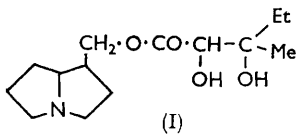
378. *Strigosine, the Major Alkaloid of* Heliotropium strigosum.

By A. R. Mattocks.

Heliotropium strigosum contains a new pyrrolizidine alkaloid, strigosine (I), which is shown to be an ester of trachelanthamidine with an αβ-dihydroxy-β-methylvaleric acid.

Hepatotoxic pyrrolizidine alkaloids have been isolated from a number of plants of the genus *Heliotropium*.[1] *H. strigosum* was studied[2] as it is known to be used in herbal medicine in West Africa. The major alkaloid, strigosine (I), has been identified as the ester of the known pyrrolizidine base, trachelanthamidine,[3] with (—)-αβ-dihydroxy-β-methylvaleric acid. This is the first pyrrolizidine alkaloid found to be an ester of a six-carbon monocarboxylic acid. The saturated character of strigosine agrees with the absence of hepatotoxic activity in this alkaloid;[4] all pyrrolizidine alkaloids so far found to be hepatotoxic have a 1,2-double bond.[5]



(I)

Crude alkaloids were obtained by continuous extraction of the plant material with cold ethanol, isolated using cation-exchange resin,[6] and reduced to convert the *N*-oxides (about

[1] Mattocks, Schoental, Crowley, and Culvenor, *J.*, 1961, 5400, and references therein.
[2] This work was initiated by Dr. R. Schoental.
[3] Men'shikoff and Borodina, *J. Gen. Chem.* (*U.S.S.R.*), 1945, **15**, 225.
[4] Schoental, unpublished results.
[5] Schoental, *Nature*, 1957, **179**, 361; Culvenor, Dann, and Dick, *Nature*, 1962, **195**, 570.
[6] Mattocks, *Nature*, 1961, **191**, 1281.

85% of the natural alkaloids) into the free bases. Further extraction of the plant residues gave more base, containing strigosine, trachelanthamidine, and minor alkaloids. Strigosine was isolated as its picrate and further characterised as the hydrochloride and methiodide. Hydrolysis of strigosine gave an acid and a base; because of the difficulty of obtaining pure strigosine most subsequent experiments were done with a hydrolysate of the crude alkaloid mixture; the major acid and base from this were identical with those from strigosine. The picrolonate of the same base was also prepared from a crude extract of the plant residues. The base was identified as trachelanthamidine by analysis and from a comparison of its properties with those reported in the literature.[3]

The acid from strigosine, recovered from its purified brucine salt, was obtained only as a gum. Analysis of the quinine and the brucine salts established the empirical formula of the acid as $C_6H_{12}O_4$. It reacted with two equivalents of metaperiodate, giving carbon dioxide, ethyl methyl ketone, and a volatile acid (which must be formic acid). The only acid satisfying these data is αβ-dihydroxy-β-methylvaleric acid, which had been reported as a precursor of isoleucine in strains of *Neurospora crassa* [7] and *Escherichia coli*.[8]

The acid was synthesised, and resolved through the quinine salt by the published method.[9] The quinine salt of the (−)-acid, and the brucine salt (which could only be partially resolved), had the same melting points and mixed melting points as the acid from strigosine. The R_F values and infrared spectra of all samples of the synthetic acid were identical with those of the acid from strigosine.

The optical rotations of the natural acid, and of its quinine salt, agreed fairly well (Table) with those of a synthetic sample (*a*). These values, and those of another synthetic sample (*b*), did not entirely agree with those quoted in the literature.[9] There are four

Optical rotations of some natural and synthetic αβ-dihydroxy-β-methylvaleric acid salts.

	Specific rotations $[\alpha]_D$			
	Present samples		Lit.[9]	
	From strigosine	Synthetic	Natural	Synthetic
Quinine salt of (−)-acid	−137° (MeOH)	(*a*) −133° (MeOH), (*b*) −140° (MeOH)	−144° (MeOH)	−144° (MeOH)
(−)-Acid from quinine salt	−21·3° (HCl; pH 1), −17·6° (EtOH)	(*a*) −22·9° (N-HCl), −16·1° (EtOH) (*b*) −28° (N-HCl), −26° (EtOH)	−16·7° (HCl; pH 1)	−15° (HCl; pH 1)

possible stereoisomers of αβ-dihydroxy-β-methylvaleric acid, and, whilst there is little doubt that the acid from strigosine is one of them, it may be a different isomer from that previously described.

