FUROQUINOLINE ALKALOIDS OF *TECLEA OUABANGUIENSIS*

J. FOYERE AYAFOR*, B. LUCAS SONDENGAM, A. NGO BILON, E. TSAMO, SAMUEL F. KIMBU

Department of Organic Chemistry, The University of Yaounde, Box 812, Yaoundé, Cameroon

and

JOSEPH I. OKOGUN

Department of Chemistry, University of Ibadan, Ibadan, Nigeria

ABSTRACT.—From the stem bark of *Teclea ouabanguiensis* Aubrev. & Perr. (Rutaceae), six furoquinoline alkaloids have been isolated and characterized. Previously known alkaloids, but new to this plant, were tecleaverdoornine (**1**), tecleine (**3**), flindersiamine (**4**), montrifoline (nkolbisine) (**5**) and kokusaginine (**6**), while tecleamine **2** is reported from a natural source for the first time. Spectroscopic and chemical evidence is presented for the structure of the new isolate **2**.

In previous papers we have reported the structure determination of new prenylated furoquinoline alkaloids and tetranortriterpenoids (limonoids) isolated from three species of the genus *Teclea* Delile (Rutaceae): *T. verdoorniana* (1, 2, 3), *T. grandifolia* (4), and *T. afzelii* (5). The interesting chemical structures of these products prompted us to investigate other species of the same genus. This paper deals with the results obtained from the chemical examination of a previously uninvestigated endemic Cameroonian species, *Teclea ouabanguiensis* Aubrev. & Perr. (6, 7).

RESULTS AND DISCUSSION

The chloroform extract of the powdered stem bark of *Teclea ouabanguiensis*, upon direct column chromatography, preparative tlc, and crystallization, afforded six alkaloids and six neutral products. By means of spectroscopic data (pmr, uv, ir and ms) and comparison with authentic samples (mmp, tlc), five of these alkaloids were identified as the already known tecleaverdoornine (**1**) (1, 2), tecleine (**3**) (2,8), montrifoline (**5**) (9) (nkolbisine (3)), flindersiamine (**4**) (10), and kokusaginine (**6**) (10).

The sixth alkaloid for which the name tecleamine **2** is proposed is new; it crystallized from petroleum ether-diethyl ether as colorless prisms, mp 112–113°. Elementary analyses and mass spectrometry established the molecular formula, $C_{18}H_{17}O_5N$, for tecleamine and thus showed that **2** was isomeric with tecleaverdoornine (**1**) (1). Tecleamine gave a positive Labat's test (11) for a methylenedioxy group; unlike **1**, **2** was nonphenolic. The ultra-violet spectrum of tecleamine (**2**) [λmax (ϵmax) 247 nm (28,000), 254 (36,500), 318 (6,100) and 340 (4,400)] suggested that **2** was a furoquinoline (12). The uv spectrum was particularly similar to that of flindersiamine (**4**) (10). The proton magnetic resonance spectrum of tecleamine confirmed its furoquinoline nature and showed resonances for a 4-methoxy group (13) at δ 4.33 (3H, s), a methylenedioxy group at δ 6.02 (2H, s), an aromatic proton at δ 7.28 (1H, s) and two olefinic furan ring protons represented by a pair of AB doublets (J 2.5 Hz) centered at δ 6.95 (1H, Hβ) and 7.50 (1H, Hα), respectively. The remaining signals, a broadened one-proton triplet at δ 5.64 (J 7Hz), a two-proton doublet at δ 4.98 (J 7Hz), and two three-proton singlets at δ 1.67 and 1.70 obviously arose from a 3-methybut-2-enyl (prenyl) ether function. All the above spectroscopic data are best accommodated by structure **2** for tecleamine.

