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THE PREPARATION OF THE BIOSYNTHETIC PRECURSOR 3,7-DIHYDROXY-2,6-DIMETHOXYPHENANTHROINDOLIZIDINE

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In order to set the stage for a study of the biosynthesis of tylophorine [7] and related phenanthroindolizidine alkaloids using callus tissues from *Cynanchum vincetoxicum* (L.) Pers. Asclepiadaceae, there was a need to prepare the key biogenetic precursor 3,7-dihydroxy-2,6-dimethoxynanthroindolizidine [**6**].

This diphenol was synthesized by a route paralleling those described by Herbert *et al.* (1) and Ban and Oishi (2) (Scheme 1). The known 3-benzyloxy-4-methoxyphenylacetaldehyde [**1**] prepared by a procedure comparable to that of Ban and Oishi (2) was condensed with the pyrrolidine **2**, which had been obtained by a route related to that of Herbert *et al.* (1). The condensation product was immediately reduced with $NaBH_4$ to provide the indolizidine **3**. This material was further characterized through its diphenolic derivative **4** obtained through acid hydrolysis.

Indolizidine **3** suffered intramolecular oxidative coupling upon treatment with thallium(III)trifluoroacetate in MeCN containing a catalytic amount of boron trifluoride etherate to produce the pentacyclic phenanthroindolizidine **5**. Acid hydrolysis of **5** then supplied the desired 3,7-dihydroxy-2,6-dimethoxyphenanthroindolizidine [**6**].

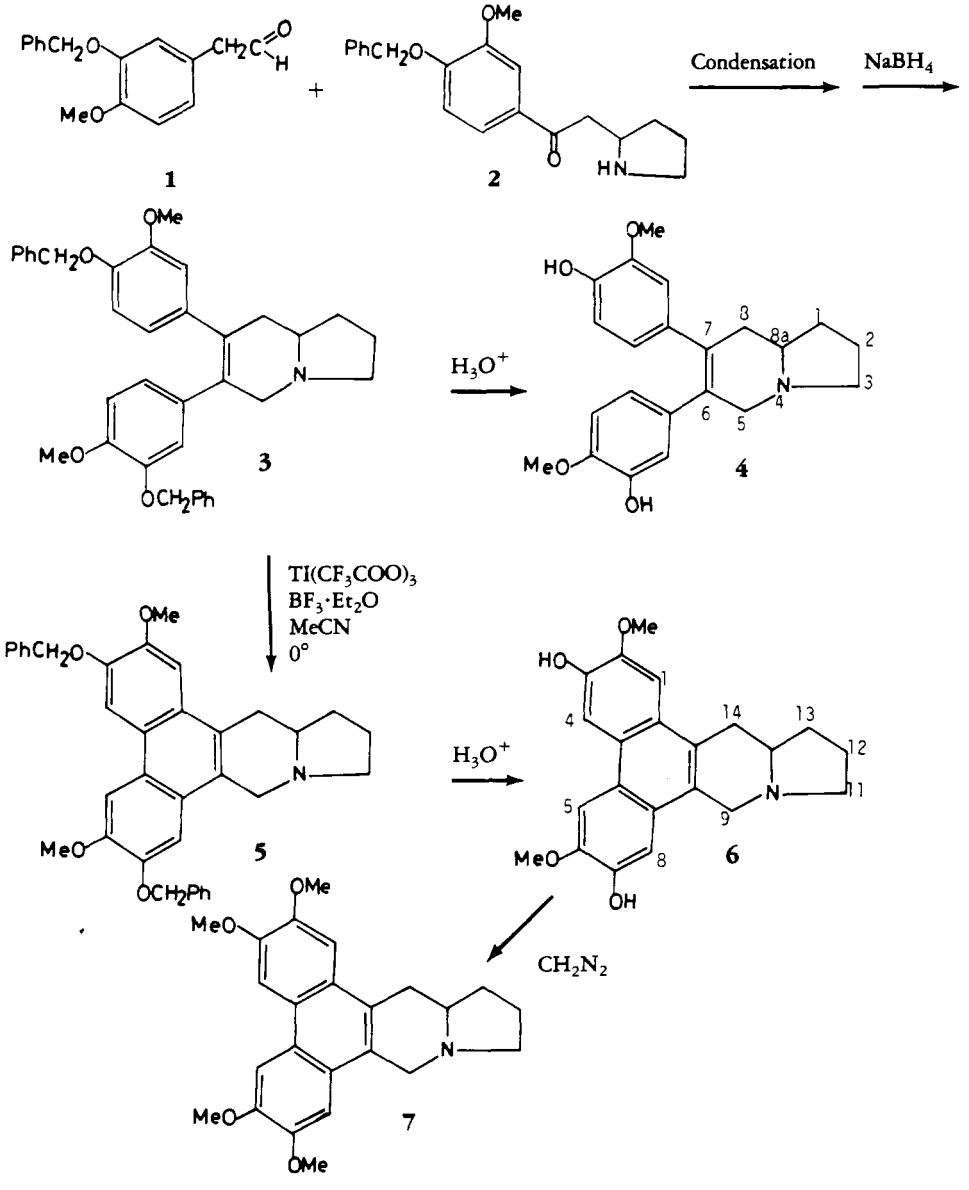
Each of our new compounds was characterized spectrally using high resolution nmr as well as ms.

