Tetrahedron. Vol. 31, pp. 2575 to 2579. Pergamon Press 1975. Printed in Great Britain

ALKALOIDS OF *COCCULUS PENDULUS* (FORSK) DIELS†

D. S. BHAKUNI* and P. P. JOSHI
Central Drug Research Institute, Lucknow

(*Received in UK* 17 *April* 1975; *Accepted for publication* 16 *June* 1975)

Abstract—Six biscoclaurine alkaloids have been isolated from *Cocculus pendulus* (Forsk) Diels. Of these cocsulin (1), cocsolin (2), cocsulinin 15) and pendulinin (21) have been assigned structures and stereochemistry by a series of chemical transformations and spectral studies.

INTRODUCTION

A programme aimed at screening Indian plants over a wide range of biological activities led to the observation of hypotensive and anticancer activities[1] in the 50% ethanolic extract of the leaves and stems of *Cocculus pendulus* (Forsk) Diels, a shrub which grows in the dry parts of North and Western India. Search of the active principles in the alkaloidal fraction in which these activities had been associated resulted in the isolation of five bisbenzylisoquinoline alkaloids pendulin, cocsulin, cocsolin, pendulinin and pendin by careful chromatography on neutral Al_2O_3 of the ethyl acetate soluble alkaloidal mixture (X). The ethyl acetate insoluble alkaloidal mixture (Y) did not furnish pure compounds by repeated column chromatography or solvent separation. It was, therefore, methylated with HCHO–HCOOH. Separation of the N-methyl bases on neutral Al_2O_3 yielded compounds A, B, C and D. Direct comparison of compound A with N-methylpendin established their identity. Compounds B and C were found identical in all respects with pendulinin (21) and cocsulin (1) respectively. Compound D was a new base and was named cocsulinin. Of the isolated bases pendulin showed hypotensive activity and cocsulinin was found active against cells derived from human carcinoma of the nasopharynx (9 KB). A preliminary report on the structures of some of these bases is communicated earlier.[2-5] A fuller account of the work leading to these structures and information regarding other alkaloidal constituents of the plant is now reported.

Consulin (1) and cocsolin (2) both gave blue colouration with a mixture of HNO_3–H_2SO_4 indicative of dibenzo-1,4-dioxin moiety.[6] 2 NMe and I OMe groups in the NMR spectrum of cocsulin were at δ 2·38, 2·56 and 3·90 respectively. There were 10 protons in the aromatic region. Of these a shielded proton was at δ 6·18 and a proton singlet was at δ 6·36. The remaining 8, *ortho*-, *meta*- and *para*-coupled aromatic protons were between δ 7·67 and 6·58. Treatment of cocsulin with CH_2N_2 and CH_3CHN_2 yielded O-methylcocsulin (3) and O-ethylcocsulin (4) respectively.

The NMR spectrum of cocsolin had signals for a lone NMe and OMe groups at δ 2·58 and 3·87 respectively. Of the 10 aromatic protons one proton singlet was at δ 6·33 and a second proton singlet was at δ 6·28. The signals for 8 *ortho*- and *para*-coupled aromatic protons were between δ 7·68 to 6·58. Treatment of cocsolin with ethereal CH_2N_2 afforded O-methylcocsolin (5) while reaction with HCHO–HCOOH formed N-methylcocsolin (1). In the NMR spectrum of I the newly generated methylimino function was at δ 2·38, N-methylcocsolin reacted with CH_2N_2 to give N,O-dimethylcocsolin(3). This compound was also obtained when O-methylcocsolin was treated with HCHO–HCOOH.

MS of both cocsulin and cocsolin were consistent with alkaloids of isotrilobine type.[7-9] The intense ions at *m/e* 350, 349, 335 and 175 in the MS of cocsulin and its O-Me and O-Et ethers indicated that the phenolic OH group was present in the benzylic residue while the OMe was in the isoquinoline moiety. Similarly the presence of phenolic OH in benzylic and OMe in the isoquinoline portion in cocsolin were located. The position of NMe group in cocsolin was fixed by analogy of the chemical shifts in the NMR spectra of cocsulin and related bases. N–Me group at N-2′ in the NMR spectra of these bases was comparatively more shielded and resonated between δ 2·50 and 2·15 whereas NMe at N-2 gave signal between δ 2·62 and 2·57. The NMe function in the NMR spectrum of cocsolin resonated at δ 2·58 and thus it should be at N-2.

Direct comparison established the identity of N-methylcocsolin with cocsulin and that of O-methylcocsulin and N,O-dimethylcocsolin with isotrilobine.[10,11] These data thus established the relationship of cocsolin, cocsulin and isotrilobine. The identity of optical activity of O-methylcocsulin and isotrilobine suggested that in cocsulin both the asymmetric centres had S, S-configuration. Final support in favour of the structure (I) for cocsulin was obtained as follows: O-Methylcocsulin was converted into its dimethiodide. Hofmann degradation of this compound yielded an optically inactive methine (6) which was found identical with isotrilobine methine.[11] Catalytic reduction of 6 gave 7. Second Hofmann elimination of 7 yielded 8 characterized by its NMR spectrum. Cleavage of 8 with OsO_4–$NaIO_4$ furnished 9. The 2 aldehydic protons and 2 OMe groups in the NMR spectrum of 9 resonated at δ 10·09 and 10·26 and at δ 3·89 and 3·98 respectively.

The position of phenolic OH and OMe groups in cocsulin (1) and O-methylcoculin (3) were located by base[12] and acid[13] catalysed deuterium exchange experiments. 1 when heated with D_2O-t-BuOK gave a deuterated product. In its NMR spectrum the signal at δ 7·05 for H-3‴ had reduced in intensity by 40%. while in the NMR spectrum of the deuterated O-methylcocsulin the singlet at δ 6·36 for H-5 had almost disappeared. H-3‴ proton in 3 was found resistant to deuterium exchange under the experimental conditions employed.

