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LIMONOIDS FROM *CLAUSENA ANISATA*

BONAVENTURE TCHALEU NGADJUI,* JOHNSON FOYERE AYAFOR, BEIBAM LUCAS SONDENGAM,

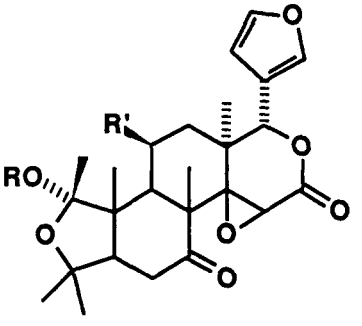
Department of Organic Chemistry, Faculty of Science, The University of Yaounde, Box 812, Yaounde, Cameroon

and JOSEPH DONALD CONNOLLY

Chemistry Department, The University, Glasgow G12 8QQ, Scotland, UK

ABSTRACT.—Five tetranortriterpenoids have been isolated from the stem bark and roots of *Clausena anisata*. The known compounds were identified spectroscopically as limonin, zapoterin [**5**], and clausenolide [**1**]. The novel compounds include clausenolide-1-ethyl ether [**2**] and clausenarin (11β-hydroxydeacetylnomilin) [**3**]. The isolation of these limonoids from *C. anisata* is of some chemotaxonomic interest.

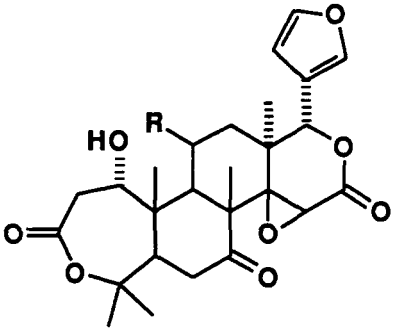
Clausena anisata (Will.) Hook. f. ex. Benth., a small tree widely distributed in West Africa, is a folkloric medicinal plant of the family Rutaceae (1). Previous work on this taxon has yielded mostly carbazole alkaloids (2,3) and coumarins (4,5). On the basis of taxonomic considerations, it became of interest to look for limonoids in *C. anisata*. We herein report the characterization of five limonoids from the combined stem bark and root extracts of this plant.



1 R=H, R′=OH
2 R=Et, R′=OH
6 R=Et, R′=OAc

RESULTS AND DISCUSSION

The finely powdered combined stem bark and root was extracted with petroleum ether and $CHCl_3$. Fractionation of the concentrated $CHCl_3$ extract on Si gel cc (see Experimental) yielded several fractions which after further preparative tlc and crystallization afforded five limonoids along with alkaloids (2) and coumarins (5). By means of spectroscopic data and direct comparison (ir, uv, ^{1}H- and ^{13}C-nmr spectra, and mmp) with authentic samples, three of the tetranortriterpenoids were identified as the known limonin (6), zapoterin [**5**], (7) and clausenolide [**1**] (8). The last two limonoids, for which the names clausenolide-1-ethyl ether [**2**] and clausenarin [**3**] are proposed, are new.



3 R=β-OH
4 R=H

Clausenolide-1-ethyl ether [**2**], $C_{27}H_{36}O_8$, mp 134–135° from Et_2O, $[\alpha]^{25}D$ −63.5°, had absorptions in the ir spectrum at ν max 1730 (δ-lactone),

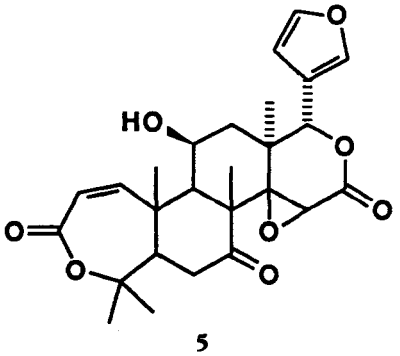


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TABLE 1. ^{1}H-nmr Assignments of Clausenolide [**1**], Clausenolide-1-ethyl ether [**2**], Clausenarin [**3**], and Zapoterin [**5**] Obtained at 90 MHz, δ TMS = 0 ppm.

