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# LIMONOIDS OF *TECLEA OUABANGUIENSIS*

JOHNSON FOYERE AYAFOR,* BEIBAM LUCAS SONDENGAM, ANASTASIE NGO BILON,

*Department of Organic Chemistry, Faculty of Science, The University of Yaounde, Box 812, Yaounde, Cameroon*

and JOSEPH DONALD CONNOLLY

*Chemistry Department, The University Glasgow G12 8QQ, Scotland, U.K.*

ABSTRACT.—The stem bark of *Teclea ouabanguiensis* has yielded six triterpenoids, two of which are new. The known compounds were identified spectroscopically as 7-deacetylazadirone (**2**), 7-deacetylproceranone (**3**), tecleanin, (**4**), and lupeol. The novel compounds include the tetranortriterpenoids, 7-deacetoxy-7-oxoazadirone (**1**), and ouabanginone (**5**). The biogenetic significance of the presence of these limonoids in *T. ouabanguiensis* is discussed.

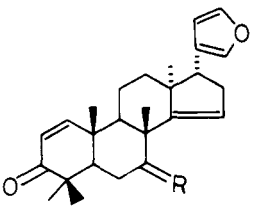
*Teclea ouabanguiensis* Aubrev. & Perr (Rutaceae) is a rare montane species of the genus *Teclea*, which has so far been located only in the uplands of North Cameroon (1). This rare plant is widely used by the local population as a remedy against cough and asthma. In a recent report from our laboratory (2) a novel alkaloid, tecleamine, and five known furoquinoline alkaloids were isolated from *T. ouabanguiensis*. In continuation of our work on this medicinal plant, we present the results of our investigations of the neutral constituents of its stem bark.

## RESULTS

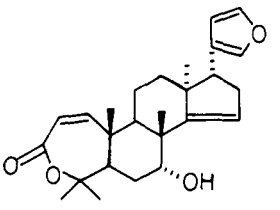
The $CHCl_3$ extract of the powdered stem bark of *T. ouabanguiensis* afforded six alkaloids and six neutral products upon successive chromatographies on silica gel and recrystallizations. The alkaloids have been reported previously (2). Two new tetranortriterpenoids, 7-deacetoxy-7-oxoazadirone (**1**) and ouabanginone (**5**), were characterized among the neutral compounds together with the known limonoids, 7-deacetylazadirone (**2**) (3), 7-deacetylproceranone (**3**) (4), and tecleanin (**4**) (4). The pentacyclic triterpene lupeol was also obtained. Whereas lupeol appears to be ubiquitous in the family Rutaceae, **2**, **3**, and **4** have so far been reported (4) only in the genus *Teclea* Delile and may constitute chemotaxonomic markers for this genus.

Ouabanginone (**5**) crystallized from MeOH/$Et_2O$ as colorless prisms, mp 269-270°, $[\alpha]^{25}D$ +35° (*C* 1, $CHCl_3$). Elemental analyses and the mass spectrum led to the molecular formula $C_{26}H_{34}O_5$ ($M^+$ at *m/z* 426), and significant fragment ions were observed at *m/z* 408 ($M^+$-$H_2O$), 411 ($M^+$-$CH_3$), and 331. The ir spectrum of **5** shows characteristic absorptions at νmax 3620 (free hydroxyl), 1725 (cyclopentanone), 1695 (enone), and 873 $cm^{-1}$ (β-substituted furan). Comparison of the $^{13}C$-nmr spectra of ouabanginone (**5**) and 7-deacetylproceranone (**3**) (Table 1) and of their 360 MHz $^1H$-nmr spectra revealed that ouabanginone lacked the $\Delta^{14}$ double bond, and had instead a 15-keto group of the type familiar from neotrichilenone and other limonoids (5). Aside from this, the structural elements are similiar in both compounds. Ouabanginone was thus provisionally assigned structure (**5**). Support for this structure was obtained from the mass spectral fragmentation of ouabanginone and a detailed analysis of its $^{13}C$-nmr spectrum in which all the carbons were assigned (Table 1). It is of interest to note that ouabanginone (**5**) is the first member of the neo-series of limonoids (5) to have an α,β-unsaturated 7-membered ring-A lactone. Since acid was used in the isolation process, **5** may be an artifact derived from the hypothetical epoxy-compound (**6**) by acid-induced isomerization of the epoxide (5). Confirmation of this proposal must however await the actual isolation of **6** from the extracts of *T. ouabanguiensis*.