As final proof of structure, the diphenol **6** was *O*-methylated with ethereal CH_2N_2 to provide (±)-tylophorine [**7**], spectroscopically identical with an authentic sample.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Uv spectra were recorded on a UVIKON 810 using MeOH as solvent. Ir spectra were recorded on a Beckman Aculab 3 spectrophotometer. ^{1}H-nmr spectra were obtained on a Varian EM 390 or Varian XL-200 or Bruker WM 250 spectrometer. Chemical shifts are reported in ppm relative to TMS as an internal standard. Except where noted, the sample for ^{1}H-nmr analyses was dissolved in $CDCl_3$. Mass spectra were determined on a Varian MAT CH7 or a Hitachi M80 at 70 eV. Isobutane gas was used in the cims. High resolution mass spectra were obtained on a Hitachi M80 or Varian MAT 311A. Preparative tlc was on Si gel 60 F-254 Merck glass plates.

PREPARATION OF 3-BENZYLOXY-4-METHOXYPHENYL ACETALDEHYDE [**1**].—Sodium 3-(3-benzyloxy-4-methoxyphenyl)glycidate was prepared starting from isovanillin according to the procedure described by Ban and Oishi (2). To a suspension of sodium glycidate (22 g) in dry C_6H_6 (280 ml) was added HOAc (5.6 ml); the mixture was stirred and refluxed gently for 3 h. After cooling the C_6H_6 solution was washed with H_2O, dried, and evaporated. The residue was dissolved in Et_2O (300 ml), and the Et_2O solution was stirred vigorously with a solution of $NaHSO_3$ (12 g) in H_2O (24 ml) overnight. The bisulfite adduct precipitated was filtered, washed with Et_2O, and dried (21 g). This adduct (11 g) was suspended in saturated $NaHCO_3$ (150 ml), extracted with Et_2O, dried, and evaporated to obtain the free aldehyde **1** (5.8 g, 74%; the yield from sodium glycidate is 60%), ir ($CHCl_3$) ν max 1730; ^{1}H nmr δ 3.45 (2H, d, J=2.2 Hz, CH_2-CHO), 3.81 (3H, s, OMe), 5.09 (2H, s, OCH_2Ph), 6.55–6.85 (3H, m, Ar-H), 7.0–7.40 (5H, m, Ar-H), 9.60 (1H, t, J=2.2 Hz, CHO). The aldehyde (500 mg) was dissolved in EtOH (10 ml) and refluxed gently with a 0.1 M solution (20 ml) of 2,4-dinitrophenylhydrazine (prepared from 2.0 g of 2,4-dinitrophenylhydrazine, 50 ml of 85% H_3PO_4, and 50 ml of EtOH) for 15 min. Upon cooling the crystallized hydrazone was



