

GOSSWEILONE: A NEW PODOCARPANE DERIVATIVE FROM THE STEM BARK OF *DRYPETES GOSSWEILERI* (EUPHORBIACEAE)

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ABSTRACT. A new podocarpane diterpenoid, named gossweilone (**1**), has been isolated from the stem bark of *Drypetes gossweileri*, along with two known friedelane triterpenoids. The structure of the new compound was elucidated using spectroscopic methods.

KEY WORDS: Gossweilone, Podocarpane diterpenoid, *Drypetes gossweileri*, Euphorbiaceae

INTRODUCTION

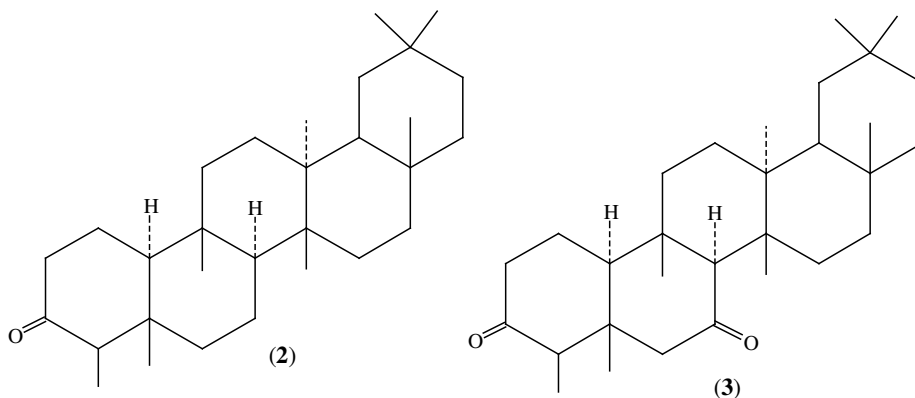
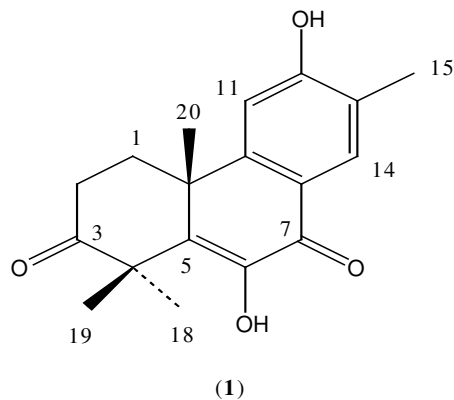
Drypetes gossweileri S. Moore (Euphorbiaceae), a large tree, is one of the many species of the genus *Drypetes* encountered in Africa. In Cameroon more than 20 species have been identified and are used by traditional healers for the treatment of many diseases [1-4], including toothache, dysentery, gonorrhoea, coryza, sinusitis, boils and swellings. Previous studies [5] on *D. gossweileri* reported the isolation of steroid and triterpenoid constituents and the toxicity of the stem bark extract to the mice. Our investigations on the methanolic extract of the stem bark of this species indicated cytotoxic and DNA damaging activities [6]. Chemical studies of the extract led to the isolation and structural elucidation of a new podocarpane diterpenoid, gossweilone (**1**), together with two known friedelane triterpenoids (**2**) and (**3**) [7, 8].

RESULTS AND DISCUSSION

Air-dried powdered stem bark of *Drypetes gossweileri* was extracted at room temperature with MeOH. The extract was concentrated to dryness *in vacuo*. Repeated column chromatography of the EtOAc-soluble portion of this MeOH extract resulted in the isolation of a new podocarpane diterpenoid, named gossweilone (**1**), together with the two known triterpenoids, friedelin (**2**) and friedelane-3,7-dione (**3**).

Compound **1**, gossweilone, m.p. 189-190 °C, was obtained as a yellow crystalline solid, and gave a positive FeCl₃ test. The molecular formula C₁₈H₂₀O₄, deduced from the ¹³C NMR spectrum (see below) and confirmed by an [M-H]⁺ ion at *m/z* 299 in the CI mass spectrum, has nine double bond equivalents. The ¹H NMR spectrum of **1** revealed the presence of three tertiary methyl groups [δ_{H} 1.24 (3H, s) and 1.58 (6H, s)], two aromatic protons [δ_{H} 6.88, 7.90 (both s)] and one aromatic methyl group [δ_{H} 2.30 (3H, s)]. The ¹³C NMR and DEPT spectra of **1** showed two carbonyl carbons at δ_{C} 217.6 and 181.4 and eight sp² carbons at δ_{C} 112.5, 121.7, 126.8, 130.5, 138.7, 144.5, 152.9 and 162.7. Other signals observed include two methylenes, two methines and two quaternary carbons (Table 1). Thus, **1** is a tricyclic bisnorditerpenoid, including an aromatic ring, two carbonyl groups and an additional tetrasubstituted double bond. The appearance of only two aromatic proton signals in the ¹H NMR spectrum suggested that the benzene ring is tetrasubstituted, bearing the methyl group at δ 2.30 and one hydroxyl group.

