Cleogynol, a Novel Dammarane Triterpenoid from *Cleome gynandra*

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Chemical examination of *Cleome gynandra* (whole plant excluding seeds) led to the isolation and identification of a novel (20*S*,24*S*)-epoxy-19,25-dihydroxydammarane-3-one hemiketal (**1**). The structure of the new compound, designated cleogynol, was determined using spectral and chemical methods.

Cleome gynandra L. (syn. *C. pentaphylla* L.; *Gynandropsis gynandra* Briq.; *G. pentaphylla* DC.) (Capparidaceae) finds use in the Indian system of medicine for the treatment of various ailments. The seed powder is used as a treatment for hemorrhages, the seed oil is used for curing skin diseases, and the aerial parts are reported to heal skeletal fractures. Anticancer activity has been observed for an alcoholic extract of the plant.[1] Previous work on the plant, primarily on the seeds, has resulted in the isolation of chromone and flavone derivatives,[2,3] as well as β-sitosterol and its glycosides. Another compound, cleomin, of undefined structure, has been obtained from the seeds of the plant.[4] The occurrence of glucocapparin in the seeds has also been reported.[5]

The present investigation has been restricted to the mature plant of *C. gynandra*, with the exception of the seeds. A novel (20*S*,24*S*)-epoxydammarane triterpenoid, cleogynol (**1**), has been isolated having a hemiketal unit. The structure of **1** was determined from chemical and spectral studies.

The molecular formula of compound **1**, mp 108−110 °C, $[\alpha]^{25}_{D}$ +115.4° (*c* 0.35, EtOH) was established as $C_{30}H_{50}O_4$ by a combination of EIMS (*m*/*z* 474 M^+), ^{13}C NMR data, and elemental analysis. It showed only UV end absorption and exhibited IR bands for hydroxyl groups (3400 and 3220 cm^{-1}). The ^{13}C NMR spectrum of **1** displayed signals for a dioxygenated nonprotonated carbon at δ 98.1, for oxygenated nonprotonated carbons at δ 86.4 and 70.4, for an oxygenated methine carbon at δ 86.5, and for an oxymethyl ($-CH_2O-$) carbon at δ 68.0. Compound **1** was resistant to acetylation at room temperature, indicating the absence of a primary or secondary hydroxyl function. However, acetylation could be carried out at elevated temperature with Ac_2O-pyridine to afford a monoacetate (**2**) and a diacetate (**3**). Though the IR and ^{13}C NMR spectra of **1** indicated the absence of a keto group, a carbonyl carbon signal at δ 216.5 in the ^{13}C NMR spectrum and a strong absorption band at 1710 cm^{-1} in the IR spectrum were discernible for the monoacetate **2** (and also for **3**). The nonprotonated carbon resonance at δ 98.1 for C-3 in the ^{13}C NMR spectrum of **1** was not observed for either **2** or **3**. Furthermore, the methylene carbon resonance at δ 68.0 in **1** shifted upfield to δ 64.3 in **2** (δ 64.5 in **3**), while the geminal proton resonances for the oxymethyl group ($-CH_2O-$) underwent minor changes during the conversions of **1** to **2** and **3**. From these observations, the presence of a hemiketal moiety such as **A** in **1** was confirmed. This conjecture was proved to be correct, as evidenced from KBH_4 reduction of cleogynol (**1**) to the triol **4**. The presence of a primary and equatorially oriented secondary and also a tertiary alcoholic function in **4** was established by its conversion to the monoacetate **5** and the diacetate **6** by the action of acetic anhydride and pyridine.

The ^{1}H NMR signal at δ 3.60 (1H, dd, J = 9.6 and 5.4 Hz) for H-24 in **1** remained more or less unaffected upon acetylation to **2**, supporting its association with an ether moiety. The presence of the oxygenated carbon resonances at δ 86.5 (d) and δ 86.4 (s) in the ^{13}C NMR spectrum of **1** suggested the presence of a 2,2,5-trisubstituted tetrahydrofuranyl system **B**.

