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STRUCTURE AND SYNTHESIS OF NUDIFLORINE

A NEW PYRIDONE ALKALOID

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Abstract—On the basis of physical and chemical studies nudiflorine, the new pyridone alkaloid isolated from the leaves of *Trewia nudiflora* Linn. has been assigned the 1-methyl-5-cyano-2-pyridone (II) structure which has been confirmed by an unambiguous synthesis. Nudiflorine is an isomer of ricinidine (I, R = H; R_1 = CN) the demethoxylation product of ricinine (I, R = OCH_3; R_1 = CN). Biogenesis of nudiflorine and ricinidine has been discussed and their simultaneous synthesis accomplished.

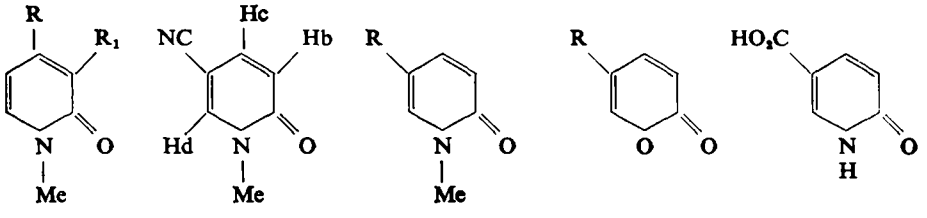
IN OUR search for new alkaloids from indigenous sources, *Trewia nudiflora* Linn. (Fam. *Euphorbiaceae*) has been chosen as it enjoys great reputation in folk medicine.[1] A preliminary report on the constitution of its seed oil[2] and the isolation of β-sitosterol and taraxerone from its stem bark[3], has been published. From the leaves of *T. nudiflora* a new pyridone alkaloid, nudiflorine (yield, 0·005%) has been isolated.

Nudiflorine,[4a] $C_7H_6N_2O$, (M = 134 from mass spectrometry), crystallizes from chloroform and petrol mixture in needles, m.p. 161°, R_f, 0·79 (butanol:formic acid: water::12:1:7) and is neutral in character. It exhibits absorption maxima in the UV spectrum, λ_{max} 206 (log ε, 4·27), 254 (log ε, 4·32) and 306 mμ (log ε, 3·89), which remain unchanged in acid and alkali (typical of a 2-pyridone nucleus)[5] and in the IR spectrum, it shows absorption peaks at 2200 (—CN), 1670 (conjugated amide) and 1610 cm^{-1} (—C=CH—). It contains one —NMe, the analysis of which was not satisfactory; but the definitive proof for this particular group has been secured from its NMR spectrum.

Nudiflorine does not dissolve in acid or alkali in the cold but does so when heated. On hydrolysis with 57% sulphuric acid it yields nudifloric acid, $C_7H_7NO_3$, m.p. 238°, λ_{max} 206 (log ε, 4·14), 255 (log ε, 4·20) and 300 mμ (log ε, 3·72), γ_{max} 3020 (—OH of α,β-unsaturated —CO_2H),[6] 1715 (—CO— of the —CO_2H), 1660 (conjugated NCO) and 1605 cm^{-1} (—C=CH—), which crystallizes from water in needles, but loses its crystalline shape during drying over P_2O_5. Nudifloric acid upon methylation with diazomethane forms a methyl ester, $C_8H_9NO_3$, m.p. 139°, λ_{max} 205 (log ε, 4·16), 264 (log ε, 4·20) and 300 mμ (log ε, 3·66), γ_{max} 1715 (conjugated carbomethoxy), 1662

[1] R. N. Chopra, S. L. Nayar and I. C. Chopra, *Glossary of Indian Medicinal Plants* p. 246 C.S.I.R., New Delhi (1956).
[2] S. Sarkar and M. M. Chakrabarty, *Sci. & Cult.* **21** 473 (1956).
[3] N. Adityachaudhury and G. Ganguli, *Indian J. Chem.* **2** 171 (1964).
[4a] R. Mukherjee and A. Chatterjee, *Chem. & Ind.* 1524 (1964).
[b] R. Mukherjee and A. Chatterjee, *J. Ind. Chem. Soc.* **42** 575 (1965).
[5] H. Specker and H. Gawrosch, *Ber. Dtsch. Chem. Ges.* **75**, 1338 (1942).
[6] L. J. Bellamy, *The infrared spectra of Complex Molecules* p. 163. Methuen, London (1956).

