CONSTITUENTS OF WEST AFRICAN MEDICINAL PLANTS. XXX[1]. TRIDICTYOPHYLLINE, A NEW MORPHINAN ALKALOID FROM *TRICLISIA DICTYOPHYLLA*

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ABSTRACT.—*Triclisia dictyophylla* Diels (Menispermaceae) is a woody climber indigenous to West Africa which has been used natively as a medicinal in the treatment of several ailments. Chromatography of an extract of the whole plant afforded tridictophylline (**3**), a new morphinan alkaloid whose structure was established by a consideration of spectral data and confirmed by x-ray crystallographic analysis. The bisbenzylisoquinoline dibenzodioxin alkaloids cocsuline (**1**) and trigilletimine (**2**) were also isolated from the same extract.

The *Triclisia* species (Fam. Menispermaceae) of West Africa are woody climbers occurring as coastal scrubs or as thickets in the interior forest (1). Extracts of these species have been used medicinally in the treatment of anemia, malaria, diarrhea, joint pains, and swelling of the extremities and as arrow poisons (1, 2). Various *Triclisia* species have been found to be excellent sources of numerous bisbenzylisoquinoline alkaloids, as well illustrated by the isolation of phaenthine (2–4), *N*,*N*-dimethylphaeanthine (2), pycnamine (4), cocsuline (4) and aromoline (5) from *T. patens*. In addition, the oxoaporphine alkaloid *O*-methylmoschatoline (5) and an uncharacterized base (6) have also been isolated from this same species. Extracts of *T. gilletii* have afforded the bisbenzylisoquinoline alkaloids stebisimine (4), isotetrandrine (4), phaenthine (7), cocsuline (4, 8, 9), trigilletimine (10), and gilletine (11) as well as the oxoaporphine *O*-methylmoschatoline (7) and an unusual indeno[1,2,3,-ij]isoquinoline (12). *T. subcordata* has been found to contain the dimeric benzylisoquinoline alkaloids fangchinoline (4), tricordatine (4, 9) and tetrandrine (5), while *T. dictyophylla* has afforded the bisbenzylisoquinoline bases phaeanthine (2) and *N*,*N*-dimethylphaeanthine (2).

A phytochemical investigation of the alkaloids of *Triclisia dictyophylla* Diels was undertaken to seek a source of compounds of potential pharmacological or phytochemical importance and to complement our studies of other *Triclisia* species (4, 5, 9–11).

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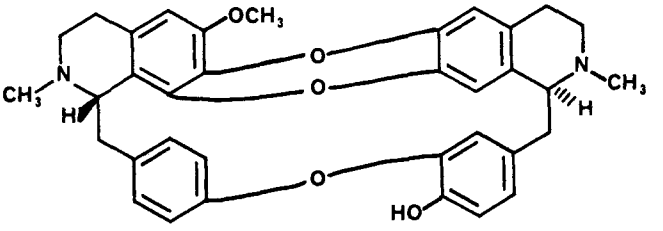
This paper reports the isolation and identification of cocsuline (**1**), trigilletimine (**2**), and the new morphinan alkaloid tridictyophylline (**3**) from extracts of the whole plant of *T. dictyophylla* Diels.

The dried, powdered whole plant was percolated with methanol, and the solvent was exaporated to afford a residue which was partitioned between dilute citric acid and ether. The acidic fraction was basified with ammonium hydroxide and extracted with ether. The ether extract was dried over anhydrous sodium sulfate, filtered, and concentrated to a residue. Chromatography of this residue over silica gel in chloroform-methanol (85:15) afforded cocsuline (**1**) and tridictyophylline (**3**) as mixtures in the earlier fractions and trigilletimine (**2**) in the later fractions. Rechromatography of the fractions containing the cocsuline-tridictyophylline mixture over silica gel in chloroform-methanol (95:5) and then over silica gel in chloroform-methanol (99:1) achieved the separation of these two alkaloids. Cocsuline (**1**) and trigilletimine (**2**) were identified by direct comparison (uv, ir, nmr [cocsuline only], ms, sp. rotn, mp, mmp) with authentic reference