First stage reductive fission of O-ethylcocsulin (4) with improved procedure[14] of Na/liq. NH_3 gave a phenolic product which was separated from the by-products and then treated with CH_2N_2 to give the O-methyl ether. Second stage fission of this compound with Na/liq. NH_3 afforded a mixture of phenolic and non-phenolic bases.

†CDRI Communication No. 1994.

Separation of the non-phenolic bases on PLC afforded **10**[14,15] and a new base **11**. In the NMR spectrum of **11** the lone NMe and OMe groups were at δ 2·61 and 3·76 respectively. The OEt group gave signals at δ 4·00 (q, 2 H, J = 7 Hz) and 1·40 (t, 3 H, J = 7 Hz). There were 7 protons in the aromatic region. The MS was consistent with the structure **11**. The mixture of phenolic bases was treated with CH_2N_2. Separation of the O-methyl ethers on PLC afforded **12**[14,15] and **13**.[16] Coclaurine derivatives with + ve rotation have been assigned S configuration.[17,18] Both the benzylisoquinolines **10** and **11** obtained from the right and left halves of O-ethylcocsulin (**4**) are dextrorotatory and should therefore, have S configuration. The configuration at the asymmetric centres in cocsulin (**1**) was thus confirmed. Cocsulin[19] has recently been shown identical with efirine,[20] trigilletine[21] and N-methyl-12′-O-desmethyltrilobine.[22]

Cocsulinin (**15**) exhibited a positive test for the dibenzo-1,4-dioxin moiety.[6] The base contained 2 NMe and 1 OMe groups. Treatment of **15** with CH_2N_2 and CH_3CHN_2 formed O,O-dimethyl (**16**) and O,O-diethylcocsulinin (**17**) respectively. While reaction with Ac_2O-C_5H_5N gave the O,O-diacetate (**18**). Cocsulinin did not react with HCHO–HCOOH indicating the absence of a secondary amine function, **16** when heated with MeI gave the dimethiodide. Hofmann degradation furnished optically inactive methine (**20**).

The MS fragments of the base (**15**), the O,O-diethyl (**17**) and O,O-dimethyl (**16**) ethers, and O,O-diacetate (**18**) were formed from double benzylic cleavage[7-9] of the respective compounds suggesting one phenolic OH and one OMe groups in the isoquinoline moieties and a second phenolic OH function in the benzylic portion of cocsulinin. The placement of the OMe group at C-6′ and not at C-6 was shown by base catalysed deuterium exchange.[12] In the NMR spectrum of the deuterated product the singlet for 5-H, was greatly reduced whereas the singlet for 5′-H remained unaffected. In addition the protons *ortho*- to the phenolic OH in benzylic half had also exchanged with deuterium. In the NMR spectrum of the deuterated product obtained by acid catalysed[13] deuterium exchange of O,O-diethylcocsulinin, the singlets at δ 6·16, 6·61 and 6·88 were reduced in intensity by 25%.

The final evidence in support of the proposed structure (**15**) and the configuration at the asymmetric centres in cocsulinin was obtained by applying the improved procedure[14] of Na/liq. NH_3 reduction to **17**. The major non-phenolic product obtained in the reaction was found to be **19**. In the NMR spectrum of **19** the signals for NMe, 2 OMe and 1 OEt were discernible and there were 6 aromatic protons signals also. MS of the compound was consistent with structure **19**.

Treatment of the phenolic fraction with ethereal CH_2N_2 and separation of the O-methyl ethers on PLC afforded **14** as the major product.

The 1-benzylisoquinoline derivatives **19** and **14** obtained from the left and right halves of **17** respectively both have positive rotation and should, therefore, have S configuration.[17] The configuration of cocsulinin, therefore be S,S at the asymmetric centres.

Pendulinin (**21**) gave a positive test for dibenzo-1,4-dioxin moiety.[6] Its NMR in TFA was not well resolved, however, one OMe resonated at δ 3·71. The base did not react with HCHO–HCOOH indicating the absence of a secondary amine function. It formed a O,O-dimethyl ether (**22**) when treated with CH_2N_2 while reaction of the base with Ac_2O-C_5H_5N afforded O,O-diacetate (**23**).

The MS fragments of pendulinin (**21**), the O,O-dimethyl ether (**22**) and O,O-diacetate (**23**) formed from double benzylic cleavage[7-9] suggested that 1 OMe and 1 OH groups were in the isoquinoline moieties and a phenolic OH was present in the benzylic portion of pendulinin.

O,O-Dimethylpendulinin (**22**) and O,O-dimethylcocsulinin (**16**) are structural isomers. MS of both the compounds were almost identical. The NMR and IR spectra of these two compounds were similar but not identical. The optical rotation of both the bases was positive and nearly of the same magnitude. Further the CD curves of these compounds were very similar. It is most likely that pendulinin has S,S configuration in its asymmetric centres as cocsulinin had. The difference in these two bases is perhaps in the ether linkage in the benzylic portion. The position of the OMe function in the isoquinoline moiety in pendulinin (**21**) remains to be settled.

EXPERIMENTAL

IR spectra were determined in KBr, UV spectra in EtOH, NMR spectra in $CDCl_3$ with TMS as internal standard and $[\alpha]_D$ in $CHCl_3$, unless otherwise, indicated.

