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Studies on Orchidaceae Alkaloids

XII.* Pyrrolizidine Alkaloids from *Phalaenopsis amabilis* Bl. and *Ph. mannii* Rchb.f.

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Two diastereomeric pyrrolizidine alkaloids (I and II) have been isolated from *Phalaenopsis amabilis* Bl. and from *Ph. mannii* Rchb.f. respectively. Both alkaloids upon acid methanolysis give (−)-dimethyl-2-benzylmalate. The methanolysis of I also gives trachelanthamidine and that of II gives the enantiomer, laburnine.

Many species belonging to the *Sarcanthinae* subfamily in *Orchidaceae* show an appreciable alkaloid content, and in the genus *Phalaenopsis* all twenty-two species hitherto tested [2,3] have been rich in alkaloids. The present paper reports the isolation of one alkaloid from each of the species *Ph. amabilis* ** (phalaenopsin T, I) and *Ph. mannii* (phalaenopsin La, II). I and II are diastereomeric esters ($C_{20}H_{27}NO_5$, Mw 361), both of which upon acid methanolysis give the same (−)-dimethyl ester ($C_{13}H_{16}O_5$, Mw 252, III). From spectral evidence III was assigned the structure dimethyl-2-benzylmalate, and this compound was synthesized by epoxidation of diethyl benzylidenesuccinate, followed by hydrogenolysis and transesterification. The synthetic racemic dimethyl ester was indistinguishable from III by IR, NMR, and MS.

From the methanolysis mixture of I an amino alcohol ($C_8H_{15}NO$, Mw 141) was isolated. Its mass spectrum,[5] melting point of the methiodide,[6] and optical rotation [6] show that the amino alcohol is trachelanthamidine. Methanolysis of II yielded laburnine, the enantiomer of trachelanthamidine.

The peaks M−73 (73 = $\cdot CH_2COOCH_3$) and M−168 (168 = $\cdot COOC_8H_{14}N$) in the mass spectrum of I and II indicate that I and II have the structures given in Fig. 1.

* For number XI in this series, see Ref. 1.

** For a preliminary report, see Ref. 4.

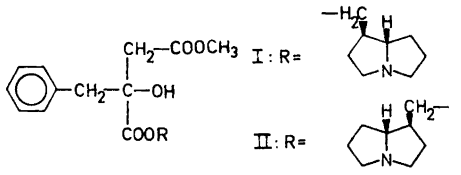

Fig. 1.

The molecular ion in the mass spectra of I and II is pertinent in spite of the presence of the tertiary hydroxyl group, whereas in that of III the molecular ion is absent.

EXPERIMENTAL

All evaporations were done under reduced pressure. Melting points are uncorrected. Preparative GLC was carried out on a column 2.9 m × 8 mm, of 20 % SE52 on Chromosorb AW DMCS, 60–80 mesh, with helium as the carrier gas. The NMR spectra were measured on a Varian A-60 A spectrometer, the mass spectra on an LKB 9000 spectrometer at 70 eV, the IR spectra on a Perkin Elmer 221 instrument and the UV spectra on a Beckman DK2 instrument using ethanol as solvent.

Isolation and characterization of the alkaloid from Phalaenopsis amabilis *Bl.* Fresh plants (0.9 kg) were minced twice with methanol, in all 5.5 l. The methanolic extract was concentrated to 50 ml, hydrochloric acid added (0.2 %, 200 ml) and the solution was washed with ether (4 × 200 ml). The aqueous solution, kept at pH 8–9, was extracted with chloroform (5 × 100 ml). The chloroform phase was dried (Na_2SO_4) and concentrated leaving the crude base as a viscous oil (0.6 g). The alkaloid was crystallized from absolute ethanol at −20°. Two further similar crystallizations gave phalaenopsin T (I, 0.4 g), m.p. 104.5–105°, $[\alpha]_D^{20}$ −15° (*c* 1.6, ethanol). (Found: C 65.9; H 7.23; N 3.92; O 22.9. Calc. for $C_{20}H_{27}NO_5$: C 66.5; H 7.53; N 3.88; O 22.1). UV: λ_{max} 210, 247, 252, 258, and 264 mμ; ε 8500, 110, 150, 190, and 140. MS: *m/e* (rel. intensity), 361 (1), 330 (1), 288 (6), 270 (1), 193 (1), 140 (2), 124 (100), 91 (24), 83 (23), 82 (13), 55 (15), m* 230. NMR ($CDCl_3$): τ 2.76 (s, 5H), τ 5.5 (s, 1H) (concentration dependent), τ 5.9 (m, 2H), τ 6.26 (s, 3H), τ 7.01 (s, 2H), τ 7.08 and τ 7.33 (AB spectrum, 2H, J=16 cps). IR: ν_{max} (KBr) 3300–2400 (weak) and 1738 cm^{-1} (shoulder at 1745 cm^{-1}). *Hydrochloride* from ethanol-ether at −20°, m.p. 155–156°. (Found: Cl 8.99. Calc. for $C_{20}H_{28}ClNO_5$: Cl 8.92).