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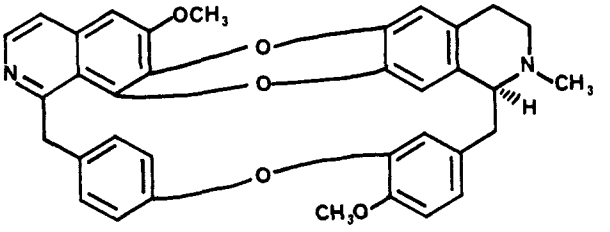

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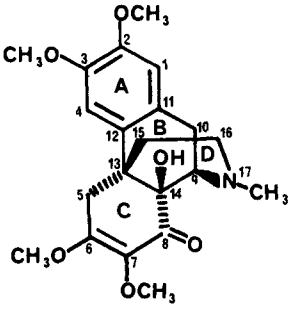

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samples available in our laboratories. Cocsuline (**1**) was first isolated from *Cocculus pendulus* in 1970 (13) but was later isolated from *Triclisia gilletii* in 1973 by two separate groups, who named the alkaloid efirine (8) and trigilletine (4, 9). A direct comparison of all three alkaloids in 1974 revealed that they were identical and were, in fact, *N*-Methyl-12'-*O*-desmethyltrilobine (14). The name cocsuline was retained because of the priority of publication. Cocsuline has also been isolated from *Triclisia subcordata* in 1973 (4). Cocsuline was found to inhibit the growth of *Mycobacterium smegmatis* in the agar dilution-streak method of Mitscher *et al.* (15). Trigilletimine (**2**) was first isolated from extracts of *Triclisia gilletii* and *T. patens* in 1974 (4) and was later characterized as the first example of a naturally occurring dibenzodioxin alkaloid containing a fully aromatized isoquinoline ring (10). The occurrence of cocsuline and trigilletimine appears restricted to the family Menispermaceae to date and, in particular, to the genera *Triclisia* (cocsuline and trigilletimine) and *Cocculus* (cocsuline).

Tridictyophylline crystallized from methanol as long colorless feathery needles or prisms (mp 204° after softening at 190°), $[\alpha]^{28}D+159°$ (c=0.16, $CHCl_3$); ir, ν max ($CHCl_3$) 3380 cm^{-1} (weak), 1665, 1610, and 1510; characteristic of an aromatic system containing an α, β-unsaturated, substituted cyclohexenone and a hydroxy group (16); uv, λ max (MeOH) 230nm(sh) (log ϵ 4.08) and 279(4.12) with no shift in alkali; characteristic of a nonphenolic, morphinan-type alkaloid (17). The nmr spectrum indicated the presence of one *N*-methyl group at δ2.45 (s, 3H), four *O*-methyl groups at δ3.43 (s, 3H), 3.85 (s, 6H) and 4.00 (s, 3H), and two aromatic protons at δ6.53 (s, 1H) and 6,64 (s, 1H). The mass spectrum showed the molecular ion at *m/e* 389 (obs. 389.1847 and calculated 389.1838 for $C_{21}H_{27}NO_6$) (62%), the base peak at 374 (100) ($C_{20}H_{24}NO_6$) and other significant ions at 372 (11) ($C_{21}H_{26}NO_5$), 358 (5) ($C_{20}H_{24}NO_5$), 356 (7) ($C_{20}H_{22}NO_5$), 303 (8) ($C_{17}H_{19}O_5$), 261 (10) ($C_{15}H_{19}NO_3$), 218 (26) ($C_{13}H_{14}O_3$), 206 (12) ($C_{12}H_{16}NO_2$) characteristic of a morphinan alkaloid (19, 20). The ^{13}C nmr spectrum of dictyophylline is summarized in table 1. These spectral data are indicative of a nonphenolic, morphinan-ene-one type of alkaloid containing one alcoholic hydroxy group, one *N*-methyl group and four methoxy groups (one of which is relatively upfield [δ3.43(19)]). Since consideration of these spectral data did not allow an unequivocal assignment of structure and since the limited amount of alkaloid available precluded an extensive chemical study, a single-crystal x-ray crystallographic study was undertaken.[4]