The proton resonances for two quaternary methyls shifted downfield (to δ 1.41 and δ 1.44) upon acetylation of **1** to **3**, while a small downfield shift of the oxygenated methine proton signal at δ 3.60 (1H, dd, J = 9.6 and 5.4 Hz, H-24) in **1** to δ 3.89 (1H, dd, J = 9.0 and 5.4 Hz) in **3** was noted. In the ^{13}C NMR spectrum the nonprotonated carbon signal at δ 70.4 (C-25) in **1** suffered an appreciable downfield shift to δ 82.8 (as observed in the changeover[6] of $-CMe_2OH$ to $-CMe_2OAc$), while the two methyl signals experienced upfield shifts (to δ 22.0 and δ 22.5, respectively), as did the methine carbon signal at δ 86.5 in **1** to δ 84.8 in **3**. These observations confirmed the presence of system **C**.

Comparison of the ^{13}C NMR resonances associated with the oxygenated carbons of the tetrahydrofuranyl unit in **1** with those of (20*S*,24*S*)-epoxydammarane-3β,11α, 25-triol,[7] ocotillol-II,[8] 3-oxo-(20*S*,24*S*)-epoxydammarane-25-ol,[9] and 3-oxo-(20*S*,24*R*)-epoxydammarane-25-ol[9] could establish the configuration of C-20 and C-24 as 20*S* and 24*S* in cleogynol (**1**), since C-20, C-21, and C-24 resonated at δ 86.4 (s), 27.5 (q), and 86.5 (d), respectively. A correlation between the ^{1}H and ^{13}C NMR signals for **1** was achieved through the HETCOR NMR experiment. Furthermore, a comparison of the ^{13}C NMR spectrum of **1** with that of **2** indicated that the methyl carbon resonances at δ 18.3 and 26.7 in **1** were affected and changed to δ 19.2 and 29.3 in **2**, respectively, in consonance with their association close to the hemiketal moiety (C-28 and C-29) (Table 1). Similarly, comparative ^{13}C NMR spectral analysis of **2** and **3** revealed that the methyl resonances at δ 23.9 and 27.6 in **2** underwent upfield shifts on acetylation of the tertiary hydroxyl group. Thus, these ^{13}C NMR signals were assigned to C-26 and C-27 in **2**, and consequently the methyl resonances at δ 24.3 and 26.9 in **1** could be attributed to

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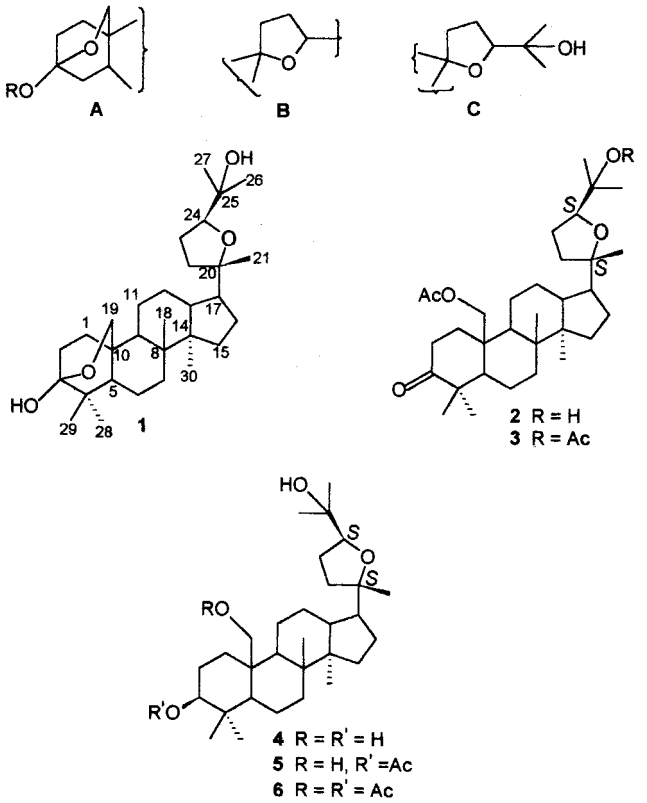

