NKOLBISINE[1], A NEW FUROQUINOLINE ALKALOID, AND 7-DEACETYLAZADIRONE FROM *TECLEA VERDOORNIANA*[2]

JOHNSON FOYERE AYAFOR

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

and

JOSEPH I. OKOGUN

Department of Chemistry, University of Ibadan, Ibadan, Nigeria

ABSTRACT.—A new furoquinoline alkaloid, nkolbisine, isolated from the chloroform extracts of the trunk bark of *Teclea verdoorniana* Exell & Mendonça, has been characterized as 6-(2,3-dihydroxy-3-methylbutyloxy)-7-methoxydictamnine (**1**) from spectral and chemical data. The limonoid, 7-deacetylazadirone (**8**), was also identified from the extracts and its structure confirmed by conversion to the known azadirone (**9**).

Previous phytochemical studies on *Teclea verdoorniana* Exell & Mendonça of Ghanaian origin have yielded mostly acridone alkaloids (1,2). Crude extracts of the stem bark of the sister species, *Teclea grandifolia* Engl., have been reported to show noteworthy activity against the KB cell culture (3,4). In the course of our own investigations on *T. verdoorniana* for possible active substances, we have already reported (5,6) the isolation and characterization of flindersiamine, kokusaginine and the new alkaloids tecleaverdoornine, tecleaverdine, and tecleine besides a new unidentified alkaloid. The latter has now been identified, and the present paper deals with details of its structural elucidation.

RESULTS AND DISCUSSION

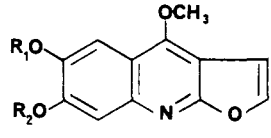
The basic fraction of the chloroform extract of the stem bark of *T. verdoorniana*, upon column chromatography, tlc and crystallization, afforded among other alkaloids (5,6), the new alkaloid nkolbisine (**1**), which recrystallized from ethylacetate-chloroform as colorless needles, mp 189–190°, $[\alpha]^{24}D - 16.5°$. The molecular formula, $C_{18}H_{21}NO_6$, was established independently by elemental analysis and high resolution mass spectrometry. Spectroscopic evidence, especially the ultraviolet and the proton magnetic resonance spectra, confirmed that the alkaloid was a 4-methoxyfuroquinoline. The 60 MHz nmr spectrum of nkolbisine in DMSO-d_6 showed two three-proton singlets at δ4.00 and δ4.42 attributable to two methoxyl groups; the lower field signal, δ4.42, was assigned to the methoxyl group at C–4 in accordance with chemical shift data recorded for alkaloids of this group (7). A pair of AB doublets ($J = 2.5$Hz) centered at δ7.02 and δ7.58 corresponded to the olefinic Hβ and Hα of the furan ring, respectively, and two singlets at δ7.33 and δ7.55, each integrating for one proton, were consistent with two isolated aromatic protons. The latter signals were well-resolved, and lack of splitting suggested that they were *para* to each other. Accordingly they were assigned to positions C–5 and C–8. In addition to these signals which are typical of 4-methoxyfuroquinolines, there were resonances for two equivalent C-methyls at δ1.35 (6H,bs), a singlet at δ3.10 (1H) which disappeared on deuteriation (tertiary OH), and another D_2O-exchangeable one-proton doublet ($J = 2$Hz) at δ3.38 (secondary aliphatic OH). A one-proton unresolved multiplet centered at δ3.65 coupled to a two-proton doublet centered at δ4.35 were attributable to an A_2X system due to $-OCH_2CH-$. This last evidence suggested the presence of a dihydroxylated isoprenyl side chain of the type found in evellerine (8) and evolatine

[1]While this paper was being edited, it was learned that nkolbisine corresponds to montrifoline, recently isolated and reported by J. Bhattacharyya and L. M. Serur, *Heterocycles*, **16**, 371 (1981).

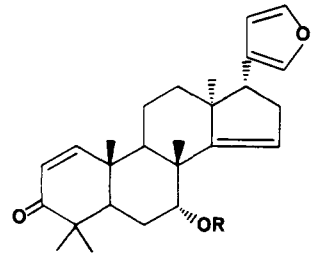
[2]Abstracted in part from the Ph.D. thesis of J.F.A., University of Ibadan, Nigeria (1978.)