(NCO) and 1610 cm^{-1} (—C=CH—). The physical constants and spectral measurements of nudifloric acid and its methyl ester are in accord with those for 1-methyl-2-pyridone-5-carboxylic acid[7,8] (III, R = CO_2H) and its methyl ester (III, R = CO_2Me).



I II III IV V

The benzenoid absorption of differently substituted and unsubstituted 2-pyridones[5,9,10] remains unaltered in the cases of ricinine[9,11] (I, R = OMe; R_1 =CN) and ricinidine (I, R = H; R_1 = CN), λ_{max} 210 (log ε, 4·02), 234 (log ε, 3·69) and 334 mμ (log ε, 3·95), but is shifted to 254 mμ in the case of nudiflorine and nudifloric acid thus showing a bathochromic shift of 17 mμ with the simultaneous increase in the values of log ε suggesting a different environment of nitrile in nudiflorine from that in ricinidine. This eventually places the nitrile in nudiflorine, and thus the carboxyl group in nudifloric acid, *para* to the lactam carbonyl group.[7]

The structure thus elaborated for nudiflorine is consistent with its NMR spectrum (Fig. 1). The latter shows a sharp singlet at 3·57 δ for —NMe and the presence of a total of six protons in the compound. The remaining three protons are found to be aromatic in character (—C=CH—) giving an 8-line spectrum, doublet at 6·55 δ for Hb, 7·31 and 7·38 δ for Hc and 7·9 δ for Hd (II). The fine structure shows that the coupling constants involved in the three spin systems of the ring protons are J = 9·5, 9·0 and 3·0 c/s and as the coupling constants in 2-pyridone ring system are not likely to be very different from benzene and pyridine ring systems, the protons are placed in an 1,2,4-trisubstituted pattern.[12] From these results coupled with the characteristic chemical shift structure II appears to be the most plausible for nudiflorine.

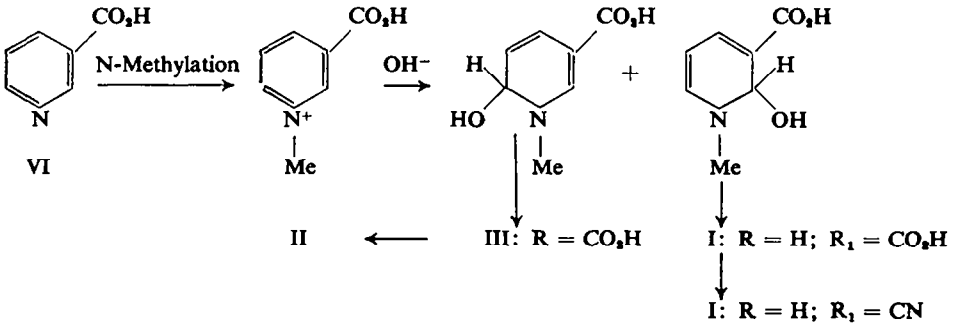
The 1-methyl-2-pyridone-5-nitrile structure for nudiflorine thus derived from spectral and chemical evidence has been finally confirmed by an unambiguous synthesis from malic acid. The latter was converted into coumalic acid methyl ester[13,14] (IV, R = CO_2Me) which on treatment with ammonia followed by hydrolysis afforded 6-oxonicotinic acid (V). N-Methylation of 6-oxonicotinic acid by methyl iodide and alkali followed by acidification yielded 1-methyl-2-pyridone-5-carboxylic acid[8] (= nudifloric acid = III, R = CO_2H), identical in all respects with the product from the natural source. The synthetic acid upon esterification gave the corresponding methyl ester, found to be identical with III (R = CO_2Me), the degraded product from

[7] R. Allouf and R. Munier, *Bull. Soc. Chim. Biol.* **34**, 196 (1952).
[8] von H. Pechmann and W. Welsch, *Ber. Dtsch. Chem. Ges.* **17**, 2384 (1884).
[9] E. Späth and G. Koller, *Ber. Dtsch. Chem. Ges.* **56**, 880, 2454 (1923).
[10] G. W. Ewing and E. A. Steck, *J. Amer. Chem. Soc.* **68**, 2181 (1946).
[11] R. Mukherjee and A. Chatterjee, Unpublished data.
[12] S. S. Dharmatti, G. Govil, C. R. Kanekar, C. L. Khetrapal and Y. P. Virmani, *Proc. Ind. Acad. Sci.* **54A**, 331 (1961).
[13] R. H. Wiley and N. R. Smith, *Organic Syntheses* **31**, 23 (1951).
[14] N. R. Campbell and J. H. Hunt, *J. Chem. Soc.* 1176 (1947).