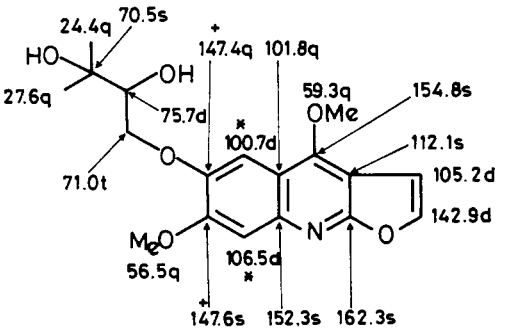
The ^{13}C-nmr spectrum was also in perfect agreement with structure **2** revealing, particularly, the ^{13}C shifts of the methylenedioxy carbon (101.6t), the 4-OMe signal (58.9 q) and the O-prenyl group carbons (59.7t, 120.9d, 139.6s, 18.2q and 25.8q) at the expected positions (14), (15). The 4-OMe signal usually occurs

between 58 and 60 ppm as against 56–57 ppm for the homocyclic MeO groups. A complete analysis of the ^{13}C-nmr spectra of **2** and nkolbisine **5** was also accomplished.

Structure **2** was also consistent with the mass spectral fragmentation which showed, in addition to the parent ion peak at *m/z* 327, important fragments at *m/z* 312 (M^+–15) and *m/z* 259 (100%) (M^+–68), the latter peak resulting from the cleavage of the prenyl ether function followed by protonation.

The structure of tecleamine (**2**) was finally confirmed by chemical correlation of **2** with tecleine (**3**) (2) and flindersiamine (**4**) (10.) Deprenylation of **2** with a hot mixture of acetic acid and concentrated hydrochloric acid in ethanol yielded a phenol, $C_{13}H_9O_5N$, mp 255–256°, identified (ir, uv, pmr and mmp) as tecleine (**3**) (2). Methylation of the phenol **3** with diazomethane afforded a methyl ether whose ir spectrum was superimposable with that of flindersiamine (**4**).

The isolation of tecleamine (**2**), tecleaverdoornine (**1**) and tecleine (**3**) from the same plant source is noteworthy and may imply that O– and C– prenylation are competing processes in *T. ouabanguiensis*. Tecleamine might be expected to derive biosynthetically from tecleine (**3**) by O– prenylation, while C– prenylation would be expected to give tecleaverdoornine (**1**).



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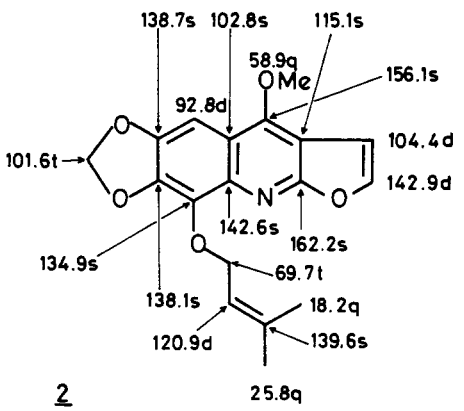

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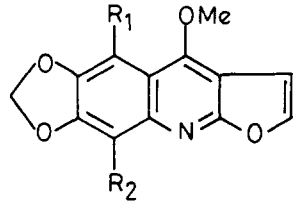
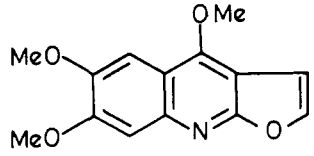
FIG. 1. ^{1}C NMR[a] Assignment of tecleamine and nkolbisine.

[a]Pulse FT spectra with 1.25Hz per data point were obtained at 25.2MHz from solutions in $CDCl_3$ for **2** and DMSO-d_6 for **5** at room temperature (*Ca* 25°C). Shifts are given as positive downfield from internal Me_4Si. Assignments are based on Chemical shift rules, multiplicities in off-resonance-decoupled spectra and comparison with published data on similar compounds (14,15).

+ x These assignments may be interchanged.



1. $R_1 = CH_2$. $CH = CMe_2$, $R_2 = OH$
3. $R_1 = H$, $R_2 = OH$
4. $R_1 = H$, $R_2 = OMe$



6

FIG. 2. Structures of the isolated alkaloids.

EXPERIMENTAL[1]

PLANT MATERIAL.—The stem bark of *Teclea ouabanguiensis* (Rutaceae) was collected on Mount Vokré, POLI (North Cameroon) during August 1981 by one of the authors (JFA) and Mr. Paul Misili. Voucher specimens have been deposited in the National Herbarium, Yaounde. These specimens were verified by Mr. Benoît Mpom of the National Herbarium, Yaounde.