Experimental

Infrared spectra were recorded on a Perkin-Elmer Infracord model 137 spectrophotometer.

Paper Chromatography.—(a) *Bases.* Descending chromatograms were run on Whatman No. 1 paper, buffered with 0·1M-sodium acetate,[10] using butan-1-ol–acetic acid–water as solvent.[11] The dried papers were sprayed with platinum iodide reagent (0·003M-platinic chloride–0·2M-potassium iodide), the bases appearing as blue spots.

(b) *Acids.* Descending chromatograms of ethylamine salts were made on Whatman No. 1 paper, using butan-1-ol–ethylamine–water as solvent.[12] In this system acetic acid had R_F 0·18.

Extraction of Alkaloids from Heliotropium strigosum.—(*a*) Dried, ground plant (1560 g.) was continuously extracted [6] with ethanol at room temperature for 15 hr., the solvent being circulated through Dowex 50 resin (100 g. damp; H^+ form). The resin was washed with ethanol

[7] Adelberg, Bonner, and Tatum, *J. Biol. Chem.*, 1951, **190**, 837.
[8] Adelberg, *J. Bact.*, 1951, **61**, 365.
[9] Sjolander, Folkers, Adelberg, and Tatum, *J. Amer. Chem. Soc.*, 1954, **76**, 1085.
[10] Munier, Macheboeuf, and Cherrier, *Bull. Soc. Chim. biol.*, 1952, **34**, 204.
[11] Crawley and Culvenor, *Austral. J. Chem.*, 1959, **12**, 694.
[12] Manganelli and Brofazi, *Analyt. Chem.*, 1957, **29**, 1441.

and water, and the bases eluted with dilute ammonium hydroxide (250 ml.), reduced with zinc and hydrochloric acid, and made alkaline with ammonia. Extraction with chloroform gave a pale brown basic gum (*A*) (1·616 g.), $[\alpha]_D$ −15·6° (in EtOH). Before reduction, only 15% of this base could be extracted from aqueous solution with chloroform, *i.e.*, 85% existed as *N*-oxide in the plant.

(*b*) Ground plant (2070 g.), extracted as above for 24 hr., when reduced, basified, and extracted with chloroform, gave a gum (*A*) (2·895 g.), R_F 0·52 (strigosine), with smaller spots, R_F 0·33 (trachelanthamidine), 0·73, 0·78, and 0·85 (unidentified alkaloids). The aqueous liquor was extracted with butan-1-ol, the extract was evaporated to dryness, and the residue (2·35 g.) re-extracted with chloroform, to give a dark brown gum (*B*) (1 g.), R_F 0·33, 0·4, 0·53, 0·78, and 0·85. The plant material was re-extracted (Soxhlet) with hot methanol for 18 hr.; the extract was concentrated and treated with dilute hydrochloric acid, and the chlorophyll removed with ether. The liquor was reduced with zinc and hydrochloric acid, concentrated under reduced pressure, made alkaline with ammonia, and extracted with chloroform to give a red-brown gum (*C*) (2·5 g.), R_F 0·33 (trachelanthamidine), with smaller spots, R_F 0·40 and 0·53.

Picrolonate from (*C*).—The gum (*C*) (1·2 g.) was neutralised with picrolonic acid in ethanol, a brown amorphous precipitate was removed, and the liquor concentrated, giving, after 2 months, crystals (0·33 g.), m. p. 147—148°. Four recrystallisations from ethanol–acetone gave pure trachelanthamidine picrolonate as deep yellow prisms, m. p. 182° (lit.,[3] 182°) (Found: C, 53·6; H, 6·2; N, 17·1. Calc. for $C_8H_{15}NO,C_{10}H_8N_4O_5$: C, 53·4; H, 5·7; N, 17·2%).

This picrolonate (156 mg.), in methanol, treated with Dowex 1 resin (OH^- form), yielded trachelanthamidine base (62 mg.), as an almost colourless viscous gum, R_F 0·33, $[\alpha]_D^{16}$ −12·0° (*c* 2·08 in EtOH) (lit.,[3] −12·94°). The base was neutralised with ethanolic hydrochloric acid, to give trachelanthamidine hydrochloride as colourless needles, m. p. 112—113° (lit.,[3] 110—112°) (from ethanol–ether), $[\alpha]_D^{16}$ −10·8° (*c* 1·7 in EtOH) and −13·5° (*c* 1·84 in H_2O) (Found: N, 8·1. Calc. for $C_8H_{16}NOCl$: N, 7·9%).

