1064. *Indicine: the Major Alkaloid of* Heliotropium indicum *L.*

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Heliotropium indicum from Ghana and from Australia contains a new pyrrolizidine alkaloid, indicine (I), and its *N*-oxide, with minor unidentified alkaloids.

In a study of potentially hepatotoxic plants, *Heliotropium indicum* was of interest as it is one of the most widely used herbs in the Ayurvedic medicine of India [1] and in some other countries in which liver diseases are common.[2] Pyrrolizidine alkaloids have previously been isolated from *Heliotropium lasiocarpum* (heliotrine, lasiocarpine),[3] *H. europaeum* (heliotrine, lasiocarpine, supinine, europine),[4] and *H. supinum* (supinine, echinatine).[5] All of these have proved to be hepatotoxic.[6]

[1] Kirtikar, Basu, and An, " Indian Medicinal Plants," Allahabad, 2nd edn., 1936, Vol. III, p. 1689; Nadkarni, " Indian Materia Medica," Nadkarni, Bombay, 1927, p. 422.

[2] Dalziel, " The Useful Plants of West Tropical Africa," Crown Agents for the Colonies, London, 1937, p. 725; Dragendorff, " Die Heilpflanzen der verschiedenen Völker und Zeiten," F. Enke, Stuttgart, 1898.

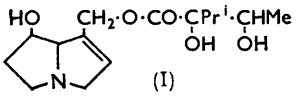
[3] Menshikov, *Ber.*, 1932, **65**, 974.

[4] Culvenor, Drummond, and Price, *Austral. J. Chem.*, 1954, **7**, 277; Culvenor, *ibid.*, p. 287.

[5] (*a*) Menshikov and Gurevich, *Zhur. obshchei Khim.*, 1949, **19**, 1382; (*b*) Crawley and Culvenor, *Austral. J. Chem.*, 1959, **12**, 694.

[6] Bull, Dick, and McKenzie, *J. Path. Bact.*, 1958, **75**, 17; Bull and Dick, *ibid.*, 1959, **78**, 483, and unpublished results; Schoental and Magee, *ibid.*, 1957, **74**, 305; 1959, **78**, 471.

Independent studies in England and in Australia (where *H. indicum* is naturalised and a potential danger to stock) have led to identification of the major alkaloid, indicine (I), as the (—)-trachelanthic ester of retronecine. Results from the two laboratories are here presented jointly.



(I)

In plant material from Ghana and from Australia, the alkaloid occurred largely as the *N*-oxide, with considerable variation in total alkaloid content. Isolation of the crude alkaloid was greatly facilitated by the use of cation exchange-resin,[7] which retained both the alkaloids and their *N*-oxides. After reduction of oxides, the crude base consisted of one major component, indicine, with small amounts of minor alkaloids. Best isolated by means of its picrate or picrolonate, indicine had the empirical formula $C_{15}H_{25}NO_5$.

Alkaline hydrolysis of indicine gave retronecine and an acid $C_7H_{14}O_4$ whose properties closely resembled those of trachelanthic acid in all except sign of specific rotation. Periodate oxidation of the acid gave acetaldehyde, isobutyric acid, and carbon dioxide, showing that it is an αβ-dihydroxy-α-isopropylbutyric acid. It was identified as the enantiomeric (—)-form of trachelanthic acid by formation of the racemate with the (+)-form obtained by hydrolysis of supinine, and by the identity of the infrared spectrum of the enantiomers. Evidence that the acid esterifies the primary hydroxyl group of retronecine was obtained by catalytic hydrogenolysis of indicine to (—)-trachelanthic acid and retronecanol.

Indicine is the first alkaloid from a *Heliotropium* species to contain retronecine as the basic moiety; the isomeric heliotridine has hitherto been found in this genus. However, esters of retronecine with monocarboxylic acids have been found in other genera of the *Boraginaceae*, namely, *Echium*, *Cynoglossum*, and *Amsinckia*.[8] (—)-Trachelanthic acid is the third isomer of αβ-dihydroxy-α-isopropylbutyric acid to occur as an esterifying acid in pyrrolizidine alkaloids. (+)-Trachelanthic acid and (—)-viridifloric acids have previously been found in several alkaloids of the *Boraginaceae*.[9,5b]

Several derivatives of indicine including the *N*-oxide, which is deliquescent, have been prepared. The picrate forms solvates with properties suggesting that they are clathrate compounds.[10]

Paper chromatography of the alkaloids in butanol–acetic acid gave more compact spots and more reproducible R_F values on paper buffered with sodium acetate.[11] *E.g.*, R_F values of indicine on unbuffered paper ranged from 0·32 to 0·39, according to the quantity spotted; on buffered paper they varied only from 0·56 to 0·58.