EXTRACTION AND CHROMATOGRAPHY.—The sun-dried milled stem bark (10 kg) was extracted with chloroform in a Soxhlet extractor for 24 hrs. After removal of the solvent, a dark green residue (303 g) was obtained. A sample (101 g) of this residue was dissolved in chloroform (150 ml) and chromatographed in a silica gel (1800 g) column. Elution was commenced with petroleum ether and continued stepwise through petroleum ether-diethyl ether mixtures, diethyl ether and diethyl ether-methanol mixtures. The eluate was collected in 500 ml fractions and the fractions were combined on the basis of tlc comparison with an appropriate solvent system. A total of 182 fractions were collected.

ISOLATION AND CHARACTERIZATION OF THE ALKALOIDS.—From the chromatographic separation above, a total of six alkaloids and six neutral products were obtained pure, in some cases with the aid of preparative tlc. The neutral products constitute another study (16). The alkaloids are presented in order of elution from the column.

TECLEAVERDOORNINE (1).—Least polar of the alkaloids, tecleaverdoornine (1) crystallized from acetone as long colorless needles (280 mg), mp 191–192° (lit. (1). mp 191°); uv λmax 248, 263, 330 and 350 nm (ε max 24,000, 48,000, 8,300 and 4,300); ir νmax 3410, 3154, 3130, 1680, 1535, 1475 and 923 cm^{-1}; ms *m/z* (%): 327 (100, M^+), 312 (57), 284 (12), 286 (9), 272 (20), 259 (32) and 41 (8); pmr δ: 1.70 (3H, s, Me), 1.80 (3H, s, Me), 3.80 (2H, d, *J*=7Hz, Ar. CH_2.CH=), 4.35 (3H, s, 4-OMe), 5.30 (1H, t, *J*=7Hz, Ar.CH_2.CH=), 6.10 (2H, s, $-OCH_2O-$), 7.10 (1H, d, *J*=2.5Hz, H-3), 7.55 (1H, d, *J*=2.5Hz, H-3) and 7.60 (1H, s, OH, washed by D_2O). The isolate was identical (tlc, mmp, uv, ir and pmr) with authentic tecleaverdoornine (1).

TECLEINE (3).—The residue from combined fractions 28–34, on treatment with ethyl acetate, yielded a white powder (28 mg). Recrystallization from boiling ethanol afforded an analytical sample, mp 256–257° (lit. (2). mp 257–258°). Tecleine gave a deep blue color in the Labat's test. Uv λmax 254.5, 320, 325 and 340 nm (ε max 52,000, 8,200, 8,200 and 7,200); λmax

[1]Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The UV spectra were obtained with a Beckman model 25 grating spectrophotometer in ethanol, and ir spectra (KBr discs) with a Perkin-Elmer model 727B spectrometer. Proton nmr spectra were recorded in $CDCl_3$, unless otherwise indicated, on a Perkin-Elmer R12B instrument operating at 60MHz. Tetramethylsilane was used as internal standard. Low resolution mass spectra were obtained at 70EV on a LKB-9000 S machine with direct inlet. Thin-layer chromatography was carried out on precoated silica gel 60 plates (0.25 mm silica gel F_{254}, E Merck). The eluent systems were benzene-ethyl acetate (3:1 and chloroform-methanol (95:5). Silica gel 60 (70–230 mesh, E Merck) was used for column chromatography.

(EtOH-0.2M NaOH) 270 nm (52,000); ir νmax 3440, 3164, 3144, 1620, 1530, 1485, 1460, 1348, 1300, 1255, 1182, 1090 and 940 cm^{-1}; pmr δ: 4.40 (3H, s, 4-OMe), 6.12 (2H, bs, OCH_2O), 7.18 (1H, d, J=2.5Hz, H-3), 7.32 (1H, s, H-5) and 7.57 (1H, d, J=2.5Hz, H-2); ms m/z (%): 259 (100, M^+), 244 (62), 343 (38), 228 (18), 172 (10) and 158 (9).

