Notes

7α,20(*S*)-Dihydroxy-4,24(28)-ergostadien-3-one from *Entandrophragma utile*

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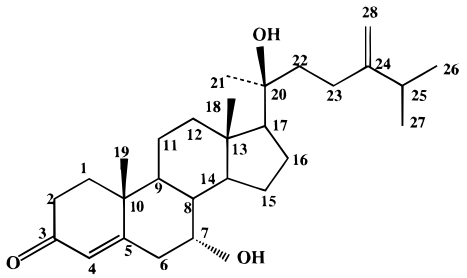
A new sterol, 7α,20(*S*)-dihydroxy-4,24(28)-ergostadien-3-one (**1**), has been isolated from the bark of *Entandrophragma utile* and its structure elucidated by ^{1}H- and ^{13}C-NMR spectroscopic methods.

Previous work on the bark of *Entandrophragma utile* Sprague (Meliaceae) has resulted in the isolation of the modified limonoids entilins A and B[1] and two ergostane derivatives.[2,3] In this paper, we describe the isolation of another new ergostane derivative, 7α,20(*S*)-dihydroxy-4,24(28)-ergostadien-3-one (**1**) from the same source. The structure and relative stereochemistry of compound **1** are based on ^{1}H- and ^{13}C-NMR studies and on NOE difference experiments.

Compound **1** was isolated as a crystalline solid that



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analyzed for $C_{28}H_{44}O_3$. CIMS gave peaks at *m/z* 410 and 390 due to successive losses of two molecules of H_2O from the molecular ion at *m/z* 428, indicating the presence of two hydroxyl groups. Fully decoupled ^{13}C-NMR and DEPT NMR spectra of **1** exhibited 28 carbon signals, consisting of five methyls, 10 methylenes, seven methines, and six quaternary carbons. The ^{1}H-NMR spectrum of **1** confirmed the presence of five methyl groups, with three tertiary methyl groups at δ_H 1.28, 1.16, and 0.88 and two secondary methyl groups at δ_H 1.00 (6H, d, J = 6.8 Hz), and revealed, in addition, an exomethylene group at δ_H 4.64 (1H, dt, J = 1.4, 1.2 Hz) and δ_H 4.70 (1H, t, J = 1.2 Hz). Other functionalities that were apparent from the ^{1}H- and ^{13}C-NMR spectral data of **1** included an α,β-unsaturated enone system with a trisubstituted double bond [δ_H 5.76 (d, J = 2 Hz); δ_C 199.0 (s), 168.1 (s), and 128.7 (d)] and two hydroxylated carbons, one tertiary at δ_C 70.5 (s) and one secondary at δ_C 68.2 (d), bearing an α-hydroxyl group[2] at δ_H 3.96 (br s). The above features are in agreement with a tetracyclic steroidal skeleton for **1**.

The presence of an exomethylene group as described above, plus a septet at δ_H 2.20 (1H, J = 6.8 Hz) showing a weak long-range coupling and two secondary methyl groups at δ_H 1.00 (6H, J = 6.8 Hz) in the ^{1}H-NMR spectrum of **1**, suggested a 24-methylene steroidal side chain. The lack of a further secondary methyl group and the presence of a tertiary methyl at δ_C 26.3 ppm suggested the attachment of one of the hydroxyl groups to C-20. The comparison of the ^{13}C-NMR chemical shifts of **1** with those of 3β,7α,20(*S*)-trihydroxy-5,24(28)-ergostadiene[3] revealed closely comparable data for the side chain and rings C and D, thus confirming the above inference. The 20*S* configuration was deduced from the ^{1}H-NMR chemical shift of Me-21 at δ_H 1.28.[3−5] Two decoupling experiments allowed the location of the axial hydroxyl group at C-7 of **1**. Thus, the irradiation of H-7 at δ_H 3.96 resulted in the loss of small couplings from H-6α at δ_H 2.39 (dd, J = 14.9, 3.3 Hz) and H-6β at δ_H 2.60 (ddd, J = 14.9, 3.1, 2.0 Hz) and from H-8 at δ_H 1.60 (obscured). Likewise, the irradiation of H-4 at δ_H 5.76 cancelled a 2 Hz coupling from H-6β. Moreover, NOE difference experiments showed signal enhancements for both H-6α and H-6β by ca. 4% by the irradiation of H-7 and an enhancement of the H-6α signal by 14% on irradiating H-4. These results are consistent with the presence of a Δ^4-3-ketone functionality with a 7α-hydroxyl group.

The ^{13}C-NMR assignments of **1** were based largely on 2D direct and long-range δ_C/δ_H correlation data. Many unambiguous assignments were made on the basis of longe-range correlations of the methyl protons. Such experiments may be exemplified by C-17 at δ_C 57.5 showing a correlation with both Me-18 and Me-21 while C-14 at δ_C 50.5 and C-9 at δ_C 44.9 correlated only with Me-18 and Me-19, respectively. The carbon doublet at δ_C 39.0 was assigned to C-8. An analysis of the H-4 correlation network helped in the assignment of C-6 at δ_C 41.0 and C-10 at δ_C 38.4, respectively, while the remaining signals were assigned by comparison with published data.[3,4]

From an analysis of all of the above data, the

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structure of compound **1** was established as 7α,20(*S*)-dihydroxy-4,24(28)-ergostadien-3-one.