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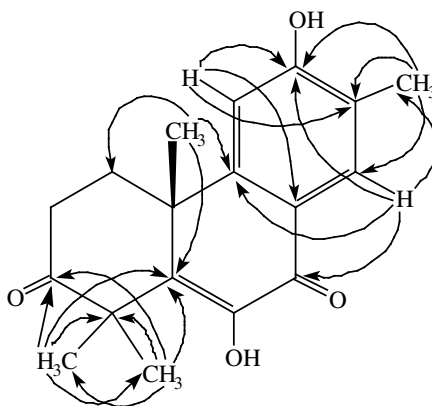


The location of the different groups on the tricyclic skeleton was deduced from the ^1H and 2D-NMR spectral data. The ^1H NMR spectrum showed the presence of two adjacent methylene groups [δ_{H} 2.89 (ddd, $J = 19.0, 9.7, 1.5$ Hz), 2.74 (dt, $J = 19.0, 9.4$ Hz), 2.50 (ddd, $J = 13.8, 9.3, 1.5$ Hz) and 2.07 (dt, $J = 13.8, 9.7$ Hz)] (Table 1), one of which is α to a carbonyl group. In the HMBC spectrum (Figure 1), the protons of Me-19 at δ_{H} 1.58 showed long-range correlations with the carbon signals at δ_{C} 271.8 (C-3), 49.0 (C-4), 138.7 (C-5) and 21.8 (C-18) while the protons of Me-20 at δ_{H} 1.24 showed long-range correlations with the carbon signals at δ_{C} 34.5 (C-1), 138.7 (C-5) and 41.0 (C-10). These correlations confirm the structure of ring A as **1**. Me-20 also showed a long-range correlation with C-9 (δ_{C} 152.9) while the aromatic proton at δ_{H} 6.88 (s, H-11) on ring C showed a long-range correlation with C-10 (δ_{C} 41.0). The other aromatic proton at δ_{H} 7.90 (s, H-14) showed long-range correlations with C-8 (δ_{C} 121.7), C-12 (δ_{C} 162.7), C-13 (δ_{C} 126.8), C-15 (δ_{C} 16.3) and C-7 (δ_{C} 181.4). This evidence enabled us to deduce the structure of rings B and C. Thus compound **1** is the new podocarpane derivative, 6,12-dihydroxy-13-methylpodocarpa-5,8,11,13-tetraene-3,7-dione. Podocarpane diterpenoids have also been isolated from *D. littoralis* [9].

In addition to **1**, two known triterpenoids were also obtained and identified as the friedelane (2) and friedelane-3,7-dione (3) [7, 8].

Table 1. ^{13}C (125 MHz) and ^1H (400 MHz) NMR chemical shift (δ) assignments for gossweilone (**1**).

Attribution	^{13}C (CD_3OD) (m)	^1H (CD_3OD), (m) J (Hz)
1	34.5 (t)	2.89 (ddd, 19.0, 9.7, 1.5); 2.74 (dt, 19.0, 9.4)
2	34.1 (t)	2.50 (ddd, 13.8, 9.3, 1.5); 2.07 (dt, 13.8, 9.7)
3	217.8 (s)	-
4	49.0 (s)	-
5	138.7 (s)	-
6	144.5 (s)	-
7	181.4 (s)	-
8	121.7 (s)	-
9	152.9 (s)	-
10	41.0 (s)	-
11	112.5 (d)	-
12	162.7 (s)	-
13	126.8 (s)	-
14	130.5 (d)	-
15	27.1 (q)	1.58 (s)
18	21.8 (q)	1.58 (s)
19	25.2 (q)	1.24 (s)
20	16.3 (q)	2.30 (s)

Figure 1. Significant long-range correlations observed in ^{13}C - ^1H HMBC for compound **1**.