TECLEAMINE (**2**).—Fractions 65 to 73 containing mainly tecleamine (**2**) were rechromatographed on a short column of silica gel and crystallized from a mixture of petroleum ether-diethyl ether; prisms (62 mg) of tecleamine (**2**) were obtained, mp 112–113°; ir νmax: 3118, 1650, 1620, 1521, 1502, 1480, 1450, 1433, 1365, 1312, 1290, 1255, 1232, 1180, 1175, 1138, 1090, 970 and 925 cm^{-1}; ms, m/z (%): 327 (M^+, 23), 312 (11), 260 (18), 259 (100), 258 (6), 244 (19), 243 (10.5), 228 (10), 214 (9), 67 (6), 53 (8) and 41 (11). The uv and pmr spectral data are given in the text, while the ^{13}C-nmr data is found in fig. 1. (Found: C, 65.98; H, 5.28; N, 4.02. $C_{18}H_{17}O_5N$ requires C, 66.05; H, 5.24; N, 4.28%).

DEPRENYLATION OF **2**.—A solution of the alkaloid (**2**) (200 mg) in ethanol (10 ml), acetic acid (4 ml) and conc. HCl (4 ml) was refluxed for 3 h then cooled and diluted with water. Most of the ethanol was removed under reduced pressure and the product allowed to precipitate. The crude material, obtained in nearly quantitative yield by filtration and purified by crystallization from hot ethanol, gave the phenol (**3**), mp 257–259°, identical (mmp, ir, pmr) with tecleine (*vide supra*). Methylation of the phenol (3) in ethanol with excess diazomethane generated from diazald gave a methyl ether whose ir spectrum was superimposable with that of flindersiamine (**4**).

FLINDERSIAMINE (**4**).—The compound gave a mp and mixture mp 207–208°; pmr and ir spectra were superimposable with those of an authentic specimen (1).

KOKUSAGININE (**6**).—Crystallized from methanol as colorless plates, mp 168–170° (lit. (10). mp 168–169), uv λmax (ε max): 246 (51,200), 253 (55,100), 298 sh (6,000), 309 (7,300) and nm (6,100); ir νmax 1625, 1560, 1088, 715 and 700 cm^{-1}; pmr δ: 4.02 (6H, s, 2 x OMe), 4.40 (3H, s, OMe), 6.98 (1H, d, J=2.5Hz, H-3), 7.27 (1H, s, H-8), 7.42 (1H, s, H-5) and 7.55 (1H, d, J=2.5Hz, H-2); ms, m/z (%): 259 (M^+ 100), 244 (12), 229 (14), 216 (29) and 173 (19). The isolate was identical in all respects (ir, uv, pmr and mp) with an authentic specimen.

MONTRIFOLINE (NKOLBISINE) (**5**).—Trituration of the combined fractions 140–147 in methanol with diethyl ether afforded a brown precipitate (152 mg) which was further purified by preparative tlc with chloroform-methanol (95:5). Crystallized from methanol-ethyl acetate, mp 190–191° [lit. (9) 191–193°]; uv λmax 245, 252, 298 sh, 310, 323 and 336 nm (ε max 38,600, 40,600, 8,200, 12,600, 12,600 and 8,400); ir νmax 3380, 3220, 3150, 3130, 1625, 1600, 1550, 1512, 1458, 1435, 1380, 1292, 1052 and 840 cm^{-1}; pmr (DMSO-d_6): δ 1.35 (6H, bs, 2 x OMe), 3.10 (1H, s, exchangeable with D_2O), 3.38 (1H, d, J=6Hz, disappears on deuteriation, OH), 3.65 (1H, m), 4.00 (3H, s, 7-OMe), 4.35 (2H, m), 4.42 (3H, s, 4-OMe), 7.02 (1H, d, J=2.5Hz), 7.33 (1H, s, H-6), 7.55 (1H, s, H-5), and 7.58 (1H, d, J=2.5Hz, H-5); ms, m/z (%): 347 (28, M^+) 288 (3), 246 (25), 245 (100), 244 (5), 230 (30), 59 (32) and 43 (25). For the ^{13}C nmr spectrum, see fig. 1.

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