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SYNTHESIS OF TYLOPHORINE

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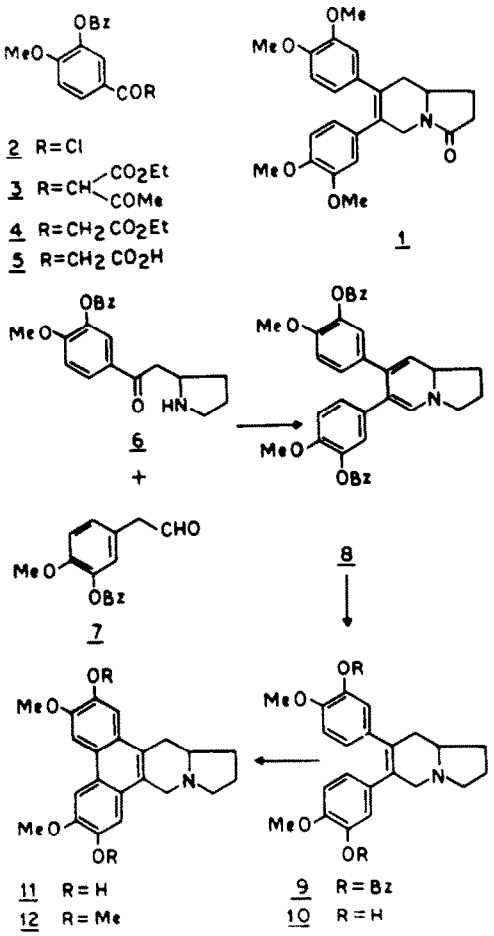
Abstract—A biogenetic type of synthesis of tylorphorine **12** (3.8% yield, established by a radio-dilution method) was achieved by ferricyanide oxidation of 6,7-di(3-hydroxy-4-methoxyphenyl)-6,7-dehydroindolizidine **10**.

Tylophorine **12**, a phenanthroindolizidine alkaloid isolated from *Tylophora asthmatica*,[1] has been synthesized by different routes.[2–4] Recently the base has also been prepared[5] by non-phenolic oxidative coupling of 6,7-di(3,4-dimethoxyphenyl)-3-oxo-1,2,3,5,8,8a-hexahydroindolizine **1** followed by reduction. We now report a biogenetic type synthesis of tylophorine **12** from 6,7-di(3-hydroxy-4-methoxyphenyl)-6,7-dehydroindolizidine **10**.

Condensation of 3-benzyloxy-4-methoxybenzoyl chloride[6] **2** with ethyl sodioacetoacetate afforded ethyl 2-(3-benzyloxy-4-methoxybenzoyl) acetoacetate **3**. Treatment of the acetoacetate **3** with NaOAc–EtOH gave ethyl (3-benzyloxy-4-methoxybenzoyl) acetate **4**. Alkaline hydrolysis of the acetate **4** furnished 3-benzyloxy-4-methoxybenzoylacetic acid **5**. Condensation of the acid **5** with Δ^1-pyrroline[7] at pH ~7.0 afforded 2-(3-benzyloxy-4-methoxyphenacyl) pyrrolidine **6**.

3-Benzyloxy-4-methoxyphenylacetaldehyde[8] **7** was condensed with the pyrrolidine **6** according to the method of Herbert *et al.*[9] to give **8** which was reduced *in situ* with $NaBH_4$ to give 6,7-di(3-benzyloxy-4-methoxyphenyl)-6,7-dehydroindolizidine **9**. Acid catalysed hydrogenolysis of the indolizidine **9** afforded 6,7-di(3-hydroxy-4-methoxyphenyl)-6,7-dehydroindolizidine **10**. The indolizidine **10** was subjected to base catalysed exchange reaction to give [2′,6′,2″,6″-$^3H_4$] 6,7-di(3-hydroxy-4-methoxyphenyl)-6,7-dehydroindolizidine. Tritium labelled indolizidine **10** was oxidised with potassium ferricyanide in a two phase system.[10] The mixture of phenolic compounds, thus formed, was treated with diazomethane to give O-methyl derivatives from which radioactive tylophorine **12** was isolated by isotopic dilution technique. The radiochemical yield of tylophorine was found to be 3.8%.



