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Studies on Orchidaceae Alkaloids

X.* A Pyrrolizidine Alkaloid from *Chysis bractescens* Lindl.

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(+)-1-Methoxycarbonylpyrrolizidine (I) has been isolated from *Chysis bractescens* Lindl. Its structure has been proved by degradation into pyrrolizidine, reduction to lindelofidine, and by synthesis. The properties of the acid obtained by hydrolysis of I did not correspond well with those reported by other authors.[2] I shows a remarkable UV band at 290 mμ ($\varepsilon_{max}^{hexane}$ 40) which disappears on protonation. The earlier concept [3] of this molecule has been shown to be incorrect.

It has long been known that several species in the Pfitzer taxon: Pleuranthae, Convolutae, Homoblastae produce alkaloids.[4–7] In *Chysis bractescens* Lindl. we have hitherto found only one tertiary alkaloid (+)-1-methoxycarbonylpyrrolizidine (I). *Catasetum* spp. contain small amounts of related compounds, and most species in *Phaius* and *Calanthe* produce indigoid substances.

The alkaloid (I) $C_9H_{15}NO_2$ is a bicyclic methyl ester (IR: 1735 cm^{-1}), NMR τ 6.27 3H singlet. Saponification of I gave an optically active acid II, $C_8H_{13}NO_2$, which upon decarboxylation afforded pyrrolizidine. The NMR spectrum of I showed, *inter alia*, a 1H quartet at τ 6.2, shifting to τ 5.6 in the acid II. It was first believed [3] that this signal derived from the α-proton in an α-amino carboxyl compound. The natural product, however, on mass spectrometry, did not give the expected base peak at *m/e* 110, given by authentic pyrrolizidine-3-carboxylates. The base peak was instead observed at *m/e* 83 (Fig. 1), implying that the carboxylate group should be attached to the 1-position of the pyrrolizidine ring system.

Reduction of I with lithium aluminium hydride gave an alcohol (III) identified as lindelofidine (optical rotation, melting points of picrate and methodide) thereby establishing the absolute configuration of I (Fig. 1). II has a higher melting point and optical rotation than reported for the acid

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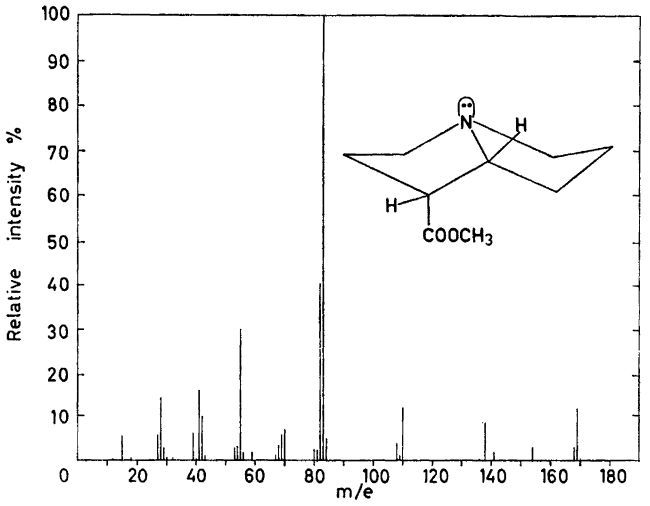

Fig. 1.

obtained by chromic acid oxidation of lindelofidine;[2] melting point of the picrates, however, showed good agreement.

Synthesis of racemic *endo*-1-ethoxycarbonyl-pyrrolizidine,[8] followed by transesterification with methanol afforded a product IV indistinguishable from I (IR, UV, mass spectrum). The NMR spectra of the methiodides of IV and I are also indistinguishable. The picrate of the racemic form (IV) has a melting point appreciably lower than that of the optically active form (I).