SCHEME 1

filtered and recrystallized from C_6H_6 (500 mg, 74%), mp 149–150° [lit. (3) mp 151–152°]; ir (Nujol) ν max 3275 (NH), 1630 (C=N); ^{1}H nmr δ 3.60 (2H, d, J=6.0 Hz, CH_2-CH=N), 3.87 (3H, s, OMe), 5.14 (2H, s, OCH_2Ph), 6.7–6.85 (2H, m, Ar-H), 6.82 (1H, s, Ar-H), 7.15–7.45 (6H, m, Ar-H), 7.90 (1H, d, J=9.0 Hz, Ar-H), 8.29 (1H, dd, J=9.0, 2.0 Hz, Ar-H), 9.1 (1H, d, J=2.0 Hz, Ar-H), 10.9 (1H, s, NH); ms *m/z* (rel. int.) $[M]^+$ 436 (5), 91 (100).

PREPARATION OF 2-(4-BENZYLOXY-3-METHOXYPHENANCYL)PYRROLIDINE [**2**].—Starting from vanillic acid, the procedure of Herbert *et al.* (1) for the preparation of 4-benzyloxy-3-methoxybenzoylacetic acid was used. The crude benzoylacetic acid (9.6 g) was dissolved in MeOH (450 ml), and phosphate buffer (45 ml, 1 M, pH 7.2) was added to a solution of 1-pyrroline (4) [freshly prepared from DL-ornithine monohydrochloride (5.36 g) and *N*-bromosuccinimide (5.6 g)]. The pH was ajusted to 7.0 (1 M KOH), and the reaction mixture was stirred under N_2 for 46 h at room temperature. The resulting mixture was concentrated under reduced pressure at 50°, and the precipitated material was filtered off. The solution was acidified with 10% HCl and extracted with Et_2O. The aqueous acidic solution was basified with K_2CO_3 and extracted with Et_2O and then with $CHCl_3$. Each extract was dried, and the

solvent was removed. The residues from the Et_2O and $CHCl_3$ extracts were dissolved in MeOH (100 ml), 37% HCl (0.1 ml) was added, the solvent was removed, and both residues were crystallized from Me_2CO to give the hydrochloride of **2** (3.0 g and 0.44 g, respectively, 52%), mp 177–180° (dec); ir (Nujol) ν max 2710–2300 ($\geqslant\overset{+}{N}$-H_2), 1680 (C=O); ^{1}H nmr δ 1.4–2.4 (4H, m), 3.1–3.5 (3H, m), 3.6–4.3 (2H, m), 3.84 (3H, s, OMe), 5.10 (2H, s, OCH_2Ph), 6.76 (1H, d, J=9.0 Hz, Ar-H), 7.2–7.5 (7H, m, Ar-H), 9.6 (2H, br, NH_2). Free base [**2**]: ir (film) ν max 3340 (br, NH), 1678 (C=O); ^{1}H nmr δ 2.10 (1H, br s, NH), 1.2–2.5 (4H, m), 2.7–3.1 (4H, m), 3.5 (1H, m), 3.92 (3H, s, OMe), 5.20 (2H, s, OCH_2Ph), 6.87 (1H, d, J=9.0 Hz, Ar-H), 7.2–7.45 (6H, m, Ar-H), 7.50 (1H, s, Ar-H); ms *m/z* (rel. int.) $[M]^+$ 325 (0.2), 256 (6.5), 91 (100); cims *m/z* $[M+1]^+$ 326.

PREPARATION OF 6-(3-BENZYLOXY-4-METHOXY)-7-(4-BENZYLOXY-3- METHOXYPHENYL)-1,2,3,5,8,8a-HEXAHYDROINDOLIZINE [**3**].—3-Benzyloxy-4-methoxyphenylacetaldehyde [**1**] (375 mg) and 2-(4-benzyloxy-3-methoxyphenyl)-pyrrolidine [**2**] (320 mg) were stirred in dry C_6H_6 (15 ml) for 1 h at room temperature. The solvent was removed under pressure, and the residue was dissolved in dry MeOH (15 ml). The solution was stirred at room temperature for 1 h. $NaBH_4$ (90 mg) was added to the MeOH solution, and the mixture was stirred for 1 h. The precipitate was collected and recrystallized from $CHCl_3$/MeOH to give **3** (125 mg, 23%), mp 133–134° [lit. (5) mp 131–132°]; uv λ max (MeOH) log ϵ) 281 (2.94), 230 (sh); ^{1}H nmr δ 1.5–2.9 (8H, m), 3.26 (1H, m), 3.20 and 3.75 (each 1H,d, J=15.5 Hz,=C-CH_2-N), 3.57 and 3.75 (each 3H, s, 2×OMe), 4.80 and 5.05 (each 2H, s, 2×OCH_2Ph), 6.45–6.85 (6H, m, Ar-H), 7.2–7.5 (10H, m, Ar-H).