The base, neutralised with picric acid in ethanol, formed trachelanthamidine picrate, needles (from ethanol), m. p. 172° (lit.,[3] 174°) not depressed an admixture with the picrate of the base obtained by hydrolysis of strigosine. The infrared spectrum of the base was identical with that of the base from pure strigosine.

Strigosine Picrate.—Crude base (*A*), in benzene, was neutralised with picric acid. Addition of light petroleum (b. p. 80—100°) gave a gum which, on tritiation with light petroleum and then with ethanol and ether, gave crystals, m. p. 102—128°. Three recrystallisations from benzene–light petroleum gave pure *strigosine picrate* as yellow leaflets, m. p. 141° (Found, for two different batches: C, 47·9, 48·3; H, 5·4, 5·8; N, 10·4, 10·3. $C_{14}H_{25}NO_4,C_6H_3N_3O_7$ requires C, 48·0; H, 5·6; N, 11·2%). No other crystalline picrate could be obtained from the mother-liquors.

Strigosine Alkaloid.—Strigosine picrate (158 mg.), in chloroform, was passed through a column of Dowex 1 resin (OH^- form; analytical grade). Removal of the solvent gave the base as a colourless gum (85 mg., 100%), $[\alpha]_D^{20}$ −19·3° (*c* 6·56 in EtOH), R_F 0·52. Base recovered from the crude picrate contained a trace of the minor alkaloid, R_F 0·78.

Strigosine Hydrochloride.—The above base (75 mg.) was neutralised with ethanolic hydrochloric acid, and the solution concentrated to dryness under reduced pressure. The residue (92 mg.) crystallised on trituration with ether–light petroleum. Four recrystallisations from ethanol–ether gave the *hydrochloride* as non-hygroscopic needles, m. p. 137·5° (Found: C, 53·9; H, 8·5; Cl, 11·4; N, 4·95. $C_{14}H_{25}NO_4$,HCl requires C, 54·6; H, 8·5; Cl, 11·5; N, 4·55%).

Strigosine Methiodide.—Crude alkaloid extract (*A*), in chloroform, was treated with an excess of methyl iodide at room temperature for 10 min., boiled down to dryness, and the residue recrystallised three times from ethanol–ether, to give the *methiodide* as colourless leaflets, m. p. 135—136°, $[\alpha]_D^{20}$ −15·5° (*c* 2·84 in EtOH) (Found: C, 44·2; H, 6·8; I, 31·7; N, 3·4. $C_{15}H_{28}INO_4$ requires C, 43·6; H, 6·8; I, 30·8; N, 3·4%).

Hydrolysis of Strigosine.—(*a*) The alkaloid (34 mg., recovered from pure picrate), barium hydroxide (80 mg.), and water (1 ml.) were heated for 1 hr. at 100°. No barium carbonate was precipitated. The solution was acidified with hydrochloric acid, extracted with ether (7 times), and the dried ($MgSO_4$) extract concentrated, to give a colourless, acidic gum (14·1 mg.), $[\alpha]_D^{20}$ −19·1° (*c* 1·67 in EtOH), R_F 0·41. This acid was identical (infrared spectrum) with that obtained from crude alkaloid [see (*b*) below], and its brucine salt had m. p. 212° (decomp.) not depressed when mixed with the brucine salt of the latter acid.

The aqueous liquor was made alkaline with sodium hydroxide and extracted with ether. The resulting base, R_F 0·33, was converted into a picrate, m. p. 172° not depressed by trachelanthamidine picrate obtained by hydrolysis of the crude alkaloid (below).

(*b*) Crude alkaloid (*B*), and base recovered (using Dowex 1 resin) from recrystallisation mother-liquors of the picrate from (*A*) (total, 2·6 g.), was hydrolysed as described above with barium hydroxide (6 g.) in water (50 ml.). The solution, acidified and continuously extracted with ether for 6 hr., yielded an acidic gum (0·823 g.).

After being made alkaline with sodium hydroxide, the aqueous liquor was continuously extracted with chloroform for 3 hr., to give a pale brown gummy base (0·772 g.), R_F 0·33. This was neutralised with picric acid in ethanol, to give trachelanthamidine picrate, needles, m. p. 172° (from ethanol) (Found: C, 45·6; H, 5·1; N, 15·1. Calc. for $C_8H_{15}NO,C_6H_3N_3O_7$: C, 45·4; H, 4·9; N, 15·1%).