Compound	H-1	H-2	H-11	H-12	H-15	H-17	H-α furan	H-β furan	Methyls	Others
Clausenolide[a] [**1**]	—	—	4.50 (m)	2.40 (m)	—	5.60 (br s)	7.40 (br s)	6.40 (br s)	1.00, 1.20, 1.35 1.40, 1.60, 1.50 (d, J = 2 Hz)	4.00 (br s, 11β-OH) 2.50 (br s, 1α-OH)
Clausenolide-1-ethyl ether [**2**]	—	—	4.55 (m)	2.30 (m)	4.03 (s)	5.58 (br s)	7.40 (br s)	6.37 (br s)	1.00 (t, J = 8 Hz) 1.10, 1.13, 1.20, 1.43, 1.46, 1.52 (d, J = 2 Hz)	4.40 (m, 11β-OH) 3.60 (2H, br t, J = 8 Hz, -OCH_2-)
Clausenarin[b] [**3**]	3.90 (m)	—	4.45 (m)	2.35 (m)	3.75 (s)	5.48 (br s)	7.64 (br s)	6.46 (s)	0.90, 1.25, 1.38, 1.45 (6H)	5.35 (d, J = 7 Hz, 11β-OH)
Zapoterin[b] [**5**]	6.82 (d, J = 12 Hz)	5.85 (d, J = 12 Hz)	4.50 (m)	2.15 (m)	3.75 (s)	5.50 (br s)	7.65 (br s)	6.47 (s)	0.95, 1.32, 1.41 1.43, 1.67	5.00 (d, J = 5 Hz, 11β-OH)

[a]Data obtained from Chakraborty *et al.* (8).
[b]Measured in DMSO-d_6.

1700 (cyclohexanone), and 870 cm^{-1} (β-substituted furan). The ^1H nmr of **2** (Table 1) proved informative and was almost superimposable with that of clausenolide [**1**] (Table 1) except for the appearance in the spectrum of **2** of two additional resonances at δ 1.00 (3H, t, $J = 8$ Hz) and 3.60 (2H, poorly resolved t, $J = 8$ Hz) which pointed to an ethyl ether function. The above data were, thus, compatible with a clausenolide ethyl ether for compound **2**. The position of etherification had to be determined, as there are two hydroxyl groups (the tertiary C-1 and the secondary C-11) in clausenolide [**1**]. Mild acetylation of compound **2** with cold Ac_2O-pyridine (1:1) readily afforded a monoacetate **6**, thus ruling out the presence of a tertiary hydroxyl group in **2**, which would not have undergone acetylation under such mild conditions. In the ^1H-nmr spectrum of **6** the acetate methyl group appeared at δ 2.04 while the acetoxymethine proton resonated at δ 5.60 compared to δ 4.45 in **2**. Compound **2** is, thus, clausenolide-1-ethyl ether.

The second new limonoid, clausenarin [**3**], $C_{26}H_{32}O_9$ ($[M]^+$ at *m/z* 488), $[\alpha]^{25}D$ −87.5°, ν max 3480–3420 (hydroxyls), 1730 (δ-lactone), 1710 (cyclohexanone) and 880 cm^{-1} (β-substituted furan), had resonances in its ^1H-nmr spectrum for five tertiary methyl groups [δ 0.90, 1.25, 1.38, and 1.45 (6H)], an oxymethine proton with appearance and chemical shift (δ 3.90, m) reminiscent of the proton on C-1 of deacetylnomilin [**4**] (9), an epoxide (δ 3.75), and the characteristic β-substituted furan (δ 6.46 and 7.64). In addition, two other protons, attributable respectively to an oxymethine proton (δ 4.45, m) and a free hydroxyl group [δ 5.35 (1H, d, $J = 7$ Hz, exchangeable with D_2O)], were clearly discernible in the ^1H nmr spectrum. All the above data are consistent with deacetylnomilin [**4**] hydroxylated in ring C. This proposed structure was supported by comparison of ^{13}C-nmr spectra (Table 2) of **3** and **4**. The SFORD (single frequency off-resonance decoupled) spectra of both compounds showed almost identical chemical shifts except for one major difference: the replacement of a methylene group in **4** by an oxygenated methine carbon (δ C 64.6) in **3**, again pointing to hydroxylation of deacetylnomilin [**4**]. Biogenetic considerations [C-12 hydroxylation is rare in Rutaceae limonoids (10)], comparison of the multiplicities and chemical shifts of the oxymethine proton and the 11-hydroxyl in the ^1H-nmr spectra of **3** and zapoterin [**5**] (7), and the co-occurrence of clausenarin with zapoterin [**5**], clausenolide [**1**], and clausenolide-1-ethyl ether [**2**] finally led to structure **3**, 11β-hydroxydeacetylnomilin, for clausenarin.