PREPARATION OF 6-(3-HYDROXY-4-METHOXYPHENYL)-7-(4-HYDROXY-3-METHOXYPHENYL)-1,2,3,5,8,8a-HEXAHYDROINDOLIZINE [**4**].—The reaction of the pyrrolidine **2** (from **2**·HCl salt 2.37 g) with the aldehyde **1** (1.6 g) followed by $NaBH_4$ reduction was carried out as described above. After reduction the MeOH was removed, H_2O was added to the residue, and it was extracted with $CHCl_3$. Combined $CHCl_3$ extracts were washed with H_2O, dried, and evaporated to leave a brown oil that was dissolved in H_2O (50 ml), 37% HCl (50 ml), and MeOH (100 ml). The mixture was refluxed for 4 h. After cooling, precipitated crystals were collected, washed with H_2O, and recrystallized from $CHCl_3$/MeOH to give **4** (330 mg, 14% from **2**), mp 250–252° (dec); ir (Nujol) ν max 3240 (br, OH); uv λ max (MeOH) (log ϵ) 285 (3.99), 236 (sh) nm, on addition of NaOH 299; ^{1}H nmr (DMSO-d_6) δ 1.3–2.4 (7H, m), 2.6–2.8 (1H, m), 3.12 (1H, m), 2.87 and 3.65 (each 1H, d, J=16.0 Hz, =C-CH_2-N), 3.48 and 3.68 (each 3H, s, 2×OMe), 6.4–6.5 (4H, m, Ar-H), 6.52 and 6.71 (each 1H, d, J=8.0 Hz, Ar-H), 8.7 (2H, br, 2×OH); ms *m/z* (rel. int.) $[M]^+$ 367 (91), 298 (100), 267 (27), 235 (27), 137 (28), 70 (54); hrms *m/z* $[M]^+$ 367.17988) $C_{22}H_{25}O_4N$ requires 367.17847), 298.12087 ($C_{18}H_{18}O_4$ requires 298.12051). The synthetic material was identical by tlc and ir spectra with an authentic sample.

PREPARATION OF 2,6-DIMETHOXY-3,7-DIBENZYLOXYPHENANTHRO[9,10-*b*]-INDOLIZIDINE [**5**].—A solution of **3** (187 mg) in MeCN (10 ml) was added to a cold (0°) and stirred solution of $Tl(CF_3COO)_3$ (205 mg) in MeCN (10 ml), followed by rapid addition of 10 drops of $BF_3 \cdot Et_2O$. The reaction mixture was stirred under N_2 for 1.5 h at 0°. The mixture was poured into H_2O and was extracted with $CHCl_3$. The combined extracts were washed with H_2O, then with 5% aqueous NH_4OH, dried, and evaporated. Crystallization of the residue from $CHCl_3$/MeOH gave **5** (111 mg, 60%), mp 199–201°; ir (Nujol) ν max 1620; uv λ max (MeOH) (log ϵ) 356 (4.84), 340 (4.56), 322 (sh), 303 (4.33), 289 (3.41), 257 (3.23), 214 (sh) nm; ^{1}H nmr δ 1.68–2.56 (6H, m), 2.90 (1H, m), 3.3–3.5 (2H, m), 3.55 and 4.49 (each 1H, d, J=15.0 Hz, =C-CH_2-N), 4.06 and 4.08 (each 3H, s, OMe), 5.33 and 5.41 (each 2H, s, OCH_2Ph), 7.22, 7.34, 7.64, and 7.88 (each 1H, s, Ar-H), 7.35–7.63 (10H, m, Ar-H); ms *m/z* (rel. int.) $[M]^+$ 545 (38), 476 (42), 385 (68), 91 (100); cims *m/z* $[M+1]^+$ 546; hrms *m/z* $[M]^+$ 545.2564 ($C_{36}H_{35}NO_4$ requires 545.2572), 476.1986 ($C_{32}H_{28}O_4$ requires 476.2010), 385.1438 ($C_{25}H_{21}O_4$ requires 385.1438), 91.0548 (C_7H_7 requires 91.0547).