The strongly deshielded proton appearing at τ 6.2 in the ester I, the resonance of which is shifted to τ 5.6 in the acid II or in the methiodide of I, should be identical with the one which appears at τ 5.8 in the NMR spectrum of pyrrolizidine methiodide, and must be the 8-H of pyrrolizidine. The extreme position of the 8-H resonance of pyrrolizidine (*cf.* τ values of bridgehead hydrogens in octahydroindolizine [9] and quinolizidines [10–12]) may be explained by the anisotropy caused by the unusual C—H bond angle relative to the direction of the nitrogen lone electron pair.

The synthetic methyl and ethyl *endo*-pyrrolizidine-1-carboxylates as well as I show a remarkable UV absorption at 290 mμ (ε 40, hexane). The band disappears on protonation, and is absent in the UV spectra of the sodium salt of the acid II and lindelofidine. We have not been able to demonstrate any cotton effect associated with the absorption band. So far, we have no explanation of this charge transfer effect.

EXPERIMENTAL

All melting points are corrected. GLC: Column A: 20 % (w/w) SE 52 on 60–80 mesh Chromosorb W HMDS, length 2.9 m width 8 mm. Column B: 20 % (w/w) SE 52 on 60–80 mesh Chromosorb W HMDS, length 2.0 m width 4 mm.

Isolation of alkaloid. Fresh plants (15.0 kg) of *Chysis bractescens* Lindl. (obtained from Orquideas Mexicanas, Mexico City) were homogenised in a mechanical mincing machine (Turmix) with 25 l of methanol. The slurry was left overnight at 4°, filtered, and the extraction repeated with another 20 l of methanol. The combined filtrates were concentrated *in vacuo* (20°C) to 1 l, and 1 M hydrochloric acid (100 ml) was added. The aqueous acidic phase was extracted with $CHCl_3$–CH_3OH, 4:1, (5×500 ml) which was rejected. After adjustment to pH 9 with sodium hydroxide, the aqueous solution was rapidly extracted with $CHCl_3$–CH_3OH, 4:1, (8×250 ml) and the combined organic phases were dried over anhydrous sodium sulphate before evaporation of the solvents. The residue was dissolved in ether and filtered through an Al_2O_3-column (200×38 mm). After evaporation of the filtrate, 9.7 g of the crude oily base was obtained.

The alkaloid was purified by preparative GLC using column A and I (retention time 7 min 50 sec, 190°C, flow rate 70 ml/min) was collected as a colourless oil, n_D^{25} 1.4763; $[\alpha]_D^{25}$ +64° (*c* 1.1, $CHCl_3$). (Found: C 64.0; H 8.53; N 8.11. Calc. for $C_9H_{15}NO_2$: C 63.9; H 8.94; N 8.28); MS: *m/e* 169 (M^+), 83 (base peak), 55; NMR (in $CDCl_3$): 6.27 τ (3H) singlet; 6.2 τ (1H) quartet (J_1=8, J_2=14 cps); 6.6–7.5 τ (5H); 7.7–8.8 τ (6H); UV: λ_{max}^{hexane} 209.5 mμ (ε 1300), 290 mμ (ε 40), both bands disappear on the addition of hydrochloric acid. *Methiodide* from acetone, m.p. 141–142°C. (Found: C 38.6; H 5.77; N 4.34; O 10.22. Calc. for $C_{10}H_{18}NO_2I$: C 38.6; H 5.79; N 4.50; O 10.29). *Picrate* from ethanol, m.p. 197–198°C. (Found: C 45.2; H 4.65; N 14.05; O 35.9. Calc. for $C_{15}H_{18}N_4O_9$: C 45.2; H 4.52; N 14.07; O 36.2).