Brucine Salt of Acid.—The crude acid (0·82 g.), in ethanol, was neutralised with brucine (1·9 g.); after adding ether, a crystalline *brucine salt* (2·15 g.) was obtained, m. p. 200—206° (decomp.). Two recrystallisations from ethanol–ether gave prisms, m. p. 215° (decomp.), $[\alpha]_D^{20}$ −28·8° (*c* 14·5 in H_2O) (Found: C, 64·1; H, 7·15; N, 5·1. $C_6H_{12}O_4,C_{23}H_{26}N_2O_4$ requires C, 64·1; H, 7·0; N, 5·2%).

Recovery of Acid from Brucine Salt.—The salt (0·35 g.) in water (3·5 ml.) was acidified with hydrochloric acid and extracted 10 times with ether. The extract was dried ($MgSO_4$) and the ether removed, leaving the acid (85 mg.) as a colourless, odourless gum, R_F 0·41, $[\alpha]_D^{20}$ −17·6° (*c* 2·02 in EtOH); −2·13° (*c* 2·3 in HCl; pH 1·0). The infrared spectrum (film) showed absorptions at 2·99 (OH), 3·39, 5·80 (CO), 6·86, 7·26, 8·20, 9·20, 9·60, 9·93, 10·80, 11·13, 12·05, and 14·08 μ.

Quinine Salt of Acid.—The acid, recovered from the brucine salt, was neutralised with quinine in ethanol, ether was added, and the salt recrystallised twice from ethanol–ether, giving colourless needles, m. p. 204—205°, $[\alpha]_D^{20}$ −137° (*c* 1·78 in MeOH) (Found: C, 66·4; H, 7·7; N, 6·1. Calc. for $C_6H_{12}O_4,C_{20}H_{24}N_2O_2$: C, 66·1; H, 7·7; N, 5·9%).

Oxidation[13] *of Acid from Strigosine.*—Acid (53·2 mg., 0·36 mmole assuming *M*, 148), recovered from the brucine salt, was treated with N-sodium metaperiodate solution (2 ml.). The solution was flushed with nitrogen which then passed in turn through 2,4-dinitrophenylhydrazine reagent and 0·1N-barium hydroxide. The reaction was rapid at first, with spontaneous warming. After 45 min. at room temperature, and 15 min. at 65—70°, 2·08 equivalents of metaperiodate were absorbed. Extraction of the acidic liquor gave a pungent, volatile acid (about 7 mg., 42%, calc. as HCO_2H). The 2,4-dinitrophenylhydrazine reagent yielded ethyl methyl ketone 2,4-dinitrophenylhydrazone (85 mg., 94%) as needles, m. p. and mixed m. p. 112—113° (from ethanol). From the barium hydroxide, barium carbonate was recovered, equivalent to 0·25 mmole of CO_2 (70%).

αβ-*Dihydroxy*-β-*Methylvaleric Acid.*—This was prepared according to Sjolander *et al.*[9] A brucine salt, after two recrystallisations from ethanol–ether, had $[\alpha]_D^{20}$ −28·4° (*c* 8·4 in H_2O), m. p. 215° (decomp.) not depressed by the brucine salt of the acid from strigosine. Acid recovered from this salt, using Dowex 50 resin (H^+ form), had R_F 0·41, $[\alpha]_D^{20}$ −3·7° (*c* 2·82 in EtOH). The acid could not be further resolved through the brucine salt.

A quinine salt from the recovered acid had m. p. 180—184° raised to 203—204° after five recrystallisations from ethanol–ether, $[\alpha]_D^{20}$ −133° (*c* 1·8 in MeOH) {lit.,[9] m. p. 203°, $[\alpha]_D^{23}$ −144° (in MeOH)}. A mixed m. p. with the quinine salt of the acid from strigosine was not depressed. Acid recovered from this salt had R_F 0·41, $[\alpha]_D^{20}$ −16·1° (*c* 3·11 in EtOH) and −22·9 (*c* 2·27 in N-HCl) (lit.,[9] $[\alpha]_D^{23}$ −15° in HCl; pH 1).

A quinine salt from the crude acid, after five recrystallisations from ethanol–ether, formed needles, m. p. 203—204, $[\alpha]_D^{20}$ −140° (*c* 1·9 in MeOH). Acid recovered from the salt had R_F 0·41, $[\alpha]_D^{20}$ −26·0° (*c* 2·58 in EtOH) and −28·0° (*c* 1·43 in N-HCl).

The infrared spectra of all samples of the synthetic acid were identical with that of the acid from strigosine.

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[13] Christie, Kropman, Novellie, and Warren, *J.*, 1949, 1703.