C-26 and C-27. ^{13}C NMR resonances in rings C and D and for C-6, C-7, C-18, and C-30 in **1** were observed at chemical shifts comparable to other 20,24-epoxydammaranes. The absence of any methyl and keto carbonyl at C-19 and C-3, respectively, gave evidence of the hemiketal unit in cleogynol (**1**), which could be opened on acetylation generating a >CO function at C-3 and a $-CH_2OAc$ group at C-19. Furthermore, the chemical shifts of the H_a-19 and H_b-19 in the ^1H NMR spectrum and of C-3 and C-19 in the ^{13}C NMR spectrum in cleogynol appeared at similar positions for the dammarane rhuslactone,[10] and more or less at comparable positions in cordialins A and B,[11,12] confirming the presence of hemiketal bridge between C-3 and C-19.

Experimental Section

General Experimental Procedures. Melting points are reported uncorrected. Optical rotations were measured in a Perkin−Elmer model 241 electronic polarimeter. IR spectra were recorded on a Perkin−Elmer 782 spectrophotometer. NMR spectra were recorded in $CDCl_3$ solution on a Bruker AM 300L spectrometer. ^1H and ^{13}C NMR spectra were measured at 300.13 and 75.47 MHz, respectively. All the chemical shifts were recorded with respect to internal TMS. EIMS were recorded at 70 eV on a JEOL AX-500 spectrometer. Column chromatography was performed over Si gel 60−100, and TLC and preparative TLC over Si gel G, using *n*-hexane−Me_2CO (4:1) as solvent system.

Plant Material. The plant material was collected locally in June 1996, and identified by Dr. S. R. Das, Survey Officer, Regional Research Institute (Ayurveda), Calcutta 700 091. A voucher specimen (no. 4201) has been deposited at the Regional Research Institute (Ayurveda), Calcutta 700 091.

Extraction and Isolation. The air-dried whole plant, *C. gynandra* (2.5 kg), (without seeds), was crushed and then extracted with petroleum ether (60−80 °C) in a Soxhlet apparatus for 15 h. After removal of solvent, the residual material (ca. 20 g) was chromatographed. Elution was performed with solvents of increasing polarity, starting with *n*-hexane. The *n*-hexane−Me_2CO (4:1) eluent yielded, after removal of solvent, the pure dammarane triterpenoid **1** as colorless crystals (350 mg): mp 108−110 °C; $[\alpha]^{25}_D$ +115.4° (*c* 0.35, EtOH); IR (KBr) ν_{max} 3400 (OH), 3220 (OH), 2980, 2960, 1665, 1465, 1380, 1105, 1075, 900 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz) δ 4.22 (1H, dd, J = 8.7, 2.5 Hz, H_a-19), 3.71 (1H, br d, J = 8.7 Hz, H_b-19), 3.60 (1H, dd, J = 9.6, 5.4 Hz, H-24), 2.81 (1H, br s, OH), 1.16 (3H, s, H_3-21), 1.11 (3H, s, H_3-26), 1.08 (3H, s, H_3-27), 1.00 (3H, s, H_3-28), 0.96 (3H, s, H_3-29), 0.87 (3H, s, H_3-30), 0.82 (3H, s, H_3-18); EIMS (70 eV) *m*/*z* 474 [M^+] (2), 456 (48), 441 (9), 415 (68), 397 (33), 385 (100), 367 (31), 329 (37), 205 (38), 189 (60), 161 (90); *anal.* C 75.81%, H 10.46%, calcd for $C_{30}H_{50}O_4$, C 75.94%, H 10.54%.