Hydrolysis of I. I (0.4 g) in 10 ml of 4 % aq. sodium hydroxide was heated at 100° for 1 h. After neutralizing with hydrochloric acid, the solution was evaporated to dryness and the residue was extracted with glacial acetic acid (4×5 ml) at room temperature for 1 h. The acetic acid solution was concentrated and the crystalline residue was sublimed at 150°C and 0.1 mm Hg, giving the amino acid II as colourless crystals, m.p. 244–245°C; $[\alpha]_D^{25}$ +82° (*c* 0.7, H_2O); Labenskii *et al.*[2] reported m.p. 228–229°C and $[\alpha]_D^{25}$ +71° for this compound. MS: *m/e* 155 (M^+), 83 (base peak); NMR (in D_2O): 5.6 τ (1H) quartet (J_1=7, J_2=16 cps). (Found: C 61.7; H 8.38; N 8.90; O 20.6. Calc. for $C_8H_{13}NO_2$: C 61.9; H 8.44; N 9.03; O 20.6). *Picrate*, yellow prisms from ethanol, m.p. 218–219°C (*cf.* Ref. 2).

Reduction of I. A solution of I (1.0 g, 0.0059 mole) in 25 ml of ether was slowly added to lithium aluminium hydride (0.17 g, 0.0044 mole) in 25 ml of ether. The reaction mixture was refluxed for 2 h and hydrolyzed successively with water (0.2 ml), 20 % sodium hydroxide (0.15 ml) and water (0.7 ml). The white granular precipitate of hydroxides was filtered off and washed with dry ether. The ether was evaporated leaving 0.8 g (0.0056 mole, 95 %) of crude amino alcohol. This was purified by preparative GLC (column: B, temp. 157°C, flow rate 30 ml/min), retention time 13 min, and collected as a viscous oil, $[\alpha]_D^{25}$ +76°; molecular weight 141 (mass spectrometry). (Found: C 61.0; H 10.71; N 9.92. Calc. for $C_8H_{15}NO$: C 61.0; H 10.71; N 9.92). *Picrate*, m.p. 193–194°C. *Methiodide*, m.p. 284–286°C. These values are in good agreement with those given for lindelofidine and its corresponding derivatives.[13]

Decarboxylation of II. Soda lime (25 mg) and II (25 mg) were ground together and the mixture was heated to 200°C for 20 h in a sealed glass tube. A colourless oil formed in a good yield, and was separated by preparative GLC into two products, retention times 3 min 15 sec, MS *m/e* 99, and 8 min 30 sec, MS *m/e* 111 (column A, temp. 140°C, flow rate 70 ml/min). The product with the longer retention time was identical with pyrrolizidine (MS, retention time) obtained by cyclization of 1,3,7-tribromoheptane with ammonia in methanol.[14] The picrates were also identical.

1-endo-Ethoxycarbonylpyrrolizidine. Synthesis of 1-*endo*-ethoxycarbonylpyrrolizidine according to Kochetkov *et al.*[8] gave the ester as a colourless oil, n_D^{20} 1.4713; MS: *m/e* 183 (M^+), 83 (base peak). (Found: C 65.5; H 9.39; N 7.78. Calc. for $C_{10}H_{17}NO_2$: C 65.5; H 9.35; N 7.65). *Picrate*, m.p. 121–122°C. These physical constants agree well with those reported earlier.[8] The same product is obtained when the catalytic hydrogenation is performed at atmospheric pressure instead of at 7 atm as used by Kochetkov *et al.*[8]

1-endo-*Methoxycarbonylpyrrolizidine IV*. 1-*endo*-Ethoxycarbonylpyrrolizidine (0.45 g, 0.0025 mole) was dissolved in 150 ml methanol containing 1 ml of concentrated sulphuric acid. The solution was refluxed for three days, and evaporated at reduced pressure to 25 ml, diluted with water (50 ml) and adjusted to pH 10 with aqueous sodium hydroxide. The ester was extracted with chloroform (6 × 10 ml) and the chloroform solution evaporated, after drying over anhydrous sodium sulphate, leaving 300 mg (0.0018 mole, 74 %) 1-*endo*-methoxycarbonylpyrrolizidine. This product contained about 1 % of the ethyl ester, which was removed by preparative GLC (column B, temp. 169°C, flow rate 30 ml/min) and IV, retention time 10.5 min, was collected as a colourless oil, n_D^{25} 1.4750. (Found: C 63.8; H 9.04; N 8.40. Calc. for $C_9H_{15}NO_2$: C 63.9; H 8.94; N 8.28); MS: *m/e* 169 (M^+), 83 (base peak). *Picrate*, m.p. 162–163°C. (Found: C 45.0; H 4.72; N 13.90. Calc. for $C_{15}H_{18}N_4O_9$: C 45.2; H 4.52; N 14.07).