In no previous study have limonoids been reported from this species, although clausenolide [**1**] has been characterized from *Clausena heptaphylla* (8). Our results are in accord with Dreyer's rule that for any limonoid-producing genus all species of that genus will contain limonoids (10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—These have been previously reported (5).

PLANT MATERIAL.—The plant material (combined stem bark and root of *C. anisata*) used for this study was collected from Oku, about 180 km from Bamenda, North-West Province of Cameroon in April 1987 by B. Mpom of the National Herbarium, Yaounde. Voucher specimens documenting this collection are deposited in the National Herbarium, Yaounde.

EXTRACTION AND CHROMATOGRAPHY.—The finely powdered sun-dried plant material (8 kg) was extracted in a Soxhlet extractor with petroleum ether (40°–60°) (13 liters) followed by $CHCl_3$ (12 liters). After removal of the solvent, dark green residues (70 g and 50 g, respectively) were obtained. A sample (40 g) of the $CHCl_3$ extract was chromatographed in a Si gel (600 g) column. Elution started with petroleum ether and continued stepwise through petroleum ether/EtOAc mixtures, EtOAc, and EtOAc/MeOH mixtures. Fractions were combined on the basis of tlc comparison with an appropriate solvent system. A total of 140 fractions were collected.

TABLE 2. ^{13}C-nmr[a] Assignments of Clausenolide [**1**], Clausenolide-1-ethyl ether [**2**], Clausenarin [**3**], Deacetylnomilin [**4**], and Zapoterin [**5**] Obtained at 25.2 MHz, δ TMS = 0 ppm.

Carbon	Compound				
	1[b]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
1	105.9	108.1	68.9	68.5	156.6
2			43.6	39.0	120.6
3			171.1	170.6	167.3
4	79.6	79.8	84.1	83.7	83.7
5	55.7	55.6	49.8	49.3	55.2
6	43.1	43.2	41.5	39.2	43.0
7	208.5	208.5	208.6	208.4	207.8
8	49.8	50.5	45.1	52.0	43.4
9	45.1	44.6	46.0	43.6	49.5
10	50.6	50.7	51.2	44.3	50.9
11	66.4	66.8	64.6	16.8	65.5
12	36.9	37.0	37.1	31.2	37.2
13	36.8	37.3	35.8	36.9	35.8
14	65.6	65.9	64.9	65.8	64.5
15	54.0	54.4	53.2	52.9	53.1
16	167.9	167.6	167.8	167.0	166.5
17	78.3	78.3	78.1	77.6	77.5
20	120.0	120.2	120.4	120.3	120.1
21	141.0	141.1	141.8	141.3	141.6
22	109.7	109.8	110.5	110.1	110.2
23	142.9	143.0	143.7	143.1	143.4
C-Me	31.7, 23.0	30.9, 23.2	33.4, 23.2	32.9, 23.3	31.6, 26.2
	19.9, 19.2	20.1, 19.1	20.0, 18.8	20.1, 16.2	19.7, 19.2
	18.4, 16.7	18.5, 16.9	17.7	16.1	18.0
		15.3			
CH_2O		55.6			

[a]Assignments are based on chemical shifts rules, multiplicities on off-resonance decoupled spectra, and comparison with published data on similar compounds (9,11).

[b]Measured in $CDCl_3$.

[c]Measured in DMSO-d_6.

ISOLATION AND CHARACTERIZATION OF LIMONOIDS.—From the chromatographic separation above, and in some cases with the aid of successive preparative tlc, a total of ten coumarins (5), six alkaloids (2), and five limonoids were obtained. The present study concerns the five uncharacterized limonoids, which are presented in order of elution from the column.

LIMONIN.—The residue from combined fractions 38–46, on treatment with $CHCl_3$/MeOH, crystallized as colorless needles (25 mg): mp 295–296° [lit (6) mp 298°]; $[\alpha]^{25}D$ −122° (c = 1.05, Me_2CO); isolate was identical (ir, ms, tlc, ^{1}H and ^{13}C nmr) with an authentic sample obtained previously in our laboratory from *Vepris louisii* (6).