The second new limonoid (**1**), $C_{26}H_{32}O_3$, mp 202-203°, ν max 1718 (cyc-



1 R=O
2 R=H, α-OH



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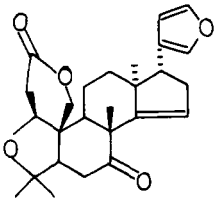

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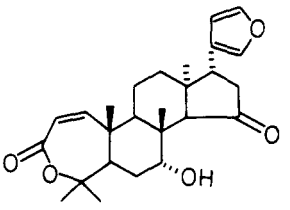

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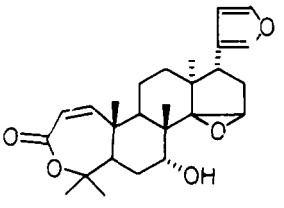

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lohexanone), 1678 (cyclohexanone), and 869 $cm^{-1}$ (Hβ-furan), was shown by its $^{13}C$-nmr spectrum (Table 1) and its $^{1}H$-nmr spectrum to be 7-deacetoxy-7-oxoazadirone (**1**), and its structure was confirmed by its partial synthesis from 7-deacetylazadirone (**2**) by oxidation with Jones' reagent.

## DISCUSSION

The occurrence of 7-deacetylazadirone (**2**) 7-deacetoxy-7-oxoazadirone (**1**), 7-deacetylproceranone (**3**), ouabanginone (**5**), and tecleanin (**4**) in *T. ouabanguiesis* is of possible biogenetic significance especially when we take into account the point put forward by Dreyer (6) that the family Rutaceae is poor at accumulating intermediates in limonoid biosynthesis. These five compounds, all from *T. ouabanguiensis*, as in the case of the limonoid constituents of *Teclea grandifolia* (6), contain the structural features which might be expected for intermediates in limonin biosynthesis (Scheme 1). The sequence of compounds involving expansion of the carbocyclic A-ring of 7-deacetylazadirone (**2**) or 7-deacetoxy-7-oxoazadirone (**1**) to a 7-membered δ-unsaturated lactone, as in deacetylproceranone (**3**), and finally oxidation of the 19-C methyl group and ring closure to give the limonin A- and A′-ring systems, as in tecleanin (**4**) are well represented, and may present circumstantial evidence for the proposed pathway.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected and were taken on a Kofler hot stage equipped with a microscope. Ir spectra were recorded on a Perkin-Elmer model 727B spectrometer, and the uv spectra, on a Beckman model 25 grating spectrophotometer. $^{1}H$-nmr measurements were made in $CDCl_3$ solutions, unless otherwise mentioned, on Perkin-Elmer R12 and R32 spectrometers, with TMS as an internal standard, and all the signals are reported as δ values. The $^{13}C$ spectra were de-

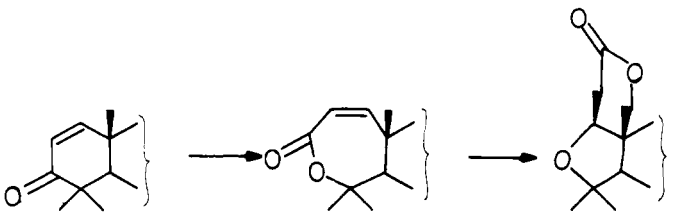
TABLE 1. $^{13}C$-nmr Spectral Data of 7-Deacetoxy-7-oxoazadirone (**1**),7-Deacetylproceranone (**3**), and Ouabanginone (**5**) Measured at 25.2 MHz in $CDCl_3$ Recorded in ppm Downfield from TMS