Extraction. The air dried powdered leaves and stems (60 kg) of *Cocculus pendulus* (Forsk) diels collected from Rajasthan in March 1970 were percolated in cold with EtOH (4 × 100 l). Solvent from the combined EtOH extract was removed *in vacuo* below 40° to yield a greenish viscous mass (6 kg) which was extracted with 5% HCl (5 × 500 ml). The acid soluble portion was extracted with $CHCl_3$ (10 × 500 ml). The $CHCl_3$ extract contained mainly nonbasic material. The aqueous acidic soln was then basified with Na_2CO_3 to pH 9·5. The liberated bases were extracted with $CHCl_3$ (10 × 500 ml) washed with H_2O, dried and solvent was removed to give a brown mass (48 g). The $CHCl_3$ insoluble basic material left in the aqueous solution was filtered off, washed with H_2O, dried and was extracted with $CHCl_3$ in a Soxhlet extractor to get more of the $CHCl_3$ soluble material (18 g). The combined $CHCl_3$ soluble material (64 g) was treated with EtOAc (6 × 100 ml) to give EtOAc soluble alkaloidal mixture (X) and EtOAc insoluble alkaloidal mixture (Y).

Isolation. Alkaloidal mixture (X) (10 g) was chromatographed over Al_2O_3 (250 g, grade III, deactivated by 1% H_2O). The column was eluted (TLC control) with solvent of increasing polarity. Elution with C_6H_6 (Fr 1–4) afforded *pendilin* (20 mg), ($C_{20}H_{19}NO_5$), (M^+353) m.p. 216–217° (MeOH); $[\alpha]_D$ + 265° (*c*, 1·0); λ_{max} 227, 283 and 308 nm, unchanged in MeOH + NaOH; ν_{max} 2920, 2825, 1660, 1520, 1387, 1260, 972 and 824 cm^{-1}; NMR δ 3·70 (OCH_3), 3·91 (OCH_3), 6·00 ($-OCH_2O$), 3·50 to 2·63 (m, 6 H), 6·66 (s, 1 H, ArH), 6·80 (s, 1 H, ArH), 8·00 (s, 1 H, ArH), 8·85 (d, J = 8 Hz, 1 H, ArH).

Pendulin. The solvent from C_6H_6 eluate (Fr 5–30) was removed to give a solid (400 mg) which was rechromatographed on neutral Al_2O_3 (8 g, grade III). Elution with C_6H_6 (TLC control) gave pendulin[2] (300 mg) m.p. 192–94° (EtOAc); $[\alpha]_D$ + 265° (*c*, 1·0); λ_{max} 277 and 284 nm, (MeOH + NaOH) 231 and 306 nm; ν_{max} 3322, 2857, 1587, 1506, 1267, 1221, 1117, 972 and 824 cm^{-1}; NMR δ 2·32 (N_2–CH_3), 2·62 (N_2–CH_3), 3·21 (2 OCH_3), 3·75 (OCH_3), 3·42 to 2·88 and 3·90 (14 H, 4 benzylic, 8 ring methylene and 2 ring methine protons). 6·05 (s, 1 H, ArH), 7·40 to 6·20 (9 H, *o*, *m*, and *p*-coupled ArH); MS *m/e* 608 (M^+), 607, 416, 396, 395, 381, 364, 349, 198, 198·5, 175·5 175 and 174; (Found: C, 72·89; H, 6·21; N, 4·20. $C_{37}H_{40}N_2O_6$ requires: C, 73·01; H, 6·62; N, 4·60%).

The C_6H_6:$CHCl_3$ (3:1) eluate (Fr 31–35) contained a mixture of bases the major component of which was pendulin.

Cocsulin (**1**). Elution with C_6H_6: $CHCl_3$ (1:3) (Fr 43–50) afforded consulin[4] (2·6 g), m.p. 272–74° (EtOAc); $[\alpha]_D$ + 280° (*c*, 1·0); λ_{max} 255, 278 (Sh) and 291 nm, (MeOH + NaOH) 224 and 300 nm; ν_{max} 3425, 1621, 1575, 1372, 1269, 1198, 1120, 831 and 778 cm^{-1}; NMR δ 2·38 (N_2'–CH_3), 2·56 (N_2–CH_3) 3·90 (OCH_3), 3·60 to 2·61 and 4·11 (14 H, 4 benzylic, 8 ring methylene and 2 ring methine protons), 6·18 (s, 1 H, ArH) 6·36 (s, 1 H, H-5), 6·58–7·67 (8 H, *o*-, *m*- and *p*-coupled ArH): MS *m/e* 562 (M^+), 350, 348, 335

and 175. (Found: C, 74·32; H, 6·69; N, 5·34. $C_{35}H_{34}N_2O_5$ requires: C, 74·71; H, 6·09; N, 4·98%). The base gave a blue coloration with H_2SO_4–HNO_3.

The $CHCl_3$ eluate (Fr 51–90) contained a mixture of bases, the major component of which was cocsulin.

Pendin. The solvent from $CHCl_3$: MeOH (19·9; 0·1) eluate (Fr 91–100) was removed and the residue (400 mg) was rechromatographed over neutral Al_2O_3 (12 g, grade III). Elution with $CHCl_3$ (TLC control) gave *pendin* (250 mg), m.p. 170–71° (MeOH–Et_2O); $[\alpha]_D$ +275° (*c*, 1·0); λ_{max} 225, 276 and 286 nm; ν_{max} 3289, 1585, 1495, 1272, 1221, 1125, 1025 and 970 cm^{-1}; NMR δ 2·58 (NCH_3). 3·93 (OCH_3), 3·90 (OCH_3), 5·84 (bh, 1 H, OH, exchanged on D_2O shake), 3·67 to 2·70 and 4·08 (15 H, 4 benzylic, 8 ring methylene and 2 ring methine), 6·20 (s, 1 H, ArH), 7·60–6·56 (8 H, *o*-, *m*- and *p*-coupled ArH); MS *m/e* 578 (M^+), 366, 365, 351, 337, 183; (Found: C, 73·10; H, 6·12; N, 5·32. $C_{35}H_{34}N_2O_6$ requires: C, 72·65; H, 5·92; N, 4·84%). It gave a blue colouration with H_2SO_4–HNO_3.

The $CHCl_3$:MeOH (19:1) eluate (Fr 101–115) contained a mixture of bases.

