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 methanolation give (−)-dimethyl-2-benzylmalate. The methanolation 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 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_{29}H_{27}NO_{5}, Mw 361), both of which upon acid methanolation give the same (−)-dimethyl ester (C_{13}H_{16}O_{5}, Mw 252, III). From spectral evidence III was 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 methanolation mixture of I an amino alcohol (C_{6}H_{15}NO, Mw 141) was isolated. Its mass spectrum, melting point of the methiodide, and optical rotation show that the amino alcohol is trachelanthamidine. Methanolysis of II yielded laburnine, the enantiomer of trachelanthamidine.

The peaks M−73 (73=−CH_{2}COOCH_{3}) and M−168 (168=−COOC_{8}H_{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.
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 evaporation 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 5.1 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 (Na₂SO₄) and concentrated leaving the crude base as a viscous oil (0.6 g). The alkaloid was crystallized from absolute ethanol at −20°C. Two further similar crystallizations gave phalaenopsin T (I, 0.4 g), m.p. 104.5 - 105°C, [α]D^20 + 45° (c 1.6, ethanol). (Found: C 65.9; H 7.3; N 3.92; O 22.9. Calc. for C₃₉H₅₅NO₄: C 66.5; H 7.53; N 3.88; O 22.1). UV: λ max 210, 247, 252, 258, and 264 μm; 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 (CDCl₃): τ 2.76 (s, 3H), 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 cph). IR: ν max (KBr) 3300 - 7000 (weak) and 1780 cm⁻¹ (shoulder at 1745 cm⁻¹). Hydrochloride from ethanol-ether at −20°C, m.p. 155 - 156°C. (Found: Cl 8.99. Calc. for C₃₉H₅₅ClNO₄: Cl 8.92).

**Isolation and characterization of alkaloid from Phalaenopsis amabilis Bl.** The alkaloid phalaenopsin La (II) was isolated and the base crystallized in the same way as I, m.p. 125 - 135°C, [α]D^20 + 10° (c 1.1, ethanol). Hydrochloride, m.p. 131 - 133°C (Found: C 60.1; H 7.20; Cl 8.83; N 3.50; O 20.0. Calc. for C₃₉H₅₅ClNO₄: 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 cph.

**Methanalysis 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 80°C 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 above. 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 (Na₂SO₄) and concentrated chloroform solution at 165°C, gas flow rate 210 ml/min yielded the amino alcohol, retention time 6 min, [α]D^20 + 15° (c 1.7, ethanol), methiodide, m.p. 304 - 306°C MS: m/e 141, 124, 83, 82, 55. These values agree well with those reported for laburnine C₃₉H₅₅NO, Mw 141 (MS, [α]D^20 + 15.45°, and its methiodide, m.p. 307 - 309°C).

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Neutral component (III). The carbon tetrachloride layer was dried (Na₂SO₄) and concentrated to 0.5 ml. Preparative GLC at 185°C, gas flow rate 230 ml/min, gave III, retention time 20 min, [α]D⁰ = 9.7° (c 1.6, ethanol). (Found: C 61.9; H 6.39; O 31.7. Calc. for C₁₃H₂₃O₄: 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 (CDCl₃): 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: \( \nu_{max} \) (film) 3500 (broad) and 1741 cm⁻¹.

A similar methanolation of I yielded III and a basic compound with a mass spectrum identical with that of laburnine, [α]D⁰ = 13° (c 0.13, ethanol) and methiodide, m.p. 303–306°.

Triphenyl-(1,2-bis-ethoxy carbonyl-ethylidene) phosphorane (IV) was prepared analogously to the corresponding dimethyl ester, m.p. 104–107° (Found: C 71.4; H 5.90; P 7.52. Calc. for C₆H₅₃P: 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 then was allowed to stand at room temperature (20 h). The solution was concentrated to 50 ml, filtered (washing with ether), kept at -20°C (15 h) and filtered again. The benzene was evaporated and the residue distilled, yielding V (29.9 g, 69%), b.p. 140°C/0.15 mm. (Found: C 68.8; H 6.47; O 24.4. Calc. for C₁₉H₂₄O₄: C 68.7; H 6.32; O 24.4). NMR (CDCl₃): 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 5.87 (t, 3H), τ 8.75 (t, 3H). UV: \( \lambda_{max} \) 210 μm (ε 8000) and 265 μm (ε 18000). MS: M⁺ = 262 (65), 117 (88), 29 (100). IR: \( \nu_{max} \) (CCl₄) 1740, 1713, 1643 cm⁻¹.

Ethyl 3-ethoxy carbonyl-3,4-epoxy-4-phenylbutyrate (VI). Peroxynitrofluorocetic acid in methylene chloride was prepared according to the method of Emmons and Pagano using 90% hydrogen peroxide (0.945 g, 0.025 mole), trifluoroacetic anhydride (0.30 g, 0.30 mole) and methylene chloride (5 ml). This solution was added over 15 min period to a well stirred refluxing mixture of disodium hydrogen phosphate (1.3 g, 0.080 mole), V (2.62 g, 0.010 mole) and methylene chloride (20 ml). After 2.5 h NMR showed a 58–90% epoxidation. The reaction mixture was then treated with water (15 ml) and chloroform (15 ml). The organic layer was washed with 10% aqueous sodium hydroxide carbonate (3 × 15 ml) and water layer (25 ml), dried (Na₂SO₄), concentrated and distilled. Yield: 1.87 g, 67%, b.p. 125°C/0.2 mm. (Found: C 64.7; H 6.49; O 28.5. Calc. for C₁₃H₂₃O₄: C 64.7; H 6.52; O 28.7). NMR (CDCl₃): 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 CDCl₃ AB spectrum, τ 7.24, τ 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: \( \lambda_{max} \) 219, 254, 260, 265 μm, ε 7900, 500, 550, 480.

Diethyl 2-benzylmalate (VII). V (385 mg, 1.38 mmole) in ethanol (10 ml) was hydrolyzed with reduced PdO/Be₄(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% SE-52 column at 184°C, ret. time 25 min. (Found: C 64.8; H 6.68; O 28.7. Calc. for C₁₉H₂₅O₄: C 64.3; H 7.19; O 28.5). NMR (CDCl₃): τ 2.82 (s, 5H), τ 5.89 (m, 2H), τ 5.94 (m, 2H), τ 5.40 (s, 1H), τ 7.08 (s, 1H), τ 7.16 (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: \( \lambda_{max} \) 210, 242, 248, 252, 258, 264 μm, ε 7000, 100, 130, 170, 230, 170. IR: \( \nu_{max} \) (film) 3510 cm⁻¹ (broad).

Dimethyl 2-benzylmaleate (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 (Na₂SO₄) and concentrated carbon tetrachloride layer were identical with those of the product from the methanalysis of phalaenopsis.

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