nudiflorine. Nudifloric acid on treatment with phosphorous oxychloride and phosphorous pentachloride formed an acid chloride, a solution of which in dry benzene was saturated with ammonia gas. Further treatment with liquor ammonia afforded nudifloramide (III, R = $CONH_2$), crystallized from water in needles, $C_7H_8N_2O_2$, m.p. 209–210°, $\lambda_{max}^{H_2O}$ 258 (log ε, 4·18) and 300 mμ (log ε, 3·70), γ_{max} 1655 (conjugated amide), 1607 (aromatic unsaturation) and a pair of split bands for —NH_2 of exocyclic —$CONH_2$ at 3448 and 3225 cm^{-1}. Nudifloramide thus prepared was dehydrated with phosphorous oxychloride at 100–110° when the corresponding nitrile (II) was formed. It crystallized from chloroform and petrol mixture in needles, $C_7H_6N_2O$, m.p. 160–161°, identical with natural nudiflorine.

Recent studies on biosynthesis of ricinine by Marion *et al.*[15,16] with isotopically labelled compounds have prompted an investigation into a plausible biogenetic route to nudiflorine, the particular reason being that both nudiflorine and ricinidine have been synthesized *in vitro* from nicotinamide as will be discussed in the sequel.

The biogenetic precursor of pyridine alkaloids is nicotinic acid[17] (VI) which has been shown to originate from (i) a $C_{(4)}$-unit, viz. succinic acid or a closely related four-carbon dicarboxylic acid providing 2, 3 and 7 carbon atoms and (ii) a $C_{(3)}$-unit, viz. glycerol or a closely related metabolite from which carbon atoms 4, 5 and 6 originate as shown in ricinine (I, R = OMe; R_1 = CN) by Marion and his research group.[15,16] The biosynthesis of nudiflorine may proceed in a similar fashion as shown in the Chart:

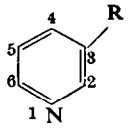


The Chart shows that both nudiflorine and ricinidine may indeed arise from the common biogenetic precursor, viz. nicotinic acid and in fact simultaneous synthesis of both these compounds has been accomplished by the present authors.[4b]

Nicotinamide (VII, R = $CONH_2$) on dehydration gave 3-cyanopyridine (VII, R = CN) (according to the method of La Forge[18]) which was converted to the methosulphate by reluxing with dimethyl sulphate. An aqueous solution of the methosulphate was oxidized with potassium ferricyanide following the method of Prill and McElvain.[19] A chloroform extract of the reaction product upon chromatographic resolution gave both nudiflorine and ricinidine. The synthetic products gave identical UV and IR spectra with the authentic samples. The total yield obtained in

[15] J. M. Essery, P. F. Juby, L. Marion and E. Trumbull, *Canad. J. Chem.* **41**, 1142 (1963).
[16] P. F. Juby and L. Marion, *Canad. J. Chem.* **41**, 117 (1963).
[17] R. A. Friedman and E. Leete, *J. Amer. Chem. Soc.* **85**, 2141 (1963); E. Leete, *Ibid.* **78**, 3520 (1956).
[18] F. B. La Forge, *J. Amer. Chem. Soc.* **50**, 2477 (1928).
[19] E. A. Prill and S. M. McElvain, *Organic Synthesis* **15**, 41 (1935).

this synthesis was 10% comprising a mixture of 25% nudiflorine and 75% ricinidine. This result conforms with theoretical expectation because the strongly electron-withdrawing character of the nitrile group (VII, R = CN) will increase the electrophilic character of the 2-position over that of the 6.



VII

It is pertinent to mention here that when the reaction was repeated separately with the methiodide instead of the methosulphate, of either 3-cyanopyridine or nicotinamide, only ricinidine and 1-methyl-2-pyridone-3-carboxamide* were the products. This result is in complete agreement with the independent findings of Holman and Wiegand.[20] Formation of only the 1-methyl-3-substituted-2-pyridones in such cases may be ascribed to (i) the poor solubility of the methiodides in water, and (ii) its consequent slow hydrolysis at low temperature, thus decreasing further the overall yield of the products. Recently simultaneous synthesis of nudiflorine and ricinidine has been reported[21] from nicotinonitrile methiodide by enzymatic oxidation.

