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DAMMARANE TRITERPENES FROM *CLEOME AMBLYOCARPA*

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Key Word Index—*Cleome amblyocarpa*; Capparaceae; dammarane triterpenes; flavonoids.

Abstract—The aerial parts of *Cleome amblyocarpa* yielded four new and two known dammarane-type triterpenes. The structures of the new compounds were elucidated by spectral methods.

INTRODUCTION

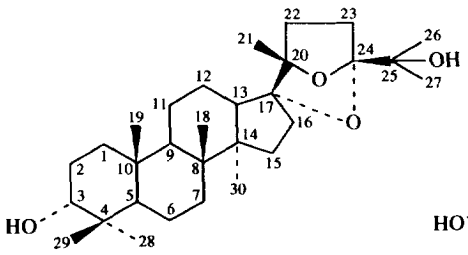
In the present study, four new dammarane triterpenes and two known compounds, cleocarpanol (**1**) [1] and cabraleahydroxy lactone (**2**) [2], were isolated from *Cleome amblyocarpa* Barr. et Murb. [3] (syn. *C. africana* Botsch and *C. arabica* auct. non L.) collected from Saudi Arabia. Although an Egyptian collection of the plant, under the name *C. africana*, was investigated previously [1], only the presence of compounds **1** and **3** together with stigma-4-en-3-one, lupeol and taraxasterol as well as a cembrane derivative [4], were reported.

Cleome amblyocarpa and *C. brachycarpa* are used as folk medicine in the treatment of scabies, rheumatic fever and inflammation [1, 4–7].

RESULTS AND DISCUSSION

The structure of the first new compound, ambylone (**4**), is quite similar to that of compound **3**, the only difference being in ring E: instead of the tetrahydrofuran ring with a hydroxyisopropyl group of **3**, there is a five-membered lactone ring in **4**. The high resolution EI-mass spectrum of **4** indicated a molecular formula $C_{27}H_{42}O_4$ (*m/z* 430.3097, calc. 430.3082). The IR spectrum showed the presence of hydroxyl (3420 cm^{-1}) and lactone carbonyl (1765 cm^{-1}) absorbances. The ^1H NMR spectrum ($CDCl_3$) revealed the structure of **4**. Signals were observed at δ1.34 (Me-21), 1.02 (Me-28), 0.97 (Me-29), 0.87 (Me-18), 0.84 (Me-30) (each 3H, *s*), 4.22 (1H, *dd*, $J = 2$ and 9 Hz, H-19) and 3.72 (1H, *dd*, $J = 1$ and 9 Hz, H-19′), and spin decoupling experiments showed the relationship between the *C*-19 protons as well as their relationship with H-1 (1.8, *m*) and H-5 (1.7, *m*). These latter signals being sharpened on irradiation of the signals at δ4.22 and at 3.72. The chemical shifts of the Me-21 group differs from those of the other methyl groups owing to the effect of the lactone group. The chemical shift and the splitting of the C-19 protons were similar in both compounds **3** and **4**. The ^{13}C NMR spectrum of **4** and an APT experiment indicated the presence of five methyl quarters, eleven methylene triplets, four methine doublets and seven quaternary carbon atoms, i.e. 27 carbons. The signals at δ98.5 (C-3, *s*), 90.2 (C-20, *s*) and 68.4 (C-19, *t*) indicated that the oxygenated carbon atoms were also similar to those of compound **3** (Table 1). The presence of a peak at *m/z* 99 (100%) in the mass spectrum of **4** clearly indicated the presence of a five-membered saturated lactone ring. All the spectral data suggested structure **4** for ambyone.