PREPARATION OF 2,6-DIMETHOXY-3,7-DIHYDROXYPHENANTHRO[9,10-*b*]-INDOLIZIDINE [**6**].—A mixture of **5** (43 mg) in MeOH (10 ml) and 10 N HCl (5 ml) was refluxed under N_2 for 3 h. The mixture was allowed to stay overnight at room temperature. The precipitated material was recrystallized from MeOH to give **6**·HCl (30 mg, 95%), mp 250–252° (dec); ir (Nujol) ν max 3530, 3480, and 3210 (br) (OH), 2720, 2640, and 2590 ($\geqslant\overset{+}{N}$-H); uv λ max (MeOH) (log ϵ) 358 (3.16), 341 (3.27), 324 (sh), 304 (4.27), 290 (4.49), 256 (4.75), 238 (sh), 222 (4.37) nm, on addition of NaOH 370, 352, 297, 266, 250; ^{1}H nmr (DMSO-d_6) δ 1.85–2.3 (3H, m), 3.0–3.95 (6H, m), 4.00 and 4.05 (each 3H, s, OMe), 4.53 and 4.98 (each 1H, d, J=15.0 Hz, =C-CH_2-N), 7.17, 7.37, 7.92, and 8.07 (each 1H, s, Ar-H), 9.54 (2H, s, OH). Free base [**6**]: mp 258–260° (dec); ir (Nujol) ν max 3410 (br, OH); ^{1}H nmr (DMSO-d_6) δ 1.90–2.50 (4H, m), 2.90–3.20 (3H, m), 3.40–3.70 (2H, m), 4.0 and 4.04 (each 3H, s, 2×OMe), 4.12–4.77 (each 1H, d,

J=15.0 Hz, =C-CH_2-N), 7.20, 7.35, 7.91, and 8.06 (each 1H, s, Ar-H), 9.48 (2H, s, 2×OH); ms *m/z* (rel. int.) $[M]^+$ 365 (22), 296 (100), 281 (14); cims *m/z* $[M+1]^+$ 366; hrms *m/z* $[M]^+$ 365.1632 ($C_{22}H_{23}O_4N$ requires 365.1626), 296.1050 ($C_{18}H_{16}O_4$ requires 296.1047).

PREPARATION OF (±)-TYLOPHORINE [7].—The hydrochloride of **6** (28 mg) was dissolved in 10% NaOH. After acidification, the solution was basified with NH_4OH, extracted with $CHCl_3$, and dried, and the solvent was removed. A solution of the residue in a mixture of $CHCl_3$ (10 ml) and MeOH (10 ml) was treated with excess ethereal CH_2N_2 (prepared from *N,N*-nitrosomethylurea), and the reaction mixture was left at room temperature for 40 h. After decomposition of excess CH_2N_2 with 10% HOAc, the solution was concentrated and H_2O was added. The solution was basified with NH_4OH, extracted with $CHCl_3$, dried, and evaporated. The residue was subjected to preparative tlc using $CHCl_3$-MeOH (19:1) to give (±)-tylophorine [7]; mp 263–265° (dec); uv λ max (MeOH) (log ε) 355 (3.05), 339 (3.29), 324 (sh), 302 (4.26), 288 (4.51), 256 (4.80), 239 (sh), 221 (4.29) nm. The synthetic material was identical by tlc ($CHCl_3$-MeOH 19:1), mp, ir, and uv spectrometry with an authentic sample.

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