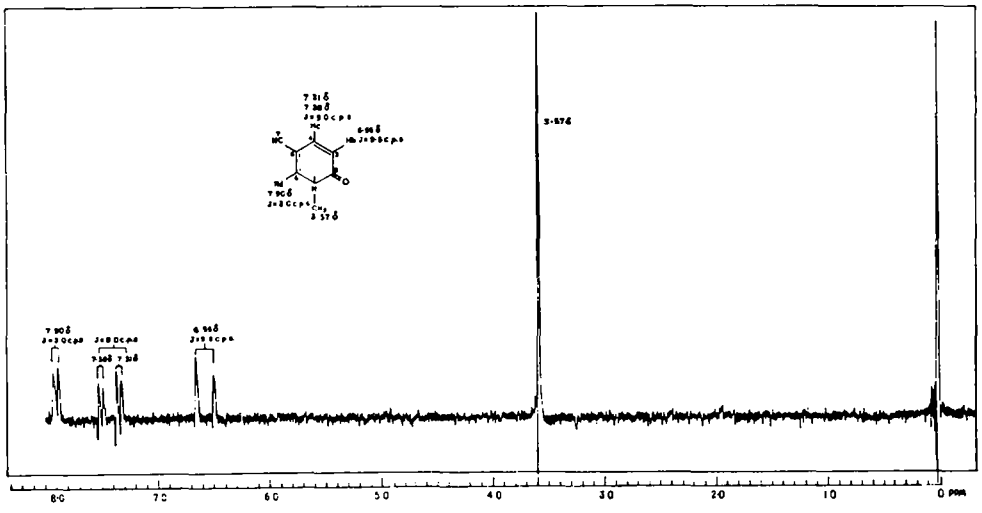


FIG. 1. NMR Spectrum of nudiflorine.

These results also support the observations of Knox and Grossman[22] about the transformation of nicotinamide to N-methyl nicotinamide and the amide of N-methyl-2-pyridone-5-carboxylic acid (nudifloramide).

* The present compound is hydrolysed according to[20] and the product formed is established as 1-methyl-2-pyridone-3-carboxylic acid, $C_7H_7NO_3$, m.p. 184°, $\lambda_{max}^{H_2O}$ 235 (log ε, 3·72) and 330 mμ (log ε, 3·98) and the values are in good agreement with the values reported.[7]

[20] W. I. M. Holman and C. Wiegand, *Biochem. J.* **43**, 423 (1948).
[21] T. Robinson, *Phytochemistry* **4**, 67 (1965); T. Robinson and C. Cepurneek, *Ibid.* **4**, 75 (1965).
[22] W. E. Knox and W. I. Grossman, *J. Biol. Chem.* **166**, 391 (1946); **168**, 363 (1947).

Thus the simultaneous synthesis[4b] of ricinidine and nudiflorine described above further emphasizes the probability of the natural occurrence of the former and the future isolation of ricinidine from the plant kingdom will further correlate the genetic pathway in plant cells with the biochemical reactions in mamalian origin.

EXPERIMENTAL

All the m.ps reported are uncorrected, the UV absorption spectra were taken in a Perkin–Elmer automatic spectrophotometer in EtOH solution unless otherwise specified, the IR absorption spectra were recorded in a Perkin–Elmer infracord machine and the NMR spectra were taken in Varian A-60 spectrometer in $CDCl_3$ solution with $SiMe_4$ as internal reference. Petrol refers to the fraction b.p. 60–80° and light petrol to the fraction b.p. 40–60°.

Isolation of nudiflorine (II). The defatted plant material (4 kg) was extracted in a soxhlet (35 hr) with $CHCl_3$ the extract concentrated (100 ml) and chromatographed over Brockmann alumina (800 g). Upon washing the chromatogram with benzene–$CHCl_3$ (4:1) nudiflorine (0·25 g) was eluted and repeatedly crystallized from $CHCl_3$–petrol (1:1) in glistening needles, m.p. 161°, $[\alpha]_D^{30°}$ ±0° ($CHCl_3$), M = 134 (Mass spectrometry). (Found: C, 62·86; H, 4·55; N, 20·87; —NCH_3, 5·50. $C_7H_6N_2O$ requires: C, 62·68; H, 4·47; N, 20·89; 1-NCH_3, 11·19%.)