Pendulinin (**21**). The solvent from $CHCl_3$:MeOH (9:1) eluate (Fr 116–130) was removed and the residue (200 mg) was rechromatographed over neutral Al_2O_3 (6 g, grade III). Elution with $CHCl_3$ (TLC control) gave pendulinin (80 mg) m.p. 272–73° ($CHCl_3$–MeOH); $[\alpha]_D$ +285° (*c*, 0·5), λ_{max} 220, 277 (Sh) and 293 nm; (MeOH + NaOH) 220 and 301 nm; ν_{max} 3135, 1616, 1502, 1274, 1115, 1070, 977 and 760 cm^{-1}; NMR (TFA) δ 3·71 (OCH_3), 6·11 (s, 1 H, ArH), 7·15–6·20 (8 H, *o*-, *m*- and *p*-coupled ArH); MS *m/e* 578 (M^+) 366, 365, 351, 183; (Found: C, 72·12; H, 6·20; N, 4·43. $C_{35}H_{34}N_2O_6$ requires: C, 72·65; H, 5·92; N, 4·84%). It gave a blue colouration with H_2SO_4–HNO_3.

The $CHCl_3$:MeOH (4:1) eluate (Fr 131–150) contained a mixture of bases.

Cocsolin (**2**). The solvent from $CHCl_3$:MeOH (4:1) eluate (Fr. 151–164) was removed and the residue (1·08 g) was rechromatographed over neutral Al_2O_3 (deactivated with 6% H_2O). The amorphous material (500 mg) obtained from $CHCl_3$:MeOH (9:1) elution was again chromatographed over neutral Al_2O_3 (15 g, grade III). Elution with $CHCl_3$: MeOH (19:1) gave cocsolin[5] (100 mg) as an amorphous powder, $[\alpha]_D$ +204° (*c*, 1·3); λ_{max} 225, 277 (Sh) and 291 nm; (MeOH + NaOH) 224 and 300 nm; ν_{max} 3300, 1605, 1575, 1270, 1110 cm^{-1}; NMR δ 2·58 (NCH_3), 3·87 (OCH_3), 4·33 (bh, 2 H, OH and NH exchanged on D_2O shake), 6·28 (s, 1 H, ArH); MS *m/e* 548 (M^+), 336, 335, 321, 168 (base peak); (Found: C, 73·91; H, 5·81; N, 5·40. $C_{34}H_{32}N_2O_5$ requires: C, 74·45; H, 5·84; N, 5·11%). The base gave a blue colouration with H_2SO_4–HNO_3.

N-Methyl bases. Alkaloidal mixture (Y) (4 g), HCHO (10 ml) and HCOOH (10 ml) were heated on a water bath for 3 hr. The excess of HCHO and HCOOH was removed, the residue was taken in 5% HCl and extracted with ether. The aqueous acidic solution was basified with $NaHCO_3$ and the liberated N-methyl bases (2·63 g) were filtered, washed with water and dried. The mixture (3·6 g) was chromatographed over neutral Al_2O_3 (50 g). Each fraction of 25 ml was collected and elution was monitored by TLC.

Alkaloid A. The solvent from C_6H_6 eluate (Fr 21–26) was removed and the product (40 mg) m.p. 152–54° (CH_2Cl_2–C_6H_6); $[\alpha]_D$ +267° (*c*, 1·0) was found identical with N-methylpendin (m.p., m. m.p., IR, MS, NMR and $[\alpha]_D$).

Alkaloid B. The solvent from C_6H_6:$CHCl_3$ (1:1) (Fr 41–50) eluate was removed and the base (180 mg) m.p. 272–74° (EtOAc); $[\alpha]_D$ +280° (*c*, 1·0) was found identical with cocsulin (1) (m.p., m. m.p., IR, UV, MS, NMR and $[\alpha]_D$).

Alkaloid C. The solvent from $CHCl_3$ eluate (Fr 61–65) was removed and the compound (80 mg) m.p. 272–73° (MeOH–$CHCl_3$); $[\alpha]_D$ +285° (*c*, 0·18) was found identical with pendulinin (**21**) (m.p., m. m.p., IR, UV, NMR and $[\alpha]_D$).

Cocsulinin (**15**). The solvent from $CHCl_3$:MeOH (99:1) eluate (Fr 91–110) was removed and the base (300 mg) m.p. 260–263° ($CHCl_3$–MeOH); $[\alpha]_D$ +312° (*c*, 0·50); λ_{max} 275 and 290 nm; (MeOH + NaOH) 302 nm; ν_{max} 3350, 1590, 1500, 1268, 1110, 974 and 850 cm^{-1}, NMR (TFA) δ 3·68 (OCH_3), 6·16 (s, 1 H, ArH), 6·23 (s, 1 H, ArH), 6·40 (s, 1 H, ArH), 7·30–6·48 (6 H, *o*, *m* and *p*-coupled ArH); MS *m/e* 578 (M^+), 366, 365, 351 and 183; (Found: C, 72·18; H, 5·30; N, 4·64. $C_{35}H_{34}N_2O_6$ requires: C, 72·65; H, 5·92; N, 4·84%). The base gave a blue colouration with H_2SO_4–HNO_3.

O-Methylcocsulin (**3**). Cocsulin (200 mg) in MeOH (20 ml) was treated with ethereal CH_2N_2 and left at room temp for 60 hr. The resulting mixture was worked up to give **3** (180 mg) m.p. 212–14° (CH_2Cl_2–MeOH); $[\alpha]_D$ +280° (*c*, 0·60); λ_{max} 278 and 286 nm; ν_{max} 1618, 1582, 1216, 1117; NMR δ 2·40 (NCH_3), 2·60 (NCH_3), 3·84 (OCH_3), 3·96 (OCH_3); MS *m/e* 576 (M^+), 350, 349, 335 and 175; (Found: C, 74·51; H, 5·89; N, 4·38; $C_{36}H_{36}N_2O_5$ requires: C, 74·98; H, 6·29; N, 4·86%).