CLAUSENOLIDE-1-ETHYL ETHER [**2**].—The combined fractions 57–72 eluted with 35% EtOAc/petroleum ether afforded a dark brown oil (2.8 g). Tlc showed that this oil was a mixture of two compounds. Ehrlich's reagent test on the mixture was positive for limonoids. Preparative tlc with $CHCl_3$-MeOH (95:5) and crystallization from Et_2O gave clausenolide-1-ethyl ether [**2**] as colorless plates (200 mg): mp 134–135°; $[\alpha]^{25}D$ −63.5° (c = 1.02, $CHCl_3$); uv λ max EtOH (log ε) 220 (3.70), 275 (3.58) nm; ir ν max ($CHCl_3$) 3600, 1730, 1700, 1590, 1495, 1450, 1385, 1370, 1340, 1285, 1270, 1160, 1145, 1070, 1025, 1000, 910, 870, 835 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ (absent), 443 (10), 365 (15), 319 (20), 277 (40), 217 (16), 125 (14), 123 (15), 97 (20), 96 (12), 95 (32), 91 (18), 83 (13), 79 (15); found C 66.50, H 7.38, $C_{27}H_{36}O_8$ requires C 66.37, H 7.43%.

ACETYLATION OF CLAUSENOLIDE-1-ETHYL ETHER [**2**].—Compound **2** (20 mg) in a mixture of Ac_2O (2 ml) and pyridine (1 ml) was allowed to stand overnight at room temperature. The usual workup afforded the monoacetate **6** (15 mg) which was purified on preparative tlc to give a pale yellow oil: ir ν max ($CHCl_3$) 1745, 1730, 1700, 1580, 1500, 1420, 875, 815 cm^{-1}; ^{1}H

nmr (60 MHz, $CDCl_3$) δ 1.10, 1.15, 1.22, 1.40, 1.45 (each 3H, s, 5 × Me), 1.50 (3H, d, $J = 2$ Hz), 1.0 (3H, t, $J = 8$ Hz, $MeCH_2O$), 2.30 (2H, m, H-12), 3.60 (2H, br t, $J = 8$ Hz, $MeCH_2O$), 4.05 (1H, s, H-15), 5.58 (1H, br s, H-17), 5.60 (1H, m, H-11), 6.40 (1H, br s, β-furan proton), 7.41 (2H, br s, α-furan protons).

ZAPOTERIN (11β-HYDROXYOBACUNONE) [**5**] (7).—Fractions 80–87 containing mainly zapoterin [**5**] were rechromatographed on a short column of Si gel, and crystallization from a mixture of DMSO/H_2O gave colorless granules (15 mg) of 11β-hydroxyobacunone [**5**]: mp 275–276°; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

CLAUSENOLIDE [**1**] (8).—The residue from combined fractions 90–100, on treatment with Et_2O, yielded a white powder (60 mg). Recrystallization from a mixture of Me_2CO/C_6H_6 afforded an analytical sample: mp 147–148° [lit. (8) mp 150°]; uv λ max EtOH (log ε) 215 (4.0), 275 (3.8) nm; ir ν max ($CHCl_3$) 3510–3480, 1730, 1700, 1530, 1300, 1250, 870, 810 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

CLAUSENARIN (11β-HYDROXYDEACETYLNOMILIN) [**3**].—Purification of the combined fractions 110–122 by preparative tlc with $CHCl_3$-MeOH (9:1) and crystallization from a mixture of DMSO/H_2O afforded colorless plates (30 mg): mp 293–294°; $[\alpha]^{25}D$ −87.5° ($c = 1.04$, Me_2CO); uv λ max EtOH (log ε) 220 (4.25) nm; ir ν max (KBr) 3480–3420, 1730, 1710, 1500, 1460, 1400, 1280, 1210, 1165, 1120, 1030, 980, 880, 810 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 488 (1), 413 (3), 365 (8), 323 (10), 305 (23), 277 (10), 241 (9), 217 (14), 199 (15), 185 (13), 175 (12), 173 (15), 159 (16), 147 (14), 135 (13), 128 (10), 123 (20), 95 (50), 43 (100); found C 63.80, H 6.50, $C_{26}H_{32}O_9$ requires C 63.92, H 6.60%.

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