(**2**) (9), both furoquinoline alkaloids obtained from plants of the family Rutaceae. Confirmation of the nature of this side chain was readily obtained from the high resolution mass spectrum of nkolbisine which showed in addition to the molecular ion peak, M^+ at m/z 347.1361 (28%) an ion fragment at m/z 245.0682 (M^+-102, 100%; $C_{11}H_{13}NO_4$) resulting from the elimination of the side chain, and the isolation of acetone as the 2,4-dinitrophenylhydrazone on periodate oxidation of nkolbisine.

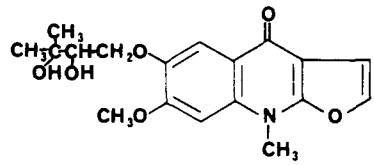
The relative positions of the substituents on the benzenoid ring were established by the following chemical transformations. Fusion of nkolbisine (**1**) with potassium hydroxide yielded an optically inactive phenol (**3**), $C_{13}H_{11}O_4N$, mp 242–244°, which on methylation formed a base which was identified as kokusaginine (**4**) (9) by direct comparison with an authentic sample. Of the two possible 6,7-hydroxymethoxydictamnines, only the 7-hydroxy-6-methoxydictamnine (**5**) (heliparvifoline), (10) is known. As the phenol was non-identical (ir, uv, mmp) with an authentic sample of heliparvifoline (**5**) on direct comparison, it must be 6-hydroxy-7-methoxydictamnine thus establishing structure **1** for nkolbisine.



1 R_1=$CH_2CH(OH)C(OH)(CH_3)_2$, R_2=CH_3
2 R_1=CH_3, R_2=$CH_2CH(OH)C(OH)(CH_3)_2$
3 R_1=H, R_2=CH_3
4 R_1=R_2=CH_3
5 R_1=CH_3, R_2=H
6 R_1=$CH_2COCH(CH_3)_2$, R_2=CH_3



8 R=H
9 R=$COCH_3$



7

More evidence for structure **1** was obtained from direct comparison of nkolbisine with an authentic sample of the isomeric evolatine (**2**) (9). Evolatine (**2**) depressed the melting point of nkolbisine (**1**) from 189–190° to 176°. The ultra-violet spectra of **1** and **2** were very similar, but their ir spectra differed mostly in the fingerprint region. Comparison of the physical properties (mp's) of some derivatives of nkolbisine with those reported (9) for the corresponding derivatives of evolatine showed that **1** and **2** were different compounds, thus supporting structure **1** for nkolbisine.

The structure **1** has also been confirmed by the following reactions modeled on those of evolatine (**2**) (9): on heating with methyl iodide under pressure, nkolbisine gave an optically active iso-compound (**7**), $C_{18}H_{21}O_6N$, mp 268–269°, $[\alpha]^{25}D-28°$ which rapidly turned pink on exposure to light (9). Also, nkolbisine underwent a pinacol-pinacolone change on heating with 20% aqueous hydrochloric acid. The product **6**, $C_{18}H_{19}O_5N$, showed a strong carbonyl band at νmax 1708 cm^{-1} but no hydroxyl absorption in the infra-red.

7-Deacetylazadirone (**8**), $C_{26}H_{34}O_3$, $[\alpha]D+10°$, was identified from its nmr spectral data, and its structure was confirmed by direct comparison with an authentic sample (12) and by acetylation to azadirone (**9**) (11).

EXPERIMENTAL[3]

PLANT MATERIAL.—The plant material, *Teclea verdoorniana* Exell & Mendonça (Rutaceae) (3 kg), was collected in Nkolbisong seven km from Yaounde, Cameroon, by Paul Misili of the National Herbarium, Yaounde, and its identity was confirmed by the Department of Forest Research, Ibadan, Nigeria, where a voucher specimen is kept.

