2N$ requires C, 65·5; H, 6·7%).

(c) *The lactone*. The above acid (1 g.) was heated (sealed tube) with 40% aqueous formaldehyde (3 ml.) at 100° for 24 hr. Excess of formaldehyde was then removed in steam. The solution was concentrated to 5 ml. and extracted repeatedly with chloroform–ether. The dried (Na_2SO_4) extracts were evaporated and the residue (0·5 g.), after being washed with ether, crystallised from methanol–ether to give needles of the *lactone*, m. p. 168—169°, $\lambda_{max.}$ 270 mμ (log ε 3·44) (Found : C, 63·3; H, 6·3. $C_{11}H_{13}O_3N$ requires C, 63·8; H, 6·3%).

Action of Formaldehyde on Dihydrogentianine.—Dihydrogentianine (0·3 g.) was heated with 40% aqueous formaldehyde (1 ml.) at 100° for 24 hr. On working up as before, a colourless solid (0·15 g.) was obtained. Recrystallisation from methanol–ether gave needles, m. p. and mixed m. p. 168—169° with the preceding lactone (Found : C, 63·8; H, 6·4%).

Synthesis of Dihydrogentianine.—The sodium salt of 5-ethyl-4-methylnicotinic acid [obtained from the acid (0·5 g.) by treatment with the calculated quantity of 2N-sodium hydroxide] was heated with 40% aqueous formaldehyde (0·3 ml.) at 100° for 15 hr., then steam-distilled, acidified, and after 1 hr. basified with solid sodium hydrogen carbonate, and extracted with chloroform–ether. The dried (Na_2SO_4) extracts were evaporated. The residue, on digestion with light petroleum, gave the lactone, m. p. 168—169°, described above. Evaporation of the light petroleum extract and crystallisation of the residue from ether–light petroleum ether gave *dihydrogentianine* (8 mg.), m. p. and mixed m. p. 74—76° (Found : C, 67·9; H, 6·2%).

Pyridine-3 : 4 : 5-*tricarboxylic Acid*.—5-Ethyl-4-methylnicotinic acid (1 g.) in water (25 ml.) and 2N-sodium hydroxide (10 ml.) was oxidised with potassium permanganate as in section (f) above, giving an acid (0·35 g.), m. p. 258° (decomp.), which left an alkaline residue on combustion. Purification of this acid by passage through Zeo-Karb 315 (Permutit) gave pyridine-3 : 4 : 5-tricarboxylic acid (0·2 g.) as needles, m. p. 262—264° (decomp.) (Found : C, 45·6; H, 2·5%). Weber [3] reported m. p. 261° (decomp.).

We are grateful to Sir Alexander Todd, F.R.S., Dr. W. Herz, Dr. Gurbakgsh Singh, and Messrs. Sadtler Research Laboratories for infrared spectra, to Dr. E. Schlittler for photostat reprints of the Russian papers, to Sojuzchimexport, Moscow, for a sample of gentianine and to the University of Madras for a studentship (to S. R.).

PRESIDENCY COLLEGE, MADRAS, INDIA. [*Received, September 21st*, 1956.]