Acid hydrolysis of nudiflorine (II). Nudiflorine (0·2 g) was refluxed with 57% H_2SO_4 (2·5 ml) for 6 hr. The reaction product was cooled, diluted with 1·5 ml water and the pH adjusted to 6 when crystals of III (R = CO_2H) appeared. This material (0·16 g) was finally crystallized from water in needles, m.p. 238, but lost its crystalline shape during drying. (Found: C, 54·89; H, 4·87; N, 8·91. $C_7H_7NO_3$ requires: C, 54·91; H, 4·58; N, 9·14%.)

Esterification of nudifloric acid to III (R = CO_2Me). To a cooled solution of nudifloric acid (0·1 g) in dry MeOH (10 ml) about 8 ml ethereal diazomethane (0·1 g) was added slowly with stirring. The reaction product was kept overnight at 0°. After the complete evaporation of the solvent III (R = CO_2Me; 0·1 g) was obtained which crystallized from $CHCl_3$–ether in needles, m.p. 139°. (Found: C, 57·59; H, 5·61; N, 8·14; —OCH_3, 18·46. $C_8H_9NO_3$ requires: C, 57·49; H, 5·39; N, 8·36; 1-OCH_3, 18·58%.)

Preparation of 6-oxonicotinic acid (V). Powdered IV (R = CO_2Me, 12 g) prepared by the known methods[13,14] was added to an ice cold aqueous ammonia solution (15%, 150 ml) with constant stirring while the temp was not allowed to rise above 5°. The brown clear solution obtained after the complete addition of the methyl ester was boiled with 20% NaOHaq (72 ml) when the excess of ammonia was completely driven off (about 5 min). The reaction product was cooled and acidified with conc. HCl and a white precipitate of 6-oxonicotinic acid obtained. This was filtered off and washed with ice cold water (15 ml). Finally the impure product (9 g) was crystallized from water in fine needles, m.p. 304–306° (dec). (Found: C, 51·94; H, 3·72; N, 10·13. $C_6H_5NO_3$ requires: C, 51·79; H, 3·59; N, 10·07%.)

Conversion of V *into nudifloric acid* III (R = CO_2H). A solution of V (4 g) in 3% NaOHaq (60 ml) was refluxed with MeI (3 ml) for 6 hr. Excess MeI was removed by warming and the reaction product cooled and acidified with conc. HCl which precipitated III (R = CO_2H). The acid thus obtained was filtered off washed with water (10 ml) and crystallized from water in needles (3·8 g), m.p. 238–239°, which showed no depression in mixed m.p. with the degraded acid (mixed UV and IR comparison also established their identity). The compound lost its crystalline shape during drying. (Found: C, 55·14; H, 4·62; N, 9·08. $C_7H_7NO_3$ requires: C, 54·91; H, 4·57; N, 9·14%.)

Esterification of the synthetic acid (III, R = CO_2Me). Synthetic nudifloric acid (0·1 g) was converted to the corresponding methyl ester similar to the method used for the degraded acid and the product was found to be identical with III (R = CO_2Me). (Found: C, 57·72; H, 5·58; N, 9·08; —OCH_3, 19·02. $C_8H_9NO_3$ requires: C, 57·49; H, 5·39; N, 8·36; 1-OCH_3, 18·58%.)

Preparation of nudifloramide (III, R = $CONH_2$). Nudifloric acid (2 g) was heated on a water bath for 3 hr with $POCl_3$ (6 ml) and PCl_5 (1 g), after which a fresh quantity of PCl_5 (1 g) was added and again heated for 3 hr. Excess of reagents were removed *in vacuo* at 40–50° and the residue taken in dry benzene which was saturated in the cold first with dry ammonia gas and then with a strong solution of ammonia (20 ml). Excess of ammonia and benzene were removed under suction and the residue (1·6 g) crystallized from water in long needles, m.p. 209–210°. (Found: C, 55·13; H, 5·16; N, 18·20. $C_7H_8N_2O_2$ requires: C, 55·26; H, 5·26; N, 18·42%.)

Dehydration of nudifloramide to II. Nudifloramide (1 g) was heated with $POCl_3$ (8 ml) at 100–110° in an oil bath for 8 hr. The reaction product was allowed to cool slowly, then dissolved in water, made alkaline with K_2CO_3 and repeatedly extracted with $CHCl_3$. The extract was first washed with 2% $NaHCO_3$aq, then with water and finally dried over Na_2SO_4. It was then concentrated and chromatographed over Brockmann alumina. The benzene–$CHCl_3$ (4:1) eluates upon evaporation furnished nudiflorine (0·25 g) which crystallized from $CHCl_3$–petrol in needles, m.p. 160–161°, which showed no depression in mixture m.p. with the natural product (and also showed identity in mixed UV and IR comparison). (Found: C, 62·78; H, 4·41; N, 21·10. $C_7H_6N_2O$ requires: C, 62·68; H, 4·47; N, 20·89%.)