Isolation and characterization of alkaloid from Phalaenopsis mannii *Rchb.f.* The alkaloid phalaenopsin La (II) was isolated and the base crystallized in the same way as I, m.p. 125–135°, $[\alpha]_D^{20}$ +10° (*c* 1.1, ethanol). *Hydrochloride*, m.p. 131–133° (Found: C 60.1; H 7.20; Cl 8.83; N 3.50; O 20.0. Calc. for $C_{20}H_{28}ClNO_5$: C 60.4; H 7.09; Cl 8.91 N 3.52; O 20.1).

The bases I and II have identical spectral properties (MS, UV, IR, NMR) except for the NMR-signal at τ 5.9. In the spectrum of II there is a doublet with J=6 cps.

Methanolysis of phalaenopsin. II (250 mg) was dissolved in a mixture of methanol (50 ml) and conc. sulphuric acid (2.5 ml). The solution was kept at 86° for six days, and the pH was then adjusted to 5 with barium hydroxide in water (0.8 l). The suspension was centrifuged, the supernatant solution was concentrated to 50 ml and extracted with carbon tetrachloride (4 × 50 ml). The combined carbon tetrachloride layers were treated as described below. The aqueous layer was made alkaline (pH 9–10) and extracted with chloroform in a mechanical shaker (2 × 200 ml, 15 h). Preparative GLC of the dried (Na_2SO_4) and concentrated chloroform solution at 165°, gas flow rate 210 ml/min yielded the amino alcohol, retention time 6 min, $[\alpha]_D^{20}$ +15° (*c* 1.7, ethanol), methiodide, m.p. 304–306° MS: *m/e* 141, 124, 83, 82, 55. These values agree well with those reported for laburnine $C_8H_{15}NO$, Mw 141 (MS,[5] $[\alpha]_D$ +15.45°,[6] and its *methiodide*, m.p. 307–309°[6]).

Neutral component (III). The carbon tetrachloride layer was dried (Na_2SO_4) and concentrated to 0.5 ml. Preparative GLC at 185°, gas flow rate 230 ml/min, gave III, retention time 20 min, $[\alpha]_D^{20} -9.7°$ (*c* 1.6, ethanol). (Found: C 61.9; H 6.31; O 31.8. Calc. for $C_{13}H_{16}O_5$: C 61.9; H 6.39; O 31.7). MS: $M^+ = 252$ lacking, 234 (28), 202 (9), 193 (18), 174 (3), 161 (32), 129 (14), 119 (21), 101 (45), 92 (43), 91 (100), 59 (20). NMR (CCl_4): τ 2.78 (s, 5H), τ 6.31 (s, 3H), τ 6.35 (s, 3H), τ 6.55 (s, 1H), τ 7.07 (s, 2H), τ 7.43 and τ 7.13 (AB spectrum, 2H, $J = 16$ cps). IR: ν_{max} (film) 3500 (broad) and 1741 cm^{-1}.

A similar methanolysis of I yielded III and a basic compound with a mass spectrum identical with that of laburnine, $[\alpha]_D^{20} -13°$ (*c* 0.13, ethanol) and *methiodide*, m.p. 303–306°.

Triphenyl-(1,2-bis-ethoxycarbonyl-ethylidene) phosphorane (IV) was prepared analogously to the corresponding dimethyl ester,[7] m.p. 104–107° (Found: C 71.4; H 5.90; P 7.52. Calc. for $C_{26}H_{27}O_4P$: C 71.9; H 6.27; P 7.13).