O-Ethylcocsulin (**4**). Cocsulin (200 mg) in MeOH (20 ml) was treated with an excess of ethereal CH_3CHN_2 to give **4** (172 mg) m.p. 214–16° (MeOH–CH_2Cl_2); $[\alpha]_D$ +230° (*c*, 1·02); λ_{max} 278 and 286 nm; ν_{max} 1626, 1590, 1212, 1116 and 871 cm^{-1}; NMR δ 2·42 (N_2–CH_3). 2·61 (N_2–CH_3), 3·90 (OCH_3), 4·26 (q, 2 H, J = 7 Hz), 1·52 (t, 3 H, J = 7 Hz) (OCH_2CH_3); MS *m/e* 590 (M^+), 350, 349, 335 and 175; (Found: C, 75·01; H, 6·44; N, 5·10. $C_{37}H_{38}N_2O_5$ requires: C, 75·32; H, 6·48; N, 4·74%).

O-Methylcocsulin dimethiodide. O-Methylcocsulin (500 mg) in MeOH (40 ml) was gently refluxed with MeI (6 ml) for 4 hr. The resulting mixture was worked up to give the dimethiodide (525 mg) m.p. 263–65° (decomp.) (MeOH–H_2O). ν_{max} 1615, 1590, 1117, 1018 and 815 cm^{-1}; (Found: C, 52·61; H, 4·96; N, 2·93. $C_{36}H_{36}N_2O_5 2CH_3I$ requires: C, 53·02; H, 4·88; N, 3·25%).

O-Methylcocsulin methyl methine (**6**). A soln of the preceding dimethiodide (300 mg) in MeOH was passed through a column of IR 410 ion exchange resin in OH form and the eluate was recycled 5 times. The solvent was then removed and the quaternary salt was heated with KOH (4·4 g in 25 ml H_2O) on a water bath for 4 hr. The resulting product was extracted with $CHCl_3$ and the solvent was removed to yield the methine (**6**) (190 mg) m.p. 112–15° (aqu. EtOH) $[\alpha]_D$ ±0·0 (*c*, 1·2); λ_{max} 230 and 276 nm; ν_{max} 1600, 1560, 1510, 1250, 1150, 1015, 850 and 790 cm^{-1}; NMR δ 2·33 (2 NCH_3). 2·25 (2 NCH_3), 3·94 (OCH_3), 4·00 (OCH_3), 3·17–2·50 (m, 8 H, $ArCH_2CH_2N_2$–; (Found: C, 75·02; H, 6·84; N, 4·15. $C_{38}H_{40}N_2O_5$ requires: C, 75·47; H, 6·67; N, 4·63%).

The methine (**6**) was found identical with isotrilobine methyl methine (m.p., IR, UV).

The preceding methine (160 mg) in MeOH (80 ml) was stirred under H_2 at atmospheric pressure with PtO_2 (100 mg) when 2 mole equivalent of H_2 was absorbed the resulting mixture was worked up to give **7**. This was treated with excess of MeI in MeOH. The dimethiodide (120 mg) was converted into its OH form by IR 410 ion exchange resin and was heated with KOH (2·2 g, in 20 ml H_2O) on a water bath to give **8** (60 mg) m.p. 301–5° (dec.) (C_6H_6–C_6H_{14}); λ_{max} 230 and 266 nm; ν_{max} 2950, 2825, 1610, 1506, 1276, 1171, 1025, 876 and 797 cm^{-1}; NMR δ 2·91 (bs, 8 H, 4 $ArCH_2$–) 3·84 (OCH_3), 3·90 (OCH_3), 6·25 (O, J = 1·5 and 16 Hz; 2 H, CH=CH_2), 5·58 (O, J = 1·5 and 16 Hz; 2 H, HC=CH_2), 7·05–5·96 (10 H, *o*-, *m*- and *p*-coupled ArH and 2 H, $ArCH$=CH_2); (Found: C, 78·63; H, 5·48. $C_{34}H_{30}O_5$ requires; C, 78·74; H,5·83%).

The dialdehyde (**9**). **8** in THF (20 ml) and H_2O (5 ml) was treated with OsO_4 (25 mg in 0·75 ml H_2O). To this mixture was added gradually $NaIO_4$ (130 mg) and additional quantity of $NaIO_4$ (20 mg) was again added after 18 hr. The resulting mixture when worked up gave the dialdehyde (**9**) (20 mg) m.p. 112–13° (EtOAc–C_6H_{14}), ν_{max} 1695; NMR δ 10·09 (s, 1 H, ArCHO), 10·26 (s, 1 H, ArCHO); (Found: C, 73·83; H, 5·12. $C_{32}H_{26}O_7$ requires: C, 73·55; H, 5·02%).

Reductive fission of O-ethylcocsulin. Liq. NH_3 (500 ml) was treated with NaH (4 g) and to it was gradually added Na (1 g) when the soln became bluish green a soln of **4** (1 g) in $C_6H_5CH_3$ (15 ml) was added dropwise. The resulting mixture was stirred at −68° and again Na (0·4 g) was added till the blue colour persisted and was left for 3 hr. NH_3 from the resulting mixure was allowed to evaporate at room temp, H_2O added and the non-phenolic material was extracted with $CHCl_3$. The aqueous alkaline soln was saturated with NH_4Cl. The biphenyl derivative from $CHCl_3$ soln was extracted with citrate-phosphate buffer pH 6·5. The remaining phenolic bases in MeOH were treated with ethereal CH_2N_2 to give the O-methyl derivatives which in $C_6H_5CH_3$ (80 ml) were added to liq. NH_3 (500 ml dried over Na) containing Na (0·4 g). More Na was added (total 1·3 g) until a permanent blue colour persisted. After stirring at −60° for 3 hr, it was allowed to stand for 16 hr at

room temp, worked up in the usual manner, and phenolic and non-phenolic bases were separated.