Table 1. ^{13}C NMR Spectral Data of Dammarane Triterpenoids **1**−**4** and **6**[a]

	compound				
carbon	**1**	**2**	**3**	**4**	**6**
C-1	33.6 t	34.2 t	34.2 t	33.8 t	34.0 t
C-2	29.8 t	34.0 t	33.8 t	28.0 t	24.2 t
C-3	98.1 s	216.5 s	216.7 s	79.0 d	80.6 d
C-4	39.6 s	45.6 s	45. 8 s	42.1 s	37.8 s
C-5	45.6 d	52.1 d	52.3 d	56.4 d	56.4 d
C-6	20.0 t	19.5 t	19.6 t	18.1 t	18.1 t
C-7	35.6 t	34.2 t	34.4 t	35.1 t	35.4 t
C-8	40.7 s	39.8 s	40.0 s	40.7 s	40.6 s
C-9	49.9 d	51.5 d	51.7 d	52.4 d	52.1 d
C-10	35.8 s	38.4 s	38.6 s	38.9 s	40.4 s
C-11	23.0 t	22.9 t	23.1 t	24.7 t	23.8 t
C-12	25.7 t	25.6 t	25.8 t	25.8 t	25.8 t
C-13	43.4 d	42.9 d	43.0 d	43.4 d	43.3 d
C-14	49.9 s	49.6 s	49.8 s	50.4 s	50.4 s
C-15	31.5 t	31.5 t	31.7 t	31.8 t	31.7 t
C-16	27.2 t	27.0 t	27.1 t	27.8 t	27.6 t
C-17	50.8 d	49.4 d	49.4 d	49.9 d	50.0 d
C-18	16.1 q	16.3 q	16.5 q	17.0 q	16.6 q
C-19	68.0 t	64.3 t	64.5 t	62.7 t	64.1 t
C-20	86.4 s	86.3 s	86.7 s	86.5 s	86.4 s
C-21	27.5 q	27.3 q	27.4 q	27.1 q	26.8 q
C-22	35.8 t	34.2 t	34.6 t	35.7 t	35.4 t
C-23	26.5 t	26.1 t	26.7 t	26.4 t	26.5 t
C-24	86.5 d	86.3 d	84.8 d	86.4 d	86.5 d
C-25	70.4 s	70.0 s	82.8 s	70.3 s	70.4 s
C-26	26.9 q	27.6 q	22.0 q	27.7 q	27.7 q
C-27	24.3 q	23.9 q	22.5 q	24.2 q	24.17 q
C-28	26.7 q	29.3 q	29.4 q	28.8 q	28.5 q
C-29	18.3 q	19.2 q	19.3 q	15.8 q	16.9 q
C-30	15.3 q	15.2 q	15.4 q	15.7 q	15.6 q
$OCOCH_3$		171.0 s	171.2 s		170.8 s
			170.5 s		170.8 s
$OCOCH_3$		20.8 q	20.9 q		21.1 q
			21.8 q		21.1 q

[a] 75.47 MHz, $CDCl_3$, δ (ppm).

Acetylation of 1. Compound **1** (200 mg) was heated with a mixture of Ac_2O (5.0 mL) and pyridine (1.0 mL) for 6 h on a boiling H_2O bath. The usual workup yielded a material composed of the products **2** and **3** (R_f 0.62 and 0.72), which were separated by preparative TLC. The monoacetate (**2**) was obtained as colorless crystals (80 mg): mp 272 °C; IR (KBr) ν_{max} 3220 (OH), 2980, 1745 (ester CO), 1710 (CO), 1480, 1400, 1250 (ester C−O−CO), 1150, 1090, 1060, 1050, 960, 900 cm^{-1}; ^1H NMR δ 4.15 (1H, d, J = 11.7 Hz, H_a-19), 3.97 (1H, d, J = 11.7 Hz, H_b-19), 3.55 (1H, dd, J = 8.1, 5.1 Hz, H-24), 1.87 (3H, s, $OCOCH_3$), 1.11 (3H, s, H_3-21), 1.07 (3H, s, H_3-26), 1.04 (3H, s, H_3-27), 1.00 (3H, s, H_3-29), 0.88 (6H, s, H_3-28 and H_3-30), 0.84 (3H, s, H_3-18).