The second new compound, cleoamblynol A (**5**) has the molecular formula $C_{31}H_{46}O_7$ (*m/z* 530.3267, calc. 530.3243). The IR spectrum indicated a hydroxyl group (3440 cm^{-1}), a lactone carbonyl group (1760 cm^{-1}) and an acetyl group (1730 cm^{-1}). In the ^1H NMR spectrum there were six methyl signals (each 3H, *s*) at δ1.47, 1.42, 1.38, 1.21, 1.10 and 0.92 and two acetyl signals at δ1.95 and 2.00 (each 3H, *s*), and the signals for an ABX system at δ2.82 (1H, *d*, $J = 15$ Hz, H-16a), 3.09 (1H, *dd*, $J = 5.5$ and 15 Hz, H-16b), 5.18 (1H, *br d*, $J = 5.5$ Hz, H-15α). Since the latter hydrogen under one of the acetyl groups was coupled only with two vicinal protons, it should be at C-15 with a β orientation. Dreiding model inspection and spin decoupling experiments indicated the relationship between H_2-16 and H-15α. The second acetyl group, for biogenetic reasons, should be at C-3, having the β position, as shown from the splitting pattern of the C-3α hydrogen at δ5.10 (1H, *dd*, $J = 5$ and 10.5 Hz, H-3α). The relationship between the axial and equatorial protons of C-2 at δ1.80 (1H, *d*, $J = 5$ and 10.5 Hz) and 1.90 (1H, *d*, $J = 10.5$ Hz) and H-3α were shown by spin decoupling experiments. Two downfield signals at δ7.36 (1H, *d*, $J = 5.5$ Hz) and 6.06 (1H, *d*, $J = 5.5$ Hz) were assigned to the unsaturated lactone ring protons at C-22 and C-23. The mass fragmentation pattern of **5** having *m/z* 97 as the base peak, indicated the presence of an α, β-unsaturated lactone ring. The relationship between



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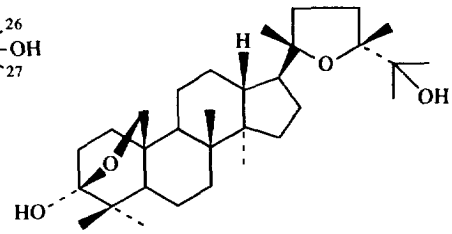

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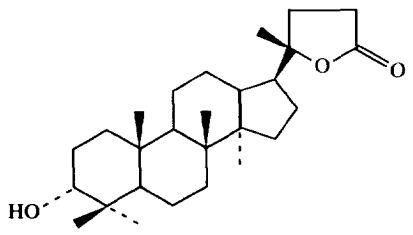

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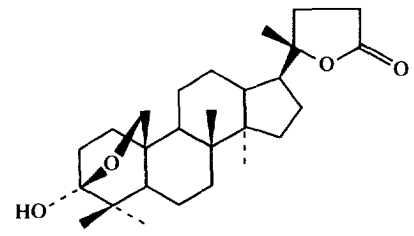

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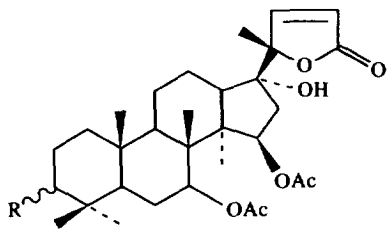

	R	R'
5	βOAc	H
6	αOAc	H
7	βOAc	βOAc

these two protons was deduced by spin decoupling experiments. The ^{13}C NMR (APT) data of **5** showed the lactone carbonyl at δ 172.0 and acetyl carbonyls at δ 170.3 and 170.5. The unsaturated carbon atoms were observed at δ 159.4 and 121.0 as doublets. Carbon atoms vicinal to oxygen functions gave signals at δ 78.7 (C-3), and 72.8 (C-15) as doublets, and at δ 84.5 (C-17), and 91.0 (C-20) as singlets (Table 1). The spectral data are in agreement with the structure shown for **5**.