The mixture of non-phenolic bases was chromatographed on basic Al_2O_3. The material from C_6H_{14}:C_6H_6 (1:3) eluate when further resolved by PLC (SiO_2) afforded **10**, m.p. 62–64°; $[\alpha]_D$ 120° (lit.[14,15] m.p. 64–65°; $[\alpha]_D$ + 129·20°, (EtOH) and **11**, $[\alpha]_D$ + 130°; NMR δ 2·61 (NCH_3), 3·76 (OCH_3), 1·36 (t, 3 H, J = 7 Hz), 4·00 (q, 2 H, J = 7 Hz OCH_2CH_3); MS *m/e* 311 (M^+), 176 (base peak), 190, 162, 161, 160, 142 and 135.

The mixture of phenolic bases in MeOH was treated with CH_2N_2 and the corresponding O-methyl ethers were separated on PLC (Al_2O_3), solvent: C_6H_6:EtOAc, 24:1) yielded **12**, m.p. 135–36°; $[\alpha]_D$ + 38° (*c*, 1·0) (lit.[14] m.p. 136–38°; $[\alpha]_D$ + 30·3°) and **13**, m.p. 105–6°; $[\alpha]_D$ 100° (lit.[16] m.p. 106–8°; $[\alpha]_D$ 108° in MeOH.

Deuterated cocsulin and O-methylcocsulin. Cocsulin (100 mg), D_2O (2 ml) and $(CH_3)_3COK$ (100 mg) were heated (sealed tube) at 100° for 120 hr. The NMR spectrum of the deuterated product was almost identical with that of cocsulin except that the multiplet at 7·05 for H-3‴ had reduced in intensity by 40%.

O-Methylcocsulin (55 mg), DCl (1 ml) and CD_3OD (0·7 ml) were heated (sealed tube) at 110° for 80 hr. The NMR spectrum of the deuterated product was almost identical with that of the parent compound except that the singlet at δ 6·36 for H-5 had reduced in intensity (19%). When the experiment was repeated and heating was continued for 125 hr the above signal had completely disappeared in the NMR spectrum of deuterated compound.

O-*Methylcocsolin* (**5**). Cocsolin (80 mg) in MeOH (20 ml) was treated at room temp with ethereal CH_2N_2 to yield **5**, m.p. 235–37° (EtOAc); $[\alpha]_D$ + 236° (*c*, 1·2); λ_{max} 215 and 285 nm; ν_{max} 3330 (NH) cm^{-1}; NMR δ 2·62 (N_2–CH_3), 3·88 (OCH_3), 3·91 (OCH_3); MS *m/e* 562 (M^+), 336, 335, 321 and 168.

N-*Methylcocsolin* (**1**). Cocsolin (50 mg), HCHO (1 ml) and HCOOH (1 ml) were heated on a H_2O bath for 2 hr to give **1**, (30 mg), m.p. 272–74°; $[\alpha]_D$ + 280° (*c*, 0·9); ν_{max} 3425 (OH) cm^{-1}; NMR δ 2·38 (N_2'–CH_3), 2·56 (N_2–CH_3) 3·90 (OCH_3); MS *m/e* 562 (M^+), 350, 349, 335 and 175; (Found: C, 74·43; H, 6·49; N, 5·34. $C_{35}H_{34}N_2O_5$ requires: C, 74·73; H, 6·05; N, 4·98%). 1 was found identical with cocsulin (m.p., m. m.p. IR, NMR, TLC and $[\alpha]_D$).

N,O-*Dimethylcocsolin* (**3**). N-Methylcocsolin (30 mg) in MeOH was treated with ethereal CH_2N_2 to give 3 (28 mg) m.p. 210–12°. (MeOH–EtOAc); $[\alpha]_D$ + 280° (*c*, 0·95); λ_{max} 286 nm; NMR δ 2·40 (N_2'–CH_3), 2·60 (N_2–CH_3), 3·84 (OCH_3), 3·96 (OCH_3); MS *m/e* 576 (M^+), 350, 349, 335 and 175; (Found: C, 74·50; H, 5·80; N, 4·38. $C_{36}H_{36}N_2O_5$ requires: C, 74·98; H, 6·29; N, 4·86%).

A mixture of **5** (30 mg), HCHO (1 ml) and HCOOH (1 ml) when heated at 100° Also gave **3**.

3,N,O-dimethylcocsolin, O-methylcocsulin and isotrilobine[10,11] were found to be identical (m.p., m. m.p., TLC, IR, NMR, MS and $[\alpha]_D$).

N,O-*Dimethylcocsolin methine* (**6**). **3** (50 mg) in MeOH (5 ml was gently heated with MeI (1 ml) to give the dimethiodide m.p. 263–65° (dec) (MeOH–H_2O and was converted into its OH form by treatment with IR-410 resin. The base hydroxide was heated with KOH to yield **6**, m.p. 112–15°.

O,O-*Dimethylpendulinin* (**22**). Pendulinin (50 mg) in MeOH (30 ml) was treated at room temp with ethereal CH_2N_2 to yield, **22**, m.p. 173–75° (EtOAc–MeOH): $[\alpha]_D$ + 300°; λ_{max} 275 and 285 nm; NMR δ 3·88 (OCH_3), 3·96 (OCH_3), 4·01 (OCH_3); MS *m/e* 606 (M^+), 380, 379, 365, 350, 190; (Found: C, 73·65; H, 6·87; N, 5·12. $C_{37}H_{38}N_2O_6$ requires: C, 73·25; H, 6·31; N, 4·62%.