Preparation of 3-*cyanopyridine methiodide.* Nicotinamide (VII, R = $CONH_2$) was dehydrated to VII (R = CN) following the method of La Forge.[18] To a solution of VII (1 g) in dry acetone (10 ml) freshly distilled MeI (2·1 g) was added and kept overnight. The solution after concentration precipitated a yellow solid (2·2 g) which was finally crystallized from EtOH–acetone in needles, m.p. 198° (dec). (Found: C, 34·52; H, 3·16; N, 11·10. $C_7H_7N_2I$ requires: C, 34·15; H, 2·85; N, 11·39%.)

Nicotinamide methiodide. Nicotinamide methiodide was prepared in the same way as 3-cyanopyridine methiodide and was crystallized from EtOH in needles, m.p. 208–209° (dec). (Found: C, 32·15; H, 3·16; N, 10·79. $C_7H_9N_2OI$ requires: C, 31·82; H, 3·41; N, 10·60%.)

Oxidation of 3-*cyanopyridine methosulphate.* 3-Cyanopyridine (2·85 g) and dimethyl sulphate (3·5 g) was refluxed for 2 hr and the methosalt dissolved in water (6 ml) and cooled to 0°. Solutions of potassium ferricyanide (18 g) in water (40 ml) and NaOH (4·5 g) in water (7·5 ml) were added to the methosulphate solution at such a rate that only half the volume of ferricyanide solution was added when the addition of NaOHaq was complete. The reaction mixture was mechanically stirred and the temp not allowed to rise above 10°. The reaction product was repeatedly extracted with $CHCl_3$, the layer washed with water till free from alkali, dried over Na_2SO_4, concentrated and chromatographed over Brockmann alumina. On successive elution with $CHCl_3$–benzene (4:1) and $CHCl_3$, II and I (R = H; R_1 = CN) were eluted out respectively. Nudiflorine (0·086 g) crystallized from $CHCl_3$–petrol in needles, m.p. 160°. (Found: C, 62·83; H, 5·05; N, 21·21. $C_7H_6N_2O$ requires: C, 62·86; H, 4·55; N, 20·89%), while ricinidine (0·25 g) crystallized from $CHCl_3$–ether in needles, m.p. 146° (mixed m.p. with the authentic sample prepared from ricinine according to the method of Späth *et al.*[9] was undepressed). (Found: C, 63·09; H, 4·73; N, 20·91. $C_7H_6N_2O$ requires: C, 62·86; H, 4·55; N, 20·89%.)

Oxidation of nicotinamide methiodide to I (R = H; R_1 = $CONH_2$). Nicotinamide methiodide (4·5 g) was dissolved in water (26 ml) in a 250 ml flask and cooled in ice. To the contents were added slowly, with constant stirring, a solution of potassium ferricyanide (19 g) in water (45 ml) and simultaneously a solution of KOH (8 g) in water (16 ml). After the complete addition of the potassium ferricyanide solution the mixture was stirred for 2 hr and extracted with $CHCl_3$ repeatedly. The extract was washed with water till free from alkali and completely evaporated yielding a pale brown solid (0·10 g). This was characterized as I (R = H; R_1 = $CONH_2$). It crystallized from water in needles, m.p. 219°. (Found: C, 55·49; H, 5·09; N, 18·56. $C_7H_8N_2O_2$ requires: C, 55·27; H, 5·27; N, 18·43%.)

Oxidation of 3-*cyanopyridine methiodide.* 3-Cyanopyridine methiodide (4 g) was dissolved in water (20 ml) in a 200 ml flask and cooled in ice. The contents were oxidized with potassium ferricyanide in alkaline medium as in the case of nicotinamide methiodide. The reaction product was extracted with $CHCl_3$ and yielded ricinidine (0·20 g) which crystallized from $CHCl_3$–ether in needles, m.p. 146°. It gave superimposable UV and IR spectra with the authentic sample. (Found: C, 62·88; H, 4·76; N, 21·10. $C_7H_6N_2O$ requires: C, 62·86; H, 4·55; N, 20·89%.)

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