Experimental Section

General Experimental Procedures. The NMR spectra were recorded at 300.13 MHz (Bruker MSL 300) for the ^{1}H NMR and 50.32 MHz for ^{13}C NMR. Chemical shifts are given on the δ (ppm) scale with TMS as internal standard. Mass spectra were obtained on a Nermag 10-10-C mass spectrometer at 70 eV. Melting point determination was performed on a Buchi-Tottoli apparatus using capillary tubes.

Plant Material. *E. utile* was collected at Awae near Akonolinga, in southern Cameroon, in January 1987. A voucher specimen (No. 29032) is deposited at the National Herbarium, Yaoundé.

Extraction and Isolation. The air-dried and finely powdered stem bark of *E. utile* (10 kg) was extracted with hexane (15 L) at room temperature. The defatted material was then extracted with $CHCl_3$ (15 L) at room temperature. After filtration and concentration of the solvent, the $CHCl_3$ extract (syrup, 60 g) obtained was chromatographed over a Si gel 60 (70−230, mesh) column using hexane−EtOAc mixtures of increasing polarity and collecting 200 mL fractions. Fractions enriched with **1** were obtained upon elution with hexane−EtOAc (8:2). Further purification of these fractions using the same solvent system yielded **1** (90 mg) along with certain previously obtained sterols.[2,3]

7α,20(*S*)-Dihydroxy-4,24(28)-ergostadien-3-one (1): powder; mp 185−188 °C; IR ν max 3450, 3300 (OH), 2990 (CH), 1670 (enone), 1635, 890 (C=CH$_2$), 1440, 1370, 1360 (*gem*-dimethyl) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H 5.76 (1H, d, J = 2.0 Hz, H-4), 4.70 [1H, t, J = 1.2 Hz, H-28(*Z*)], 4.64 [1H, dt, J = 1.4 and 1.2 Hz, H-28(*E*)], 3.96 (1H, br s, H-7), 2.60 (1H, ddd, J = 14.9, 3.1, 2.0 Hz, H-6β), 2.39 (1H, dd, J = 14.9, 3.3 Hz, H-6α), 2.20 (1H, septet, J = 6.8 Hz, H-25), 1.28 (3H, s, Me-21), 1.16 (3H, s, Me-19), 1.00 (6H, d, J = 6.8 Hz, Me-26 and Me-27), 0.88 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ_C 13.4 (q, C-18), 16.9 (q, C-19), 20.6 (t, C-11), 21.9 (q, C-26, C-27), 22.3 (t, C-16), 23.0 (t, C-15), 26.2 (q, C-21), 28.9 (t, C-23), 33.8 (t, C-2), 33.9 (d, C-25), 35.3 (t, C-1), 38.4 (s, C-10), 39.0 (d, C-8), 39.5 (t, C-12), 41.0 (t, C-6), 42.4 (t, C-22), 42.6 (s, C-13), 44.9 (d, C-9), 50.5 (d, C-14), 57.5 (d, C-17), 65.8 (d, C-7), 75.0 (s, C-20), 106.2 (t, C-28), 128.7 (d, C-4), 156.1 (s, C-24), 168.1 (s, C-5), 199.0 (s, C-3); EIMS (70 eV) m/z [M]$^+$ 428, [M − H_2O]$^+$ 410, [M − 2H_2O]$^+$ 392; HRMS m/z 410.3198 ($C_{28}H_{42}O_2$), 392.3067 ($C_{28}H_{40}O$). Anal. Calcd for $C_{28}H_{44}O_3$: C, 78.45; H, 10.34. Found: C, 78.32; H, 10.10.

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References and Notes

(1) Tchouankeu, J. C.; Tsamo, E.; Sondengam, B. L.; Connolly, J. D.; Rycroft, D. S. *Tetrahedron Lett.* **1990**, *31*, 4505−4508.
(2) Garcia, J.; Morin, C.; Nyassé, B.; Sondengam, B. L.; Tchouankeu, J. C.; Tsamo, E. *J. Nat. Prod.* **1991**, *54*, 136−142.
(3) Tchouankeu, J. C.; Nyassé, B.; Tsamo, E.; Sondengam, B. L.; Morin, C. *Phytochemistry* **1992**, *31*, 704−705.
(4) Nes, W. R.; Varkey, T. E. *J. Org. Chem.* **1976**, *41*, 1652−1653.
(5) Drew, J.; Letellier, M.; Morand, P.; Szabo, A. G. *J. Org. Chem.* **1987**, *52*, 4047−4052.

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