EXPERIMENTAL

All m.ps are uncorrected. IR spectra (ν_{max} in cm^{-1}) were recorded on a Perkin-Elmer model 157 spectrophotometer and the NMR spectra on a Varian A-60D spectrometer using TMS as internal standard; chemical shifts are expressed in δ values. For labelling of phenolic compound **10** and counting method see earlier paper.[10]

Ethyl 2-(3-*benzyloxy*-4-*methoxybenzoyl*) *acetoacetate* **3**. To a stirred suspension of ethyl sodioacetoacetate [prepared from Na (4 g) and ethyl acetoacetate (25.4 g)] in dry ether (200 ml) was added dropwise a solution of **2** (18 g) in a mixture of dry C_6H_6–ether (1:1, 180 ml) (N_2 atmosphere). The resulting mixture was refluxed for 5 hr and left overnight. The sodium salt of **3**, thus obtained, was filtered, washed with ether, dissolved in cold H_2O, acidified with 10% HCl. The product was extracted with ether, washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent removed to give **3** (21 g, 90%), mp 104 5° (from abs EtOH), IR (KBr): 1715, 1705 (C=O), 1660 (aryl C=O), 1590; (Found: C, 68.50; H, 6.24. Calc. for $C_{21}H_{22}O_6$: C, 68.10; H, 5.94%).

Ethyl 2-(3-*benzyloxy*-4-*methoxybenzoyl*) *acetate* **4**. A mixture of **3** (20 g), AcONa (0.15 g) and EtOH (95%, 100 ml) was refluxed for 7 h. The solvent from the resulting mixture was removed, H_2O added to the residue, which was then extracted with ether. The ether extract was washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent removed to give **4** (16.1 g, 91%), mp 79–80° (from EtOH) (lit[11] 61 62°), IR

(KBr): 2900, 1720 (C=O), 1665 (aryl C=O), 1580; NMR ($CDCl_3$): 7.66–7.21 (m, 7 H, Ar-H), 6.86 (d, J = 9 Hz, 1 H, Ar-H), 5.15 (s, 2 H, $-OCH_2$), 4.18 (q, 2 H, CH_2 CH_3), 3.91 (s, 3 H, $-OCH_3$), 3.88 (s, 2 H, $COCH_2$), 1.25 (t, 3 H, CH_2 CH_3) (Found: C, 69.20; H, 6.20. Calc. for $C_{19}H_{20}O_5$: C, 69.51; H, 6.10%).

2-(3-*Benzyloxy*-4-*methoxyphenacyl*) *pyrrolidine* **6**. An aqueous solution (pH ~7.0) of Δ^1-pyrroline[7] (1.4 g) was added dropwise to an ice-cooled stirred solution (pH ~7.0) of **5** (6 g) (obtained from **4** by alkaline hydrolysis) in a mixture of MeOH (120 ml) and phosphate buffer (pH ~7.0, 200 ml) (N_2 atmosphere). The resulting mixture was left for 48 hr at room temp. It was then acidified with 10% HCl and extracted with ether. The aqueous acidic solution was basified with 28% NH_3 solution and the liberated base was extracted with ether, washed with H_2O, dried (anhyd Na_2SO_4) and the solvent removed. The residue, so obtained, was chromatographed over Al_2O_3. Elution with C_6H_6–$CHCl_3$ (1:1) afforded **6** (3.2 g, 50%), m.p. 94 95° (from C_6H_6–hexane), IR (Neat): 2900, 1660 (aryl C=O), 1585; NMR (CCl_4): 7.82–7.22 (m, 7 H, Ar-H), 6.82 (d, J = 9 Hz, 1 H, Ar-H), 5.1 (s, 2 H, $-OCH_2$), 4.3 (bs, 1 H, –NH), 3.86 (s, 3 H, OCH_3), 3.20 (m, 1 H, –CH–), 2.7–2.23 (m, 4 H, $COCH_2$, N CH_2), 2.1–1.6 (m, 4 H, remaining protons) base fumarate, m.p. 165 66° (d) (from MeOH-ether) (Found: C, 65.45, H, 6.70; N, 3.23. Calc. for $C_{20}H_{23}NO_3$: 1/2 $(C_4H_4O_2)$.H_2O: C, 65.68; H, 6.73; N, 3.49%).