A crystal of dimensions 0.15 x 0.4 x 0.7 mm was used to collect intensity data on a Syntex $P2_1$ diffractometer system. The unit cell is monoclinic with a=27.245(13), b=10.442(6), c=7.206(3) Å, β=105.32°, V=1977(2) $Å^3$ and d_c=1.214 gcm^{-3} for Z=4. Systematic absences were consistent with space group $P2_1$. The structure was solved by direct methods and refined by least-squares procedures to an intermediate R value of 0.105. The structure represented here is relative and not absolute. Complete refinement and structural comparisons will be reported elsewhere.

Tridictyophylline exhibits the same general conformational features as oxymorphone (21) and 14-hydroxyazidomorphine (22). The A–B ring plane is essentially perpendicular to the plane of the C–D rings. The 14-hydroxy group extends from the face of the C–D plane opposite to the A–B system and is readily accessible for binding.

[4]The study was performed in the laboratories of authors V.Z. and W.H.W.

To our knowledge this is the first reported occurrence of a morphinan alkaloid in the genus *Triclisia*. Furthermore, it appears to be only the second report of the isolation of a morphinan alkaloid containing a 14β-hydroxy group from nature, as 14β-hydroxycodeine and 14β-hydroxycodeinone were only recently isolated from the capsules of *Papaver bracteatum* (19). Finally, tridictyophylline[5] was found to be identical to an incompletely characterized alkaloid which was simply designated TGL-3 and isolated from extracts of *Triclisia gilletii* in 1973 (4).

Due to the limited amount of tridictyophylline available, a complete pharmacological evaluation of the alkaloid was not possible. However, because of the presence of the morphinan nucleus, screening of the alkaloid for analgesic activity was undertaken. At three dosage levels (8 mg/kg, 16 mg/kg and 32 mg/kg),

TABLE 1. Tentative ^{13}C-nmr chemical shift assignments for tridictyophylline (**3**).[a]

Carbon Atom	3
1	107.2
2	148.1
3	148.1
4	110.5
5	34.6
6	135.0
7	161.9
8	[a]
9	60.3
10	29.6
11	129.4
12	129.4
13	40.5
14	66.7
15	34.6
16	45.8
OCH_3	55.8 (2), 57.6, 58.7
NCH_3	42.6

[a]The small sample size made it impractical to run a coupled spectrum, and unambiguous line assignments could not be made. However, comparisons with other morphine-type alkaloids (23, 24) indicate the above assignments are reasonable. The slow relaxation time and the small sample size prevented observation of the carbonyl carbon. One additional resonance was missing; however, the line at 34.6 ppm was broadened slightly and may contain two carbon signals.

tridictyophylline did not show significant analgesic activity when tested by the acetic acid writhing test described in Experimental. Control mice exhibited 10–15 writhing responses during each 5 minute observation period. Administration of tridictyophylline tended to reduce the number of responses (range = 6–11 responses), however, the differences were not statistically significant nor were they dose-related. By way of comparison, a dose of 5 mg/kg of morphine sulfate completely abolished the writhing associated with intraperitoneal administration of acetic acid (75 mg/kg). The mice receiving tridictyophylline did not demon-

[5]Dr. Kurt L. Loening, Director of Nomenclature, Chemical Abstracts Service, Columbus, Ohio 43210 has kindly informed us that the preferred IUPAC name for tridictyophylline is 6,7-didehydro-14-hydroxy-2,3,6,7-tetramethoxy-17-methylmorphinan-8-one.

strate any significant behavioral changes such as sedation, respiratory depression, or tail erection (a response often seen in morphine treated mice). Although these results were essentially negative, they do not preclude the possibility that tridictyophylline is an analgesic, albeit considerably less potent than morphine. The limited amount of pure tridictyophylline available did not allow for testing at higher dosage levels or use of other analgesic screening models.