EXPERIMENTAL

General. All melting points were recorded with a Reichert microscope and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in CD_3OD or in CDCl_3 using a Bruker 400 AMX spectrometer. The chemical shifts (δ) are reported in ppm with the solvent signals, (δ_{H} 7.25 and δ_{C} 77.0 for CDCl_3 or δ_{H} 3.30 and δ_{C} 49.0 for CD_3OD) as reference, while coupling constants (J) are given in Hz. HMQC and HMBC experiments were recorded with gradient enhancement using standard Bruker programmes. Mass spectra (CIMS) were recorded by direct inlet at 70 eV. Column chromatography was run on Merck Silica gel 60. TLC analyses were carried out on Silica gel GF₂₅₄ pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C.

Plant material. The stem bark of *D. gossweileri* was collected from Bankomo, Central Province, Cameroon in November 2001 by Dr Zapfack of the University of Yaoundé I, who authenticated the voucher specimens (N°32811 NHC) that have been deposited in the National Herbarium, Yaoundé, Cameroon.

Extraction, isolation and characterization. Air-dried powdered stem bark (6 kg) was extracted with MeOH (10 L x 3) at room temperature. After the removal of the solvent by concentration under reduce pressure, the methanolic residue (80 g) was triturated successively with hexane and EtOAc to give a hexane-soluble fraction (3 g) and an EtOAc-soluble fraction (10 g). The hexane and EtOAc extracts were qualitatively very similar on TLC analysis and were thus combined. The whole combined extract (13 g) was subjected to flash chromatography on Silica gel 60 using mixture of hexane-EtOAc to furnish five fractions: A (1 g), B (2.5 g), C (1 g), D (1.5 g) and E (2 g). Fraction B (2.5 g) was subjected to column chromatography using the same eluents to yield compounds **2** (25 mg) and **3** (80 mg). Further column chromatography of fraction D (1.5 g) using a hexane-EtOAc mixture afforded compound **1** (20 mg).

6,12-Dihydroxy-13-methylpodocarpa-5,8,11,13-tetraene-3,7-dione (1). R_f 0.6 [(EtOAc-hexane (3:7)]; m.p. 189-190 °C; ^1H NMR (CD_3OD) see Table 1; ^{13}C NMR (CD_3OD) see Table 1; HMBC data (CD_3OD): H-11 to C-10, C-12, C-13 and C-8; H-14 to C-7, C-12, C-9 and C-18, Me-15 to C-13, C-12 and C-14, Me-18 and Me-19 to C-4, C-5 and C-3, Me-19 to C-18, Me-18 to C-19; CIMS (70 eV) m/z (rel. int.) $[\text{M}-\text{H}]^+$ 299 (100), 284 (37), 269 (17).

Friedelin (2). White powder; m.p. 215-216 °C; ^{13}C -NMR spectral data were in agreement with those reported by Mahato and Kundu [8].

Friedelane-3,7-dione (3). White powder; m.p. 286 °C; ^{13}C -NMR spectral data were in agreement with those reported by Mahato and Kundu [8].

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REFERENCES

1. Dalziel, J.M. *The Useful Plants of West Tropical Africa*, Crown Agents for the Colonies: London; **1937**; p 140.
2. Irvine, R.F. *Woody Plants of Ghana*, Oxford University Press: London; **1961**; p 223.
3. Bouquet, A. *Féticheurs et Médecine du Congo*, Mémoires O.R.S.T.O.M. (Ed.); O.R.S.T.O.M.: Paris; **1969**; p 113.
4. Bouquet, A.; Debray, L. *Plantes Médicinales de la Côte d'Ivoire*, Travaux et documents de l'O.R.S.T.O.M. (Ed.); O.R.S.T.O.M.: Paris; **1974**; p 83.
5. Dupont, M.P.; Llabrés, G.; Delaude, C.; Tchissambou, L.; Gastmans, J.P. *Planta Medica* **1997**, 63, 282.
6. Nougoue, D.; Ngoupayo, J.; Lenta, N.B.; Ngouela, S.; Tsamo, E.; Tanvir, N.; Aziz, N.; Khan, M.T.H. *Bangladesh J. Integr. Med.* **2000**, 6, 19.
7. Patra, A.; Chaudhuri, S.K. *Magn. Reson. Chem.* **1987**, 25, 95.
8. Mahato, S.B.; Kundu, A.P. *Phytochemistry* **1994**, 37, 1517.
9. Lin, M.T.; Chen, L.C.; Chen, C.K.; Liu, K.C.S.C.; Lee, S.S. *J. Nat. Prod.* **2001**, 64, 707.