6,7-*Di*(3-*benzyloxy*-4-*methoxyphenyl*)-6,7-*dehydroindolizidine* **9**. To a solution of 3-benzyloxy-4-methoxyphenylacetaldehyde[8] **7** (0.9 g) in dry MeOH (5 ml) was added a solution of **6** (1 g) in dry MeOH (10 ml) and left for 12 hr at room temp The precipitated material was dissolved by adding C_6H_6 and the clear solution was treated with $NaBH_4$ (0.5 g). After 2 hr, the solvent was removed, H_2O added, acidified with 5% HCl and extracted with ether. The aqueous acidic solution was basified with 28% NH_3 solution and the liberated base was extracted with $CHCl_3$, washed with H_2O, dried (anhyd Na_2SO_4) and the solvent removed. The residue, thus obtained, was chromatographed over SiO_2. Elution with $CHCl_3$ MeOH (98:2) gave **9** (0.36 g, 21.3%), m.p. 136 37° (from MeOH); IR (KBr): 2900, 1590; NMR ($CDCl_3$): 7.2 (s, 10 H, Ar-H), 6.67–6.35 (m, 6 H, Ar-H), 4.77 (s, 2 H, $-OCH_2$), 4.73 (s, 2 H, $-OCH_2$), 3.71 (s, 6 H, 2 × $-OCH_3$), 3.65–1.42 (m, 11 H, rest protons) (Found: C, 78.70; H, 6.97; N, 2.30. Calc. for $C_{36}H_{37}NO_4$: C, 78.97; H, 6.76; N, 2.55%).

6,7-*Di*(3-*hydroxy*-4-*methoxyphenyl*)-6,7-*dehydroindolizidine* **10**. A mixture of **9** (0.2 g), MeOH (20 ml) and 12N-HCl (12 ml) was heated on a water bath for 3 hr. The solvent from the resulting mixture was removed, the residue dissolved in H_2O, basified with 25% $NaHCO_3$ solution and the liberated base extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent removed to afford **10** (95 mg, 70%), m.p. 229–30° (from MeOH); UV: ν_{max}^{MeOH} 233, 288 nm, on addition of NaOH 231, 297.5 nm; IR (KBr): 3350, 2900, 1580; NMR ($CDCl_3$ + DMSO-d_6): 6.78 6.38 (m, 6 H, Ar-H), 3.91 (s, 6 H, 2 × $-OCH_3$), 3.70 1.57 (m, 11 H, rest protons); MS: M^+ 367, 298 (M^+–69) (Found: C, 71.45; H, 6.63; N, 3.70. Calc. for $C_{22}H_{25}NO_4$: C, 71.93; H, 6.81; N, 3.81%).

(±)-[2′,6′,2″,6″-$^3H_4$]6,7-*Di*(3-*hydroxy*-4-*methoxyphenyl*)-6,7-*dehydroindolizidine* **10**. A mixture of **10** (75 mg) KOBu^t (70 mg) and ^3H_2O (activity = 70 mCi) was heated on a water bath for 110 hr (N_2 atmosphere). The resulting mixture was diluted with H_2O, saturated with NH_4Cl and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent removed. The residue was passed through a column of SiO_2. Elution with $CHCl_3$ MeOH (92:8) gave **10** (52 mg), m.p. 227–28° (from MeOH) [identical with authentic radioinactive sample (Co and mixed m.p.)].

(±)-[1,8-$^3H_2$] *Tylophorine* **12**. A mixture of **10** (17 mg, activity = 2.89×10^{-1} mCi) and inactive tylophorine (100 mg) was added to a stirred mixture of $K_3Fe(CN)_6$ (100 mg), 28% NH_3 solution (0.3 ml), $CHCl_3$ (100 ml) and 8% NH_4OAc solution (1.5 ml) at 0° (N_2 atmosphere). The resulting mixture was further stirred for 4 hr. The $CHCl_3$ layer was separated, washed with H_2O, dried (anhyd Na_2SO_4) and the solvent removed. The residue, so obtained, in MeOH (200 ml) was treated with excess of diazomethane [prepared from N,N-nitroso-methylurea (0.5 g)] and left at room temperature for 36 hr. The solvent from the resulting mixture was removed and the crude product was subjected to preparative tlc [solvent system, $CHCl_3$–MeOH (96:4)] and crystallized from $CHCl_3$–MeOH to give radioactive tylophorine **12** (45 mg, spec. act. = 1.098×10^{-4} mCi/mg, 3.8%), m.p. 285–86° (d) [lit[1] 286 87° (d)] [identical with authentic radioinactive sample of tylophorine (Co-tlc and mixed m.p.)].

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