Experimental

Paper Chromatography of Alkaloids.—Except where otherwise stated, descending chromatograms were run on Whatman no. 1 paper, buffered with 0·1M-sodium acetate,[11] with butan-1-ol–acetic acid–water as solvent.[5b] Spots were detected by exposing the dried paper to iodine vapour.

Isolation of Alkaloids from Heliotropium indicum.—(a) *From Ghana.* Dried ground plant (2·04 kg.) was continuously percolated for 9 hr. with ethanol at room temperature, a column of Dowex 50 resin (200—400 mesh; acid form; 100 g. damp) being included in the circuit.[7] The Dowex column was washed with ethanol and with water, and the bases were eluted with ~0·8N-aqueous ammonia (300 ml.). The eluate was reduced with sulphuric acid and zinc dust to convert *N*-oxides into bases, and extracted from alkaline solution with chloroform, to give a pale brown gum (8 g.). Paper chromatography showed a large spot, R_F 0·58 (indicine), and weak spots, R_F 0·26 (retronecine) and 0·69 (unidentified alkaloid).

When the plant (11 kg.) was twice soaked in ethanol (total 20 l.), and the extract passed through a column of Dowex 50, the crude alkaloids, obtained as described above, formed an

[7] Mattocks, *Nature* 1961, **191**, 1281.
[8] Culvenor, *Austral. J. Chem.*, 1956, **9**, 512, and unpublished results.
[9] Leonard, " The Alkaloids," ed. Manske, Academic Press, 1960, Vol. VI, p. 35.
[10] Powell, *J.*, 1948, 61.
[11] Munier, Macheboeuf, and Cherrier, *Bull. Soc. Chim. biol.*, 1952, **34**, 204.

almost colourless gum (15·6 g.). This partly crystallised during several weeks at room temperature. Recrystallisation from light petroleum (b. p. 80—100°) or from benzene–hexane gave colourless hexagonal prisms of *indicine*, m. p. 97—98°, $[\alpha]_D^{20}$ +22·3° (*c* 1·65 in EtOH), R_F 0·58 (Found: C, 60·1; H, 8·3; N, 5·0. $C_{15}H_{25}NO_5$ requires C, 60·2; H, 8·3; N, 4·7%). On a larger scale this alkaloid was best recrystallised from diethyl ether.

By chromatography on a column of Hyflo Supercel (140 g., depth 43 cm.) buffered with M/15-disodium hydrogen phosphate (70 ml.) and elution with various chloroform–carbon tetrachloride mixtures, the crude reduced material (3 g.) yielded pure indicine (1·4 g.; R_F 0·58 only) and a mixture of indicine with the substance, R_F 0·69.

By chloroform extraction of aqueous extracts before and after reduction, two batches of *H. indicum* from Ghana were found to contain tertiary base, 0·009%, *N*-oxide 0·08%, and base, 0·045%, *N*-oxide 0·345%, respectively.

(b) *From Australia.* Preliminary assay [12] showed that the plant contained 0·23% of tertiary base and 0·18% of *N*-oxide (2 earlier samples from different parts of Queensland contained base 0·09%, oxide 0·37%, and base 0·09%, oxide 1·33%, respectively). A hot methanol extract of dried plant tops (10 lb.) was evaporated and the residue extracted with dilute sulphuric acid. Reduction and extraction with chloroform gave the crude alkaloid as a gum (24 g.), apparently nearly all one base (R_F 0·34 on unbuffered paper with ascending chromatography). A portion of this gum (8 g.) was treated with a slight excess of picrolonic acid in ethanol, the solution evaporated, and the residue extracted with hot water. *Indicine picrolonate* (9·5 g.), m. p. 108—109°, crystallised on cooling. Recrystallisation from aqueous ethanol gave crystals, m. p. 108—109°. The m. p. was raised to 115—117° by drying for 3 hr. at 100° but the picrolonate still contained water of crystallisation (Found: C, 52·0; H, 6·0; N, 12·1. $C_{15}H_{25}NO_5,C_{10}H_8N_4O_5,H_2O$ requires C, 51·6; H, 6·0; N, 12·0%). Base, recovered from the picrolonate by means of Deacidite FF resin, was a gum.