EXPERIMENTAL[6]

PLANT MATERIAL.—The plant material used in this study was collected from the Aponmu Forest Reserve in West Nigeria (Ondo State) in January, 1979. A voucher specimen is on deposit at the Moore Plantation, Ibadan, Nigeria.[7] The whole plant was dried and ground to a coarse powder.

EXTRACTION, FRACTIONATION AND CHROMATOGRAPHY.—Powdered whole plant (750 g) was percolated with methanol (20 liters). The methanolic extract was evaporated to a syrup, which was partitioned between citric acid (2%) (1 liter) and ether (1 liter) (2x). The acidic solution was basified with ammonium hydroxide to pH 8–9 and extracted with ether (1 liter) (3x). The combined ether extracts (1.9 g) were chromatographed over silica gel-Celite[8] (4:1) (50 g) (Column A) in chloroform-methanol (85:15). The sample was dissolved in chloroform-methanol (25 ml), applied to the column, and 10 ml fractions were collected via an automatic fraction collector. Fractions 9–11 (1.4 g) were rechromatographed over silica gel-Celite[8] (4:1) (50 g) (Column B) in chloroform-methanol (95:5). Ten milliliter fractions were again collected.

ISOLATION OF TRIDICTYOPHYLLINE (**3**).—Fractions 13–14 (420 mg) were rechromatographed over silica gel-Celite[8] (4:1) (50 g) in chloroform-methanol (99:1). Ten milliliter fractions were collected. Treatment of fractions 1–3 (26 mg) with methanol afforded colorless prisms of tridictyophylline (**3**) (11 mg), mp 204° (after softening at 190°), $[\alpha]^{25}D$ +159° (c 0.16, $CHCl_3$); R_f 0.42 (system 1), 0.30 (system 2); ir, ν max ($CHCl_3$) 3380 cm^{-1} (weak), 2930, 1665, 1610, 1510, 1495, 1450, 1360, 1338, 1295, 1250, 1205, 1145, 1118, 1075, 1045, 990, 945, 920, 885, and 855; ν max (KBr) 3390 cm^{-1}, 2925, 2830, 1655, 1600, 1510, 1445, 1425, 1365, 1355, 1330, 1310, 1255, 1240, 1230, 1205, 1195, 1180, 1160, 1140, 1115, 1110, 1070, 1045, 995, 980, 920, 880, 860, 850, 805, and 780, λ max (MeOH) 230 nm (sh) (log ϵ 4.08) and 279 (4.12) with no shift in alkali; nmr, δ($CHCl_3$), 2.45 (s, 3H), 3.43 (s, 3H), 3.85 (s, 6H), 4.00 (s, 3H), 6.53 (s, 1H) and 6.64 (s, 1H); ms, M^+ *m/e* 389 (obs. 389.1847 and calculated 389,1838 for $C_{21}H_{27}NO_6$) (62%), 374 (100) ($C_{20}H_{24}NO_6$), 358(5) ($C_{20}H_{24}NO_5$), 356(7) ($C_{20}H_{22}NO_5$), 303(8) ($C_{17}H_{19}O_5$), 261(10) ($C_{15}H_{19}NO_3$), 281(26) ($C_{13}H_{14}O_3$), and 206(12) ($C_{12}H_{16}NO_2$); ^{13}C nmr, 29.6, 34.6(2), 40.5, 42.6, 45.8, 55.8(2), 57.6, 58.7, 60.3, 66.7, 107.2, 110.5, 129.4(2), 134.9, 148.1(2) and 161.9.