EXTRACTION AND ISOLATION OF CONSTITUENTS.—The air-dried stem bark of *T. verdoorniana* was extracted successively with chloroform (7.5 liters) and methanol (7.5 liters) in a Soxhlet extractor. The concentrated chloroform extract (128 g) was dissolved in ethyl acetate and exhaustively extracted with 10% HCl. The aqueous solution was made alkaline with conc NH_3 and further extracted with chloroform. Evaporation of the chloroform solution gave the total alkaloid fraction (25 g) which was repeatedly chromatographed over silica gel. Elution with chloroform-methanol (8:2) furnished a gum which on further purification (prep. tlc on silica gel, eluent methylene chloride-methanol (9:1) and crystallization from ethyl acetate-chloroform (7:3) afforded nkolbisine (**1**) as colorless needles (400 mg). The neutral ethyl acetate extract resulting from the above extraction on concentration and chromatographic purification furnished *lupeol* and *7-deacetylazadirone* (**8**). Subsequent extractions were made on larger quantities of plant material from which more nkolbisine was obtained for the reactions described below.

PHYSICAL AND CHEMICAL PROPERTIES OF ISOLATED COMPOUNDS

NKOLBISINE.—Colorless needles, mp 189–190°, $[\alpha]^{24}D = -16.5°$ (MeOH, *c* 2.0) (Found: C, 62.01; H, 6.35; N, 4.23. $C_{18}H_{21}O_6N$ requires: C, 62.24; H, 6.10; N, 4.03%); uv λ max (EtOH) (ε): 245 (38800), 252 (41000), 298 sh (8500), 310 (13800) and 336 (9500) nm; ir νmax (KBr); 3380, 3220, 3150, 3130, 1625, 1600, 1550, 1512, 1458, 1435, 1380, 1292, 1255, 1210, 1170, 1155, 1100, 1078, 1052, 1020, 990, 980, 950, 930, 880, 840, 820, 790, 745, 735, 710, 700, 660, 630, and 575 cm^{-1}; ^1H nmr (DMSO-d_6): δ 1.35 (6H, bs), 3.10 (1H, s, exchangeable with D_2O), 3.38 (1H, d, *J*=6 Hz, disappears on deuteration), 3.65 (1H, m) 4.00 (3H, S), 4.35 (2H, m), 4.42 (3H, s) 7.02 (1H, d, *J*=2.5 Hz), 7.33 (1H, s) 7.55 (1H, s) and 7.58 (1H, d, *J*=2.5 Hz); ms *m/z* (%): 347.1361 (M^+, 28); calc. for $C_{18}H_{21}O_6N$: 347.1369; 288 (3), 246 (25), 245.0682 (100), 244 (5), 230 (30), 59 (32) and 43 (25).

PERIODATE OXIDATION OF NKOLBISINE (**1**).—Oxidation of nkolbisine (**1**) (300 mg) with periodic acid (0,1N, 20 ml) under the conditions used for the oxidation of evolatine (**9**) afforded acetone which was isolated as its crystalline 2,4-dinitrophenylhydrazone (190 mg), light orange prisms, mp 126°, from ethanol. The identity of the products was confirmed by comparison (ir, mmp) with an authentic sample of acetone 2,4-dinitrophenylhydrazone. Other products of the reaction were not characterized.

ALKALI FUSION OF NKOLBISINE.—Nkolbisine (500 mg) was heated in a melt of potassium hydroxide under the conditions described for the alkali fusion of evolatine (**9**). The melt was cooled, diluted with water, and extracted with chloroform to remove unreacted starting material. The aqueous solution was neutralized with acetic acid, and the precipitate was extracted with chloroform. The concentrated extract was filtered through a short column of silica gel, and the recovered product (**3**) was crystallized from methanol as cream colored plates (200 mg), mp 242–244°. (Found: C, 63.27; H, 4.91; N, 5.46. Calc. for $C_{13}H_{11}O_4N$: C, 63.70; H, 5.70%); uv λmax (MeOH) (ε): 246 (42200), 311 (9400), 324 (9000), 337 (6200) and 298 (sh) (6000) nm; λmax (MeOH+0.1N NaOH) 260 (35000) nm; ir νmax (nujol mulls): 3500–3000 (OH), 1618, 1580, 1370, 1325, 1290, 1267, 1205, 1190, (sh), 1158, 1088, 1050, 1016, 940, 873, 864, 854, and 806 cm^{-1}; ^1H nmr (DMSO-d_6): δ 3.95 (3H, s, OMe), 4.40 (3H, s, OMe), 7.25 (1H, s, H-8), 7.33 (1H, d, *J*=2,5 Hz, β-furan-H), 7.45 (1H, s, H-5), 7.90 (1H, d, *J*=2.5 Hz, α-furan-H) and 9.57 (1H, s, OH; disappeared upon addition of D_2O); ms *m/z* (%): 245 (M^+, 100), 230 (90), 202 (85), 187 (60) and 159 (50).