| Carbon | Compound | | |
|---|---|---|---|
| | **1** | **3** | **5** |
| 1 | 156.4 | 155.8 | 159.3 |
| 2 | 126.0[a] | 119.8[a] | 121.7 |
| 3 | 203.4 | 167.8 | 168.2 |
| 4 | 45.0 | 85.2 | 85.7 |
| 5 | 47.8 | 47.8 | 47.6 |
| 6 | 29.7 | 28.4 | 29.3 |
| 7 | 209.5 | 71.6 | 69.2 |
| 8 | 44.7[b] | 44.2 | 42.7 |
| 9 | 39.7 | 39.5 | 37.8 |
| 10 | 45.0[b] | 44.2 | 41.7 |
| 11 | 17.3 | 17.7 | 19.5 |
| 12 | 33.4 | 32.7 | 34.6 |
| 13 | 47.8 | 47.1 | 43.3 |
| 14 | 152.6 | 160.6 | 61.2 |
| 15 | 126.5[a] | 120.4[a] | 221.5 |
| 16 | 34.9 | 34.4 | 47.6 |
| 17 | 51.8 | 51.7 | 43.3 |
| 20 | 124.5 | 124.2 | 122.8 |
| 21 | 142.6 | 142.7 | 142.9 |
| 22 | 111.1 | 111.0 | 110.8 |
| 23 | 139.8 | 139.7 | 140.2 |
| CMe | 28.0 | 32.1 | 32.2 |
| | 26.7 | 26.4 | 27.7 |
| | 21.7 | 27.1 | 27.2 |
| | 21.0 | 20.7 | 18.1 |
| | 18.0 | 15.7 | 15.5 |

[a,b]These values may be interchanged.

termined in $CDCl_3$ solution (which also provided the lock signal) on a Varian XL-100 spectrometer with VFT-100 accessory, and the chemical shifts are reported in ppm downfield from TMS. Tlc of compounds was accomplished on Merck silica gel 60H, and the compounds were visualized by spraying with Ehrlich's Reagent. Column chromatography was conducted on Merck silica gel 60H-254 (70-230 mesh). The solvent system used for tlc and ptlc was $CHCl_3$-MeOH (98:2).

PLANT MATERIAL.—The plant material (stem bark, *T. ouabanguiensis*) used for this study was collected on Mount Vokré Poli (North Cameroon) in August 1981, by one of the authors (JFA) and Paul Misili of the National Herbarium, Yaounde. Voucher specimens documenting this collection are deposited in the National Herbarium, Yaounde.



SCHEME 1

EXTRACTION AND CHROMATOGRAPHIC SEPARATION.—The sun-dried, milled stem bark of *T. ouabanguiensis* (10 kg) was extracted, partitioned, and chromatographed as previously reported (2). The present study concerns the six uncharacterized neutral products reported in the previous study (2). The triterpenoids are presented in order of elution from the column.

LUPEOL.—Combined fractions 6-10 eluted with 10% $Et_2O/n$-$C_6H_{14}$ afforded copious amounts of a crude solid which crystallized from $C_6H_{14}$/EtOAc to give lupeol (26 g) mp 214-215° (4).

7-DEACETOXY-7-OXOAZADIRONE (**1**).—Recovered from fractions 12 and 13 crystallized from $CHCl_3/n$-$C_6H_{14}$ as colorless needles (128 mg) mp 202-203°; uv λ max (EtOH) 218 and 257 sh nm (12,000 and 3,600); ir ν max (KBr) 3145, 1718, 1678, 1460, 869 $cm^{-1}$; $^1H$ nmr δ 0.76 (3H, s, Me), 1.08 (3H, s, Me), 1.11 (3H, s, Me), 1.32 (3H, s, Me), 1.40 (3H, s, Me), 5.72 (1H, d, $J$=11 Hz, H-1), 6.00 (1H, t, $J$=3 Hz, H-15), 6.31 (1H, m, Hβ-furan), 7.07 (1H, d, $J$=11 Hz, H-2), 6.98 (1H, m, Hα-furan), and 7.38 (1H, m, H-α-furan); ms *m/z* (%) 392 ($M^+$, 27), 377 (16.7), 328 (21), 327 (100), 312 (54), 41 (15); Found: C, 79.22; H, 8.53. $C_{26}H_{32}O_3$ requires C, 79.55; H, 8.22%). The $^{13}C$-nmr spectral data are given in Table 1.

OXIDATION OF 7-DEACETYLAZADIRONE (**2**).—7-Deacetylazadirone (**2**) (100 mg) was dissolved in $Me_2CO$ (20 ml) and the solution cooled to 0°. Jones reagent (2 ml) was added; after 10 min. the reaction mixture was diluted with $H_2O$ (40 ml) and extracted with $CHCl_3$. The $CHCl_3$ solution was further washed successively with a saturated solution of NaCl and $H_2O$. The product, after recrystallization from hexane/$CHCl_3$, gave 7-deacetyl-7-oxoazadirone (1) (68 mg), identical in all respects (ir, uv, $^1H$ nmr, and mixed mp) with the natural sample.