Diethyl benzylidene succinate (V). A solution of IV (71.5 g, 0.165 mole) and benzaldehyde (19.0 g, 0.180 mole) in benzene (350 ml) was refluxed (19 h) and was then allowed to stand at room temperature (20 h). The solution was concentrated to 50 ml, filtered (washing with ether), kept at −20° (15 h) and filtered again. The benzene was evaporated and the residue distilled, yielding V (29.9 g, 69 %), b.p. 140°/0.15 mm. (Found: C 68.8; H 6.74; O 24.4. Calc. for $C_{15}H_{18}O_4$: C 68.7; H 6.92; O 24.4). NMR ($CDCl_3$): τ 2.10 (s, 1H), τ 2.63 (s, 5H), τ 4.71 (m, 2H), τ 4.81 (m, 2H), τ 5.47 (s, 2H), τ 8.67 (t, 3H), τ 8.75 (t, 3H). UV: λ_{max} 210 mμ (ε 8000) and 265 mμ (ε 18000). MS: $M^+ = 262$ (65), 117 (88), 29 (100). IR: ν_{max}(CCl_4) 1740, 1713, 1643 cm^{-1}.

Ethyl 3-ethoxycarbonyl-3,4-epoxy-4-phenylbutyrate (VI). Peroxytrifluoroacetic acid in methylene chloride was prepared according to the method of Emmons and Pagano [8] using 90 % hydrogen peroxide (0.945 g, 0.025 mole), trifluoroacetic anhydride (6.30 g, 0.030 mole) and methylene chloride (5 ml). This solution was added over a 15 min period to a well stirred refluxing mixture of disodium hydrogen phosphate (11.3 g, 0.080 mole), V (2.62 g, 0.010 mole) and methylene chloride (20 ml). After 2.5 h NMR showed a 98–99 % epoxidation. The reaction mixture was then treated with water (15 ml) and chloroform (15 ml). The organic layer was washed with 10 % aqueous sodium hydrogen carbonate (3 × 15 ml) and water (25 ml), dried (Na_2SO_4), concentrated and distilled. Yield: 1.87 g, 67 %, b.p. 125°/0.2 mm. (Found: C 64.7; H 6.49; O 28.5. Calc. for $C_{15}H_{18}O_5$: C 64.7; H 6.52; O 28.7). NMR (CCl_4): τ 2.62 (s, 5H), τ 4.62 (s, 1H), τ 5.77 (m, 2H), τ 5.97 (m, 2H), τ 7.45 (s, 2H, in $CDCl_3$ AB spectrum, τ_1 7.24, τ_2 7.47, $J = 17$ cps), τ 8.72 (t, 3H), τ 8.86 (t, 3H). MS: $M^+ = 278$ (4), 135 (100), 107 (51), 43 (38), 29 (32). UV: λ_{max} 219, 254, 260, 265 mμ, ε 7900, 500, 550, 480.

Diethyl 2-benzylmalate (VII). V (385 mg, 1.38 mmole) in ethanol (10 ml) was hydrogenolyzed with reduced PdO/$BaSO_4$ (70 mg) for 30 min. Hydrogen uptake: 35 ml (1.56 mmole). NMR showed a 99–100 % conversion to VII. The product was purified by preparative GLC on a 2 m 20 % SE52 column at 184°, ret. time 28 min. (Found: C 64.8; H 6.68; O 28.7. Calc. for $C_{15}H_{20}O_5$: C 64.3; H 7.19; O 28.5). NMR (CCl_4): τ 2.82 (s, 5H), τ 5.89 (m, 2H), τ 5.94 (m, 2H), τ 5.40 (s, 1H), τ 7.08 (s, 2H), τ 7.16 and τ 7.42 (2H, AB spectrum, $J = 16$ cps), τ 8.82 (t, 6H). MS: $M^+ = 280$ lacking, 262 (51), 207 (56), 189 (43), 115 (58), 91 (100). UV: λ_{max} 210, 242, 248, 252, 258, 264 mμ, ε 7000, 100, 130, 170, 230, 170. IR: ν_{max} (film) 3510 cm^{-1} (broad).

Dimethyl 2-benzylmalate (VIII). A solution of VII (226 mg) in methanol (50 ml) and conc. sulphuric acid (2.7 ml) was refluxed for 7 days. The solution was concentrated to 15 ml, water was added (40 ml) followed by extraction with carbon tetrachloride (3 × 25 ml). The NMR and mass spectrum of the dried (Na_2SO_4) and concentrated carbon tetrachloride layer were identical with those of the product from the methanolysis of phalaenopsin.

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