The diacetate (**3**) was obtained as colorless crystals (90 mg): mp 135 °C; IR (KBr) ν_{max} 2920, 2850, 1730 (ester CO and CO), 1420, 1335, 1075 cm^{-1}; ^1H NMR δ 4.21 (1H, d, J = 11.7 Hz, H_a-19), 4.03 (1H, d, J = 11.7 Hz, H_b-19), 3.89 (1H, dd, J = 9.0, 5.4 Hz, H-24), 1.95 (3H, s, $OCOCH_3$), 1.94 (3H, s, $OCOCH_3$), 1.44 and 1.41 (3H each, s, H_3-26 and H_3-27), 1.14 (3H, s, H_3-21), 1.06 (3H, s, H_3-29), 0.94 (6H, s, H_3-28 and H_3-30), 0.89 (3H, s, H_3-18).

Triol 4. Cleogynol (**1**) (20 mg) in 10% aqueous MeOH (2 mL) was treated with KBH_4 (ca. 100 mg) and kept at room temperature for 2 days. The solution was diluted with H_2O

(ca. 100 mL) and extracted with $CHCl_3$ (3 × 20 mL). The usual workup of the $CHCl_3$ layer and solvent removal gave a residue that gave crystals of triol **4** (18 mg) as needles from $CHCl_3$−petroleum ether, mp 215 °C; $[\alpha]^{25}_D$ +28.0° (*c* 0.18, $CHCl_3$); IR (KBr) ν_{max} 3460 (OH), 2960, 2840, 1470, 1460, 1370, 1340, 1320, 1300, 1240, 1180, 1170, 1160, 1150, 1120, 1090, 1060, 1040, 980, 950, 900, 810 cm^{-1}; ^1H NMR δ 4.02 (1H, d, J = 12.0 Hz, H_a-19), 3.97 (1H, d, J = 12.0 Hz, H_b-19), 3.62 (1H, dd, J = 9.6, 5.3 Hz, H-24), 3.24 (1H, dd, J = 10.7, 5.6 Hz, H-3), 1.17 (3H, s, H_3-21), 1.13 (3H, s, H_3-26), 1.11 (3H, s, H_3-27), 1.10 (3H, s, H_3-28), 0.99 (3H, s, H_3-29), 0.88 (3H, s, H_3-30), 0.77 (3H, s, H_3-18).

Acetylation of Triol 4. Triol **4** (12 mg) in pyridine (0.4 mL) was treated with Ac_2O (0.3 mL) and kept at room temperature overnight. The usual workup and subsequent preparative TLC over Si gel using $CHCl_3$-MeOH (99:1) afforded the monoacetate **5** (2 mg): amorphous; ^1H NMR δ 4.51 (1H, dd, J = 8.4, 5.0 Hz, H-3), 4.04 (1H, d, J = 12.0 Hz, H_a-19), 3.98 (1H, d, J = 12.0 Hz, H_b-19), 3.63 (1H, dd, J = 8.7, 4.3 Hz, H-24), 2.04 (3H, s, 3−OCOCH_3), 1.18 (3H, s, H_3-21), 1.14 (3H, s, H_3-26), 1.10 (6H, s, H_3-27 and H_3-29), 0.89 (6H, s, H_3-28 and H_3-30), 0.88 (3H, s, H_3-18), and the diacetate (**6**) (10 mg): amorphous; IR (KBr) ν_{max} 3460 (br, OH), 2970, 2880, 1740 (ester CO), 1470, 1450, 1370, 1245 (ester C−O−CO), 1030, 760 cm^{-1}; ^1H NMR δ 4.50 (1H, m, H-3), 4.46 (1H, d, J = 12.2 Hz, H_a-19), 4.32 (1H, d, J = 12.2 Hz, H_b-19), 3.62 (1H, m, H-24), 2.06 (3H, s, CH_3COO-19), 2.04 (3H, s, CH_3COO-3), 1.18 (3H, s, H_3-21), 1.14 (3H, s, H_3-26), 1.10 (3H, s, H_3-27), 1.01 (3H, s, H_3-29), 0.88 (6H, s, H_3-28 and H_3-30), 0.87 (3H, s, H_3-18).

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