O,O-*Diacetylpendulinin* (**23**). Pendulinin (50 mg) in C_5H_5N (0·7 ml) was treated with Ac_2O (0·5 ml) to yield, **23**, m.p. 210–11° (C_6H_6:$C_{16}H_{14}$): $[\alpha]_D$ + 203·3°; ν_{max} 1766, 1712, 1267, 1214 cm^{-1}; NMR δ 2·34 and 2·34 ($OCOCH_3$); (Found: C, 70·12; H, 5·23; N, 3·84. $C_{39}H_{38}N_2O_8$ requires: C, 70·68; H, 5·78; N, 4·33%).

N-*Methylpendin.* Pendin (50 mg), HCHO (2 ml) were heated on a water bath for 10 min, cooled, diluted with MeOH (15 ml) and treated with $NaBH_4$ (300 mg) to yield N-methylpendin, m.p. 152–54° (C_6H_6–CH_2Cl_2); $[\alpha]_D$ + 267°; NMR δ 2·48 and 2·58 (NCH_3); (Found: C, 72·61; H, 6·40; N, 5·12. $C_{36}H_{36}N_2O_6$ requires: C, 72·95; H, 6·12; N, 4·73%).

O-*Methylpendin.* Pendin (80 mg) in MeOH (30 ml) was treated at room temp with ethereal CH_2N_2 to yield, O-methylpendin, as an amorphous yellow powder; $[\alpha]_D$ + 253°; λ_{max} 276 and 288 nm; NMR δ 3·84; 3·91 and 3·95 (one OCH_3 each).

N,O-*Dimethylpendin.* N-methylpendin (30 mg) in MeOH (20 ml) at room temp was treated with an excess of ethereal CH_2N_2 to yield N,O-dimethylpendin, m.p. 173–75° (EtOAc–MeOH); $[\alpha]_D$ + 300°; NMR δ 3·88; 3·96 and 4·01 (one OCH_3 each); (Found: C, 73·84; H, 6·58; N, 5·13. $C_{37}H_{38}N_2O_6$ requires: C, 73·25; H, 6·31; N, 4·62%).

O,O-*Dimethylcocsulinin* (**16**). Cocsulinin (100 mg) in MeOH (40 ml) was treated at room temp with an excess of ethereal CH_2N_2 to yield **16**, m.p. 145–46° (EtOAc–MeOH); $[\alpha]_D$ + 295°; λ_{max} 275 and 285 nm; NMR δ 3·85, 3·93 and 3·98 (one OCH_3 each); (Found: C, 72·84; H, 5·98; N, 4·32. $C_{37}H_{38}N_2O_6$ requires: C, 73·25; H, 6·31; N, 4·62%).

O,O-*Dimethylcocsulinin dimethiodide.* **16** (100 mg) in MeOH (40 ml) was gently refluxed with MeI (6 ml) for 4 hr. The resulting mixture was worked up to give the dimethiodide (105 mg) as yellow amorphous solid; m.p. 259–63° (dec.); $[\alpha]_D$ + 159°; λ_{max} 221, 276 and 300 nm; λ_{min} 363 nm; ν_{max} 1695, 1590, 1130, 1075 and 960 cm^{-1}; (Found: C, 53·32; H, 4·62; N, 2·82. $C_{37}H_{38}N_2O_6 \cdot 2 \cdot CH_3I$ requires: C, 53·81; H, 4·94; N, 3·14%).

O,O-*Dimethylcocsulinin methine* (**20**). A soln of the preceding dimethiodide (100 mg) was converted to OH form as before and the base hydroxide was heated with KOH (2 g in 20 ml H_2O) on a water bath for 4 hr and worked up as before to yield **20** m.p. 120° (MeOH); $[\alpha]_D$ + ± 0·0; λ_{max} 228 and 283 nm; ν_{max} 1600, 1577, 1259, 1192, 1097 and 804 cm^{-1}; NMR δ 2·27 (2 NCH_3), 2·36 (2 NCH_3); 3·91 (OCH_3), 4·01 (OCH_3); 4·07 (OCH_3); (Found: C, 73·54; H, 6·33; N, 4·10. $C_{39}H_{42}N_2O_6$ requires: C, 73·79; H, 6·67; N, 4·41%).

O,O-*Diacetylcocsulinin* (**18**). Cocsulinin (50 mg), in C_5H_5N (0·7 ml) at room temp was treated with Ac_2O (0·5 ml) to yield **18**, m.p. 217° (C_6H_6–C_6H_{14}); $[\alpha]_D$ + 225·9°; ν_{max} 1757, 1723, 1272 and 1214 cm^{-1}; NMR δ 2·34 ($OCOCH_3$), 2·37 ($OCOCH_3$); (Found: C, 70·52; H, 5·43;N, 3·85. $C_{39}H_{38}N_2O_8$ requires: C, 70·68; H, 5·78; N, 4·23%).

O-O-*Diethylcocsulinin* (**17**). Cocsulinin (100 mg) in MeOH (30 ml) was treated at room temp with an excess of ethereal CH_3CHN_2 to give, **17**, m.p. 152°(EtOH); $[\alpha]_D$ + 320°; NMR δ 1·38 (t, J = 7 Hz, OCH_2CH_3); 1·50 (t, J = 7 Hz, OCH_2CH_3); 3·92 (OCH_3); 4·24 (q, J = 7 Hz, OCH_2CH_3) 4·20 (q, J = 7 Hz, OCH_2CH_3); (Found: C, 73·30; H, 6·05; N, 4·31. $C_{39}H_{42}N_2O_6$ requires: C, 73·79; H, 6·67, N, 4·41%).

Deuterated cocsulinin and O,O-dimethylcocsulinin. Cocsulinin (55 mg), D_2O (2 ml) and $(CH_3)_3COK$ (100 mg) were heated (sealed tube) at 110° under N_2 for 86 hr. The NMR spectrum of the deuterated product was almost identical with that of cocsulinin except that the signals at δ 6·50 and 6·46 were reduced in intensity by 40%.