Indicine Salts.—Pure indicine, in ethanol, was neutralised with ethanolic hydrogen chloride, excess of dry ether was added, and the product was recrystallised from ethanol–ether to give hygroscopic, rectangular leaflets of the *hydrochloride*, m. p. 131—132°, $[\alpha]_D^{20}$ +11·25° (*c* 4·54 in EtOH) (Found: C, 53·9; H, 7·9; N, 4·4; Cl, 10·9. $C_{15}H_{25}NO_5,HCl$ requires C, 53·6; H, 7·8; N, 4·2; Cl, 10·6%).

Aqueous indicine hydrochloride, treated with saturated ammonium reineckate solution, yielded the *reineckate*, pale pink blades (from water), m. p. 141—142° {Found: C, 36·8; H, 5·6. $C_{15}H_{26}NO_5[Cr(NH_3)_2(SCN)_4]$ requires C, 36·9; H, 5·2%}.

Indicine (0·3 g.) in benzene was neutralised with picric acid; the *picrate* (0·5 g.), after several recrystallisations from benzene formed leaflets, m. p. 88—90° (efferv.). The recrystallised picrate was redissolved in hot benzene, and portions of the solution were cooled, (*a*) very slowly, giving leaflets, m. p. 90° (efferv.) (Found: C, 53·4; H, 5·3; N, 9·5. $C_{15}H_{25}NO_5,C_6H_3N_3O_7,C_6H_6$ requires C, 53·5; H, 5·6; N, 9·2%), and (*b*) rapidly, giving leaflets, m. p. 81—86° (efferv. above 90°) (Found: C, 51·0; H, 5·5; N, 10·3. $C_{15}H_{25}NO_5,C_6H_3N_3O_7,0{\cdot}62C_6H_6$ requires C, 51·3; H, 5·5; N, 9·7%).

The slowly recrystallised picrate (68·3 mg.) was dried *in vacuo* for 3 days at room temperature over P_2O_5, KOH, and paraffin wax, without loss in weight. It was then heated *in vacuo* during 8 min. from 60° to 90°, and then to 100°. Vigorous effervescence occurred at first, but ceased completely after ½ hr. at 100°. The volatile product was collected in a cold trap, and diluted with hexane; benzene (8·0 mg.) was identified and estimated by its ultraviolet spectrum.[13] The loss in weight was 8·1 mg. (11·9%. Calc. for 1 mol. of benzene of crystallisation: 12·9%).

In a similar experiment the rapidly recrystallised picrate lost 6·1% by wt.

The glassy residue, recrystallised twice from water, gave a *hydrate* as platelets, m. p. 66—68°, without effervescence up to 140° (Found: C, 46·6; H, 5·4; N, 10·5. $C_{15}H_{25}NO_5,C_6H_3N_3O_7,H_2O$ requires C, 46·2; H, 5·5; N, 10·3%).

Samples of indicine, recovered from the picrate before and after heating, had identical R_F values (0·58).

Indicine (0·2 g.) in chloroform was treated with methyl iodide for 5 min. at room temperature, the solvent removed, and the residue recrystallised from ethanol–ether to give the *methiodide* as blades (0·21 g.), m. p. 159—160°, $[\alpha]_D^{20}$ +12·5° (*c* 4·32 in EtOH) (Found: C, 44·4; H, 6·15; N, 3·2; I, 29·4. $C_{16}H_{28}INO_5$ requires C, 43·5; H, 6·35; N, 3·2; I, 28·8%).

[12] Culvenor and Smith, *Austral. J. Chem.*, 1955, **8**, 556.

[13] Tunnicliff, Brattain, and Zumwalt, *Analyt. Chem.*, 1949, **21**, 890.

When the methiodide was hydrolysed with dilute alkali, an acid was obtained, m. p. 86°, not depressed by the acid obtained by hydrolysing indicine.