METHYLATION OF ALKALI FUSION PRODUCT (**3**).—An ethereal solution of diazomethane was slowly added to a cooled stirred solution of **3** (50 mg) in methanol (100 ml). After removal of the solvent, the residue was crystallized from methanol as white platelets (35 mg) of kokusaginine (**4**), mp 168—169° [lit. (9), 170–171°]. The mp was not depressed when the compound was admixed with a reference sample of kokusaginine (**4**); the uv, ir, ^1H nmr and ms were identical with those of **4**.

ISOMERIZATION OF NKOLBISINE WITH MeI: ISONKOLBISINE (**7**).—Nkolbisine (200 mg) and methyl iodide (5 ml) were heated in a sealed tube at 100° for 4 hr. The tube was cooled in liquid N_2 and broken. The product (180 mg) obtained after evaporation of excess MeI crystallized from aqueous methanol in colorless needles, mp 268–269° $[\alpha]^{25}D$ −28° (*c*, 2.5, MeOH), became pink on exposure to light. The crystals were dried in an oven at 120° for 8 hr before satisfactory analyses were obtained. (Found: C, 62.25; H, 6.02; N, 3.46%. Calc. for $C_{18}H_{21}O_6N$: C, 62.24; H, 6.10; N, 4.03%; uv λmax (MeOH) (εmax): 255 (48000), 264 (52000), 310 (8400), 330 (10500) and 343 (10500) nm; ir νmax (nujol mulls): 3500–3400, 1615, 1595, 1450, 1368, 1275, 1255, 1210, 1155, 1035, 975, 895 and 825 cm^{-1}; ^1H nmr (DMSO-d_6): δ 1.15 (6H, bs, 2 x C-Me).

[3]Melting points were determined on a Kofler block, and are uncorrected. Ir spectra were determined on a Perkin-Elmer 337 instrument, and uv spectra were recorded on a Beckmann model 25 spectrophotometer. ^1H nmr spectra were recorded on a Varian T 60 for $CDCl_3$ solutions unless otherwise stated. Ms were measured at 70 eV by direct inlet on a Hitachi-Perkin Elmer RMU 6 or AEI MS 9 machine.

3.97 (3H, s, =NMe), 4.00 (3H, s, OMe), 3.60–4.40 (5H, m, 2 x OH, C*H*–C*H*$_2$–OAr), 7.05 (1H, d, J=2.50 Hz, β-furan-H), 7.15 (1H, s, H-8), 7.74 (1H, d, J=2.5 Hz, α-furan-H) and 7.75 (1H, s, H-5); ms m/z (%): 347 (M^+, 35) 262 (30), 245 (100), 244 (10), 230 (15), 59 (43) and 43 (22).

ACTION OF DIL HCL ON NKOLBISINE (**1**).—A solution of nkolbisine (400 mg) in dil HCl (20%, 15 ml) was heated on a water-bath for 2 hr then poured into excess aqueous KOH solution. The solution was extracted with chloroform (4 x 20 ml). The chloroform extract, on evaporation, left a white solid. Recrystallization from aqueous ethanol gave colorless needles of (**6**), mp 126–127°, $[\alpha]^{25}D$=0° (EtOH) (Found: C, 64.98; H, 5.99; N, 3.92%. Calc. for $C_{18}H_{19}O_5N$: C, 65.50; H, 5.80; N, 4.30%); uv λmax (MeOH) (εmax): 247 sh (55000) 253 (56200), 298 (8900) 310 (12600), 322 (12600) and 334 (10500) nm; ir νmax (nujol mulls): 1708, 1600, 1580, 1500, 1370, 1310, 1250, 1215, 1170, 1154, 1125, 1080, 1055, 1040, 1020, 945, 850, 820 and 745 cm^{-1}; ^1H nmr ($CDCl_3$): 1.17 (6H, d, J=7 Hz, 2 x C-Me), 3.05 (1H, m, $(CH_3)_2CH$), 4.02 (3H, s, OMe), 4.40 (3H, s, OMe), 4.63 (2H, s, Ar–O–C*H*$_2$–C=O) 6.99 (1H, d, J=2.5 Hz, β-furan-H), 7.34 (1H, s, H-8), 7.54 (1H, s, H-5) and 7.55 (1H, d, J=2.5 Hz, α-furan-H); ms m/z (%): 329 (M^+, 70), 258 (100), 244 (90), 243 (75), 201 (60), 200 (65), 140 (80), 71 (90) and 43 (95).