ISOLATION OF COCSULINE (**1**).—Treatment of the residue (934 mg) obtained from fractions 15–22 (Column B) with methanol-chloroform afforded white needles of cocsuline (**1**) (575 mg), mp 271–274° (dec) $[\alpha]^{25}D$ +292° (c 0.76, $CHCl_3$): $[\alpha]^{25}D$ +337° (c 0.86, pyr); R_f 0.53 (system 1), 0.39 (system 2); uv, λ max (MeOH) 225 nm (log ϵ 4.87), 278 (sh) (3.55), 291 (3.58) and 313 (sh) (3.35); ν max ($CHCl_3$) 3560 cm^{-1}, 2940, 1590, 1500, 1455, 1440, 1365, 1275, and 1120; nmr, δ2.40 (s, 3H, NCH_3), 2.57 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3) and 6.10–7.61 (m, 10H, ArH); ms, M^+ *m/e* 562 (45%), 350(32), 349(100), 335(34) and 175(59), identical by direct comparison (uv, ir, nmr, ms, sp. rotn., mp, mmp) with an authentic sample.

ISOLATION OF TRIGILLETIMINE (**2**).—Fractions 14–20 (144 mg) (Column A) were rechromatographed over silicic acid (10 g) in chloroform (25 ml). Elution with chloroform-methanol (95:5) afforded a residue (35 mg) which, upon treatment with acetone, gave white needles of trigilletimine (**2**) (13 mg), mp 274°, $[\alpha]^{27}D$ −292° (c 0.25, $CHCl_3$); R_f 0.28 (system 1), 0.55 (sys-

[6]Melting points were taken on a Fisher-Johns Apparatus and are uncorrected. The uv spectra were obtained on a Perkin-Elmer model 202 recording spectrophotometer in methanol and the ir spectra were determined on a Perkin-Elmer model 257 recording spectrophotometer in chloroform or potassium bromide pellets. The ^1H nmr spectra were recorded in deuterated chloroform on a Hitachi Perkin-Elmer model R-24 high resolution spectrometer with tetramethylsilane as internal standard and chemical shifts recorded in δ(ppm) units. The ^{13}C nmr spectra were determined on a JEOL FX-60 with $CDCl_3$ as solvent and TMS as internal standard. The mass spectra were taken with a LKB-900 mass spectrometer or a Hitachi Perkin-Elmer model RMU-6 spectrometer. The optical rotations were measured on a Perkin-Elmer model 241 polarimeter. Silicic acid (100 mesh) (Mallinckrodt) and silica gel G (Camag) were used for column chromatography, while silica gel G (Camag) was used for thin-layer chromatography. The thin-layer solvent system 1 was chloroform-methanol (21:9), and solvent system 2 was benzene-acetone-ammonium hydroxide (20:16:0.3). All solvents were evaporated under reduced pressure at 40°.

[7]The plant material was collected and identified by Mr. George Adesida, Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

[8]Celite 545 (acid-washed), Fisher Scientific, Pittsburgh, PA.

tem 2); uv, λ max (MeOH) 237 nm (log ε 4.59), 275 (sh) (4.19), 281 (sh) (4.15), 304 (sh) (3.71) and 350 (sh) (3.35); ir, ν max (KBr) 1630 cm^{-1}, 1503, 1129 and 1112; ms, M^+ *m/e* 558(91%), 557(100), 543(32) and 279 (41) identical by direct comparison (uv, ir, ms, sp. rotn., mp, mmp) with an authentic sample.

SCREENING FOR ANALGESIC ACTIVITY.—The acetic acid writhing test (25, 26) was used to test for potential analgesic activity of tridictyophylline. Twenty-four male Swiss mice (Hilltop Laboratories, Scottdale, PA) weighing approximately 20 g were divided into four groups of six mice each. Group 1 received a subcutaneous injection of 0.1 ml of 0.1M KH_2PO_4, while the other groups received equal volumes of tridictyophylline (dissolved in 0.1M KH_2PO_4) at dosage levels of 8 mg/kg, 16 mg/kg and 32 mg/kg. Fifteen minutes later, each animal was given an intraperitoneal injection of approximately 0.15 ml of 1% acetic acid (75 mg/kg). Each mouse was placed in individual plexiglass cages and observed for 30 minutes after the acetic acid injection. The number of writhes for each animal was recorded for five minute intervals during this time. Writhing is defined as a stretch response that includes full extension of the hind limbs and lowering of the abdomen to the floor of the cage. The results have been previously summarized in the last paragraph of the text of this paper.

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