Indicine N-*Oxide*.—Indicine (0·5 g.) in ethanol (4 ml.) was treated with 30% hydrogen peroxide (2 ml.) for 3 days at room temperature. The excess of reagent was destroyed with manganese dioxide, and the filtered solution was evaporated under reduced pressure. The deliquescent residue was dissolved in a little methanol, reprecipitated with acetone and ether, cooled, and rubbed to induce crystallisation. Recrystallisation from methanol–acetone gave colourless, deliquescent needles, which were isolated by centrifugation and washing with ether and light petroleum (b. p. 30—40°) in the centrifuge tube, and were dried by slow evacuation in a desiccator over P_2O_5. The solvated *N*-oxide had m. p. 130—131° (decomp. 165—166°), $[\alpha]_D^{20}$ +34·0° (*c* 7·0 in EtOH) (Found: C, 56·1; H, 8·25; N, 3·75. Calc. for $C_{15}H_{25}NO_6$: C, 57·1; H, 7·9; N, 4·4. Calc. for $C_{15}H_{25}NO_6,CH_3{\cdot}OH$: C, 55·3; H, 8·35; N, 4·0%).

Hydrolysis of Indicine.—Indicine (1·2 g.) was heated in 2N-sodium hydroxide at 100° for 2 hr., and the solution was acidified and extracted with ether to give an acid (0·55 g.) which crystallised from benzene–light petroleum in rosettes, m. p. 94°, $[\alpha]_D$ −3·4° (Found: C, 51·9; H, 8·7. Calc. for $C_7H_{14}O_4$: C, 51·8; H, 8·7%). A mixture of equal quantities of this acid with (+)-trachelanthic acid, after melting and cooling, remelted at 118—119° (racemic trachelanthic acid has m. p. 119—121° [14]). The *brucine salt* of the acid from indicine formed needles (from ethanol), m. p. 197—198° (Found: C, 63·1; H, 7·2; N, 4·9. $C_7H_{14}O_4,C_{23}H_{26}N_2O_4$ requires C, 63·7; H, 7·2; N, 5·0%). Adams and van Duuren [14] and Dry and Warren [14] record 183—187° and 196—198°, respectively, for the m. p. of the brucine salt of synthetic (−)-trachelanthic acid.

The basic product of hydrolysis was isolated by neutralising the solution from which acid has been extracted, evaporating it to dryness, extracting the residue with ethanol, and passing the extract, diluted with an equal volume of water, through Deacidite FF resin. Evaporation of the eluate gave a base (0·55 g.) (R_F 0·26) which crystallised from acetone in prisms, m. p. 118—119°, undepressed on admixture with retronecine (Found: C, 62·1; H, 8·5; N, 9·2. Calc. for $C_8H_{13}NO_2$: C, 62·0; H, 8·4; N, 9·0%).

Oxidation of the Acid from Hydrolysed Indicine.—(*a*) The acid (62·3 mg.), oxidised with 0·5M-sodium metaperiodate [15] (2 ml.) at room temperature for 30 min., absorbed 1·91 mol. of periodate. After an additional 1 hour's heating at 80—90°, the total periodate absorbed was 1·95 mol.

(*b*) The acid (115 mg.) was oxidised as above, the solution was flushed with nitrogen, and this was passed in turn through 2,4-dinitrophenylhydrazine reagent and barium hydroxide solution. This gave much barium carbonate and the acetaldehyde 2,4-dinitrophenylhydrazone (45 mg.), m. p. and mixed m. p. 151—152°.

The oxidation mother liquor, when saturated with calcium chloride and extracted with ether, yielded a pungent acid whose anilide formed colourless leaflets (from water), m. p. 103—104° alone or mixed with isobutyranilide.

Hydrogenolysis of Indicine.—In dilute hydrochloric acid, in the presence of platinum oxide, indicine absorbed 2 mol. of hydrogen. Filtration and extraction with ether gave (−)-trachelanthic acid, m. p. 94° (from benzene–light petroleum), identical with the acid obtained by hydrolysis. The residual aqueous solution was made alkaline and extracted with chloroform to give retronecanol, crystallising from light petroleum in prisms, m. p. and mixed m. p. 92— 93° (Found: C, 68·1; H, 10·6; N, 10·3. Calc. for $C_8H_{15}NO$: C, 68·1; H, 10·6; N, 9·9%). It formed a picrate, m. p. 211—213°, from ethanol, undepressed on admixture with retronecanol picrate.[16]

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[14] Adams and Van Duuren, *J. Amer. Chem. Soc.*, 1952, **74**, 5349; Dry and Warren, *J.*, 1952, 3445.
[15] Christie, Kropman, Novellie, and Warren, *J.*, 1949, 1703.
[16] Barger, Seshadri, Watt, and Yabuta, *J.*, 1935, 11; Adams and Rogers, *J. Amer. Chem. Soc.*, 1939, **61**, 2815.