The aqueous solution resulting from the above extraction on acidification and then purification gave a compound, mp 242–244°, identical in all respects with the alkali fusion product **3**.

7-DEACETYLAZADIRONE (**8**).—Compound **8** (180 mg) was obtained as granular crystals from methanol-hexane mp 203–205°, $[\alpha]^{21}D$+10° ($CHCl_3$ *c* 0.3); λmax ($CHCl_3$) 240 nm (8000); νmax (KBr) 3560 (OH), 1676 (cyclohexanone), and 870 (β-substituted furan) cm^{-1}; ^1H nmr: δ ($CDCl_3$): 0.75 (3H, s, C-Me), 1.05 (3H, s, C-Me), 1.12 (9H, s, 3 x Me), 2.10 (1H, bs disappeared on deuteriation, OH), 4.00 (1H, m, H-7), 5.06 (1H, m, H-15), 5.80 (1H, d, J=10 Hz, H-2), 6.25 (1H, bs, Hβ-furan), 7.08 (1H, d, J=10 Hz, H-1) and 7.32–7.20 (2H, m, H$_2$-α-furan (Found: C, 79.0; H, 8.50. $C_{26}H_{34}O_3$ requires C, 79.15; H, 8.70%).

AZADIRONE (**9**).—7-Deacetylazadirone (**8**) (50 mg) was treated in pyridine (3 ml) with acetic anhydride (3 ml) on a steam-bath for 1 hr. After the usual work up, the product, when purified by preparative tlc, gave azadirone (**9**) as a gum (36 mg). Its nmr data were identical with those reported (11) for azadirone.

ACKNOWLEDGMENTS

We thank Dr. W. C. Taylor (University of Sydney) for the sample of evolatine, Dr. G. A. Cordell (University of Illinois at the Medical Center) for that of heliparvifoline, and Dr. Wolff (Strasbourg) for exact mass determination. Microanalyses were done at the Microanalytical Laboratory, University of Ibadan, by Mr. Mowete. The research program was partially supported by the University of Yaounde research fund.

Received 22 July 1981

LITERATURE CITED

1. P. G. Waterman, *Phytochemistry*, **14**, 2092 (1975).
2. F. Fish, I. A. Meshal and P. G. Waterman, *J. Pharm. Pharmacol.*, **28**, 72P (1976).
3. F. D. Popp and D. P. Chakraborty, *J. Pharma. Sci.*, **53**, 968 (1964).
4. F. D. Popp, J. M. Wefer, D. P. Chakraborty, D. Rosen and A. C. Casey, *Planta Medica*, **16**, 343 (1968).
5. J. I. Okogun and J. F. Ayafor, *J.C.S. Chem. Comm.*, 652 (1977).
6. J. F. Ayafor, J. I. Okogun, *J. Chem. Soc. Perkin I*, (1981) (in press).
7. A. V. Robertson, *Austral. J. Chem.*, **16**, 451 (1963).
8. S. R. Johns, J. A. Lamberton and A. A. Sioumis, *Austral. J. Chem.*, **21**, 1897 (1968).
9. R. G. Gell, G. K. Hughes and E. Ritchie, *Austral. J. Chem.*, **8**, 114 (1955).
10. P. T. O. Chang, G. H. Aynilian, G. A. Cordell, M. Tin-Wa, H. H. S. Fong, R. E. Perdue and N. R. Farnsworth, *J. Pharm. Sci.*, **65**, 561 (1976).
11. D. Lavie and M. K. Jain, *Chem. Comm.*, 379 (1967).
12. J. F. Ayafor, B. L. Sondengam, J. D. Connolly and D. S. Rycroft, *J.C.S. Perkin I*, 1750, (1981).