7-DEACETYLAZADIRONE (**2**).—Compound (**2**) crystallized from MeOH/*n*-hexane as white granules (600 mg), mp and mixture mp 203-204°. Compound (**2**) had $^1H$ nmr and ir spectra superimposable with those of an authentic specimen (3).

7-DEACETYLPROCERANONE (**3**).—The combined fractions 110-115 on further purification by filtration through a short column of silica gel and crystallization from $CH_2Cl_2/n$-hexane afforded (3 g) of 7-deacetylproceranone (**3**), mp 168-170°; $[\alpha]^{22}D$ + 31° (c, 0.5 in $CHCl_3$); uv λ max (EtOH) 213 nm (16,000); ir ν max (KBr) 3560, 3140, 1698, 1460, 1340, 1300, 1260, 1115, 1080, 940, 870, 800, 600 $cm^-$; $^1H$ nmr δ 0.78 (3H, s, Me), 1.13 (3H, s, Me), 1.25 (3H, s, Me), 1.42 (6H, s, 2Me), 2.77 (1H, bs, exchanged with $D_2O$, 7-OH), 3.98 (1H, m, H-7), 5.54 (1H, t, $J$=3 Hz, H-15), 5.82 (1H, d, $J$=12 Hz, H-1), 6.26 (1H, m, Hβ-furan), 6.44 (1H, d, $J$=12 Hz, H-2), 7.35 (1H, m, Hα-furan), and 7.28 (1H, m, Hα-furan); ms *m/z* (%) 410 ($M^+$, 30), 377 (10), 315 (35), 270 (10), 257 (25), 138 (100), 131 (20), 119 (15).

TECLEANIN (**4**).—Preparative tlc separation of combined fractions 118-124 afforded an unidentified gum and tecleanin (**4**) (180 mg), which crystallized as needles from $CH_2Cl_2/n$ hexane, mp254-256°. The identity of tecleanin was confirmed as **4** by direct comparison (ir, uv, $^1H$ nmr, and mixed mp) with an authentic specimen obtained from *T. grandifolia*.

OUABANGINONE (**5**).—Combined fractions 130-135 eluted with $CHCl_3$-MeOH (98:2) was evaporated to give a brown gum (2.8 g). Tlc showed that this gum was a mixture of flindersiamine and a limonoid (Ehrlich's reagent positive). Repeated column chromatography on silica gel failed to resolve the mixture. The gum was finally dissolved in $CHCl_3$ and washed several times with 8% HCl. Repeated recrystallization in MeOH/$Et_2O$ afforded ouabanginone (**5**) as colorless prisms (75 mg) mp 269-270°; uv λ max (EtOH) 217 nm (18,000); ir ν max ($CCl_4$) 3620, 1725, 1695, 873 $cm^{-1}$; $^1H$ nmr ($CDCl_3$, 360 MHz δ 0.77 (3H, s, Me), 1.09 (3H, s, Me), 1.27 (3H, s, Me), 1.43 (6H, s, 2Me), 2.37 (1H, d, $J$=5 Hz, exchangeable with $D_2O$, OH), 2.50 (2H, d, $J$=10 Hz, 2H-16), 2.60 (1H, dd, $J$=14 and 3 Hz, H-9 or H-5), 2.70 (1H, s, H-14), 3.51 (1H, t, $J$=10 Hz, H-17), 3.86 (bs, 1H, H-7), 5.88 (1H, d, $J$=12 Hz, H-1), 6.28 (1H, bs, Hβ-furan), 6.53 (1H, d, $J$=12 Hz, H-2), 7.28 and 7.39 (both m, of 1H each, 2Hα-furan); ms *m/z* (%) 426 ($M^+$, 51), 411 (34), 393 (27), 344 (26), 331 (100), 245 (43), 107 (33), 95 (36), 91 (33), 81 (56), 43 (62), 41 (39); Found C, 72.98; H, 8.32. $C_{26}H_{34}O_5$ requires C, 73.21; H, 8,04%). The $^{13}C$-nmr spectral data are found in Table 1.

## ACKNOWLEDGMENTS

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