The mass spectrum, IR spectrum, and UV spectrum of IV were identical with those of I. The NMR spectra of the methiodides were also identical.

2,3-Dihydro-5-ethoxycarbonyl-1-H-pyrrolizine (*V*). Trimethylenepyrrole [15] (0.9 g, 0.0084 mole) in 2 ml of ether was added to ethylmagnesium bromide prepared from 0.28 g (0.0115 mole) Mg and 1.2 g (0.0110 mole) ethyl bromide in 5 ml of ether. After the evolution of ethane had ceased, the reaction mixture was refluxed for 45 min, and then 1.16 g (0.0107 mole) ethyl chloroformate in 2 ml of ether was added and the reaction mixture was refluxed for another 1.5 h. The solution was kept overnight and then it was hydrolyzed with a mixture of 4.0 ml of saturated ammonium chloride solution and 2 ml of water. The ether phase was separated, and the aqueous layer was extracted with ether (2 × 20 ml). The combined ether solutions were washed six times with 4 M hydrochloric acid to remove unreacted trimethylenepyrrole. The ether solution was evaporated and the residue separated by preparative GLC (column A, temp. 174°C, flow rate 200 ml/min) and V, retention time 12 min, was collected in a yield of 0.195 g (0.0011 mole, 13 %), n_D^{25} 1.5280. (Found: C 66.9; H 7.36; O 17.9; N 7.63. Calc. for $C_{10}H_{13}NO_2$: C 67.0; H 7.26; O 17.9; N 7.82); IR: ν_{max}^{film} 1693 cm^{-1}, 750 cm^{-1} (pyrrole CH bending); UV: λ_{max}^{hexane} 265 mμ (ε 16 000), 269 mμ (ε 15 900), 275 mμ (ε 15 300); MS: *m/e* 179 (M^+), 150, 134, 106, 79; NMR: 3.20 τ (1H) doublet (J=4 cps); 4.32 τ (1H) doublet (J=4 cps); 5.85 τ (2H) quartet (J=7 cps); 5.97 τ (2H) triplet (J=7 cps); 7.15–8.00 (4H) multiplet; 8.78 (3H) triplet (J=7 cps).

3-endo-*Ethoxycarbonylpyrrolizidine* (*VI*). 2,3-Dihydro-5-ethoxycarbonyl-1-H-pyrrolizine (195 mg, 0.0011 mole) was hydrogenated at atmospheric pressure in glacial acetic acid (50 ml) for 120 h. The catalyst was filtered off and the solvent removed *in vacuo* leaving a residue which was taken up in water. After adjusting the pH to 9 the base was taken up in chloroform and purified by preparative GLC (column B, temp. 174°C, flow rate 26 ml/min) and VI, retention time 11.3 min, was collected as a colourless oil. Yield 75 mg (0.0004 mole, 36 %), n_D^{25} 1.4670. (Found: C 65.4; H 9.28; N 7.86. Calc. for $C_{10}H_{17}NO_2$: C 65.5; H 9.35; N 7.65); IR: $\nu_{max}^{CCl_4}$ 1735 cm^{-1}; MS: *m/e* 183 (M^+), 110 (base peak).

3-exo-*Methoxycarbonylpyrrolizidine*. Synthesis according to Seiwerth *et al.*[16] gave a product with the same retention time as I. Molecular weight 169 (mass spectrometry). IR: $\nu_{max}^{CCl_4}$ 1735 cm^{-1}. *Hydrochloride*, m.p. 114–117°C. The aminoester had the base peak *m/e* 110 in the mass spectrum, and the fingerprint region of the IR spectrum was distinguishable from that of I.

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