O,O-Diethylcocsulinin (100 mg), DCl (1 ml) and CH_3OD (1 ml) were heated (sealed tube) under N_2 at 110° for 110 hr. The NMR spectrum of the deuterated product was almost similar with that of the parent compound except that the singlets each at δ 6·20, 6·63 and 6·88 had reduced in intensity by 25%.

Reductive fission of O,O-diethylcocsulinin. Liq. NH_3 (30 ml) was treated with NaH (5 g) and Na metal was gradually added to it till the solution acquired a blue colouration. To this was added dropwise a solution of O,O-diethylcocsulinin (1·07 g) in $C_6H_5CH_3$ (15 ml). After 2 hr liq. NH_3 (300 ml) was replenished and the blue colour was maintained by adding further quantity of Na metal (0·2 g). NH_3 from the resulting mixture was allowed to evaporate at room temp. H_2O added and the non-phenolic material was extracted with $CHCl_3$. The aqueous alkaline solution was treated as before to separate biphenyl derivative (45 mg) from phenolic products (700 mg). The phenolic bases in MeOH were treated with ethereal CH_2N_2 to give O-methyl derivatives which in $C_6H_5CH_3$ (80 ml) were added to liq. NH_3 (700 ml) containing Na. More Na (1 g) was added (total 2 g) until a permanent blue colour persisted. After stirring at − 80° for 3 hr, it was allowed to stand for 14 hr at room temp, worked up in the usual way and phenolic (40 mg) and non-phenolic (150 mg) bases were separated.

The major non-phenolic base **19** was obtained by PLC (basic, Al_2O_3, solvent: C_6H_6: EtOAc, 94·6); $[\alpha]_D$ + 65°; NMR: δ 2·50 (NCH_3), 3·77 (OCH_3), 1·40 (t, J = 7 Hz, OCH_2CH_3), 6·07 (q, J = 7 Hz, OCH_2CH_3); MS *m/e* 341 (M^+).

The mixture of phenolic bases in MeOH was treated with CH_2N_2 and the corresponding O-methyl ethers were separated on PLC (base Al_2O_3; solvent: C_6H_6: EtOAc, 96:4) to afford **14** as the major compound.

REFERENCES

[1]D. S. Bhakuni, M. L. Dhar, M. M. Dhar, B. N. Dhawan and B. N. Mehrotra, *Indian J. Expt. Biology* **7**, 250 (1969).

[2]N. C. Gupta, D. S. Bhakuni and M. M. Dhar, *Experientia* **26**, 12 (1970).

[3]P. P. Joshi, D. S. Bhakuni and M. M. Dhar, *Indian J. Chem.* **12**, 517 (1974).

[4]D. S. Bhakuni, N. C. Gupta and M. M. Dhar, *Experientia* **26**, 241 (1970).

[5]P. P. Joshi, D. S. Bhakuni and M. M. Dhar, *Indian J. Chem.* **12**, 649 (1974).

[6]H. Kondo and M. Tomita, *J. Pharm. Soc., Japan* **52**, 139 (1932); I. R. C. Bick and A. R. Todd, *J. Chem. Soc.* 1606 (1950).

[7]J. Baldas, I. R. C. Bick, T. Ibuka, R. S. Kapil and Q. N. Porter *J. Chem. Soc.* Perkin I, 592 (1972).

[8]J. Baldas, Q. N. Porter, I. R. C. Bick and M. J. Vernengo *Tetrahedron Leters* 2059 (1966).

[9]M. Tomita, T. Nikuch, K. Fujitani, A. Kato, H. Furwkawa, Y. Aoyagiy, M. Kitano and T. Ibuka, *Ibid.* 857 (1966).

[10]M. Tomita and S. Ueda, *J. Pharm. Soc. Japan* **79**, 977 (1969).

[11]M. Tomita and Y. Inubushi, *Pharm. Bull. Japan* **2**, 6 (1964).

[12]G. W. Kirby and L. Orgunkoya, *J. Chem. Soc.* 6914 (1965).

[13]Y. Inubushi and T. Kikuchi, T. Ibuka and I. Saji, *Tetrahedron Letters* 423 (1972); T. Ibuka, T. Konoshima and Y. Inubushi, *Ibid.* 4001 (1972).

[14]Y. Inubushi and K. Normura, *Ibid.* 1133 (1962); Y. Inubushi, K. Normura and M. Miyawaki, *J. Pharm. Soc. Japan* **83**, 282 (1963).

[15]Y. Sasaki, H. Ohnishi and N. Sato, *Pharm. Bull. Tokyo* **3**, 178 (1955).

[16]M. Tomita and Y. Okamoto, *J. Pharm. Soc. Japan* **85**, 456; (1965); E. Fujita and T. Tomimatsu, *Ibid.* **79**, 1260 (1959).

[17]M. Tomita and J. Kunitomo, *Ibid.* **82**, 734, 741 (1962).

[18]A. R. Battersby, I. R. C. Bick, W. Keyne, J. P. Jennings, P. M. Scopes and M. J. Verengo, *J. Chem. Soc.* 2239 (1965).

[19]N. Weber, M. M. Dhar, R. Huls, J. E. Knapp, D. J. Slatkia, P. L. Schiff, A. N. Tackie, D. Dwuma-Badu and T. Okarter, *Phytochemistry* **13**, 2326 (1974).

[20]R. Huls and C. Detry, *Bull. Soc. Roy. Sci. Liege* **42**, 73 (1973).

[21]A. N. Tackie, D. Dwuma-Badu, T. Okarter, J. E. Knapp, D. J. Slatkin and P. L. Schiff, *Phytochemistry* **12**, 2509 (1973).

[22]E. Schlittler and N. Weber, *Helv. Chem. Acta* **55**, 2061 (1972).