Compound **6** was assigned the molecular formula $C_{31}H_{46}O_7$ HRMS: *m/z* 530.3255, calc. 530.3243). Its IR and UV spectra were similar to those of compound **5**. The ^{1}H NMR spectrum also showed similar signals to those of **5** at: δ 7.32 (1H, *d*, J = 5.5 Hz, H-22) and 6.03 (1H, *d*, J = 5.5 Hz, H-23); δ 1.47 (3H, *s*), 1.44 (3H, *s*), 1.34 (6H, *s*), 1.10 (3H, *s*) and 0.98 (3H, *s*) for five Me groups; δ 2.05 (3H, *s*) and 1.94 (3H, *s*) for two acetyl groups, and δ 3.00 (1H, *dd*, J = 5 and 13 Hz, H-16a), 2.84 (1H, *br d*, J = 13 Hz, H-16b) and 5.20 (1H, *d*, J = 5 Hz, H-15α) for an ABX system. The relationship between the C-16 axial and equatorial protons and H-15α were shown by spin decoupling experiments. The signal at δ 5.48 (1H, *t*, J = 2 Hz) was attributed to H-3β, indicating the presence of an acetoxy group at the C-3α position. The mass fragmentation pattern of **6** was also quite similar to that of compound **5** (see Experimental). The ^{13}C NMR (APT) data of **5** and **6** were also quite similar, the only difference between them was the chemical shift and the splitting pattern of H-3. Therefore **6** was deduced to be the 3α isomer of compound **5**.

The IR spectrum of the fourth new compound, cleoambblynol B (**7**), was also similar to those of compounds **5** and **6**, but the ^{1}H NMR spectrum of **7** indicated the presence of three acetyl groups instead of two. There were slight chemical shift differences for the methyl signals (each 3H, *s*) at δ 1.47, 1.42, 1.39, 1.19, 1.16 and 1.08. Other signals were more or less similar to those of **5** and **6**: δ 7.37 (1H, *d*, J = 5.5 Hz, H-22), 6.11 (1H, *d*, J = 5.5 Hz, H-23), 5.16 (1H, *br d*, J = 5 Hz, H-15α), 5.05 (1H, *dd*, J = 5 Hz and 11 Hz, H-3α), 3.18 (1H, *dd*, J = 5 and 15 Hz, H-16a), 2.90 (1H, *br d*, J = 15 Hz, H-16b), 2.00 (6H, *s*, 2 × OAc) and 1.94 (3H, *s*, OAc). The signal at δ 4.9 (1H, *dd*, J = 7 and 10 Hz) was assigned to H-7α for the following reason. The third acetoxy group could be

Table 1. ^{13}C NMR spectral data compounds 3–7

C	3*	4	5	6	7
1	35.9	35.9	34.7	34.7	35.2
2	29.5	29.5	30.4	30.6	30.8
3	98.1	98.5	78.7	78.7	79.1
4	40.4	40.9	41.4	41.5	40.2
5	49.3	50.2	51.1	51.1	51.1
6	19.7	20.2	23.1	23.4	32.9
7	30.3	30.1	28.6	31.3	76.8
8	39.2	39.7	32.1	32.1	42.8
9	45.2	45.6	48.7	48.2	51.1
10	35.4	35.9	36.6	34.7	35.2
11	22.6	23.0	23.4	23.5	23.8
12	27.4	27.4	30.2	30.5	30.8
13	43.1	40.9	41.6	43.8	42.4
14	49.6	50.6	46.4	48.2	51.1
15	31.3	31.6	72.8	73.8	73.8
16	25.5	25.4	48.7	47.9	51.6
17	49.9	49.7	84.5	84.9	84.7
18	15.2	15.7	14.3	14.3	14.7
19	68.1	68.4	15.7	15.6	17.7
20	86.2	90.2	91.0	90.6	90.7
21	23.3	23.0	17.0	16.9	17.7
22	35.5	35.9	159.4	159.5	158.6
23	26.1	27.3	121.0	120.8	122.3
24	83.3	176.8	172.0	172.1	172.2
25	71.4	—	—	—	—
26	26.8	—	—	—	—
27	24.2	—	—	—	—
28	27.5	27.3	30.3	30.0	30.8
29	18.5	18.8	21.6	21.2	21.5
30	15.9	16.2	21.4	21.6	21.8
C=O	—	—	170.5	170.7	170.7
CH_3	—	—	23.1	21.2	22.0
C=O	—	—	170.3	170.3	170.8
CH_3	—	—	24.8	21.8	23.8
C=O	—	—	—	—	171.8
CH_3	—	—	—	—	21.6

*Data taken from ref. [1].

placed at C-6, C-7, C-11 or C-12. However, if the third acetyl was situated between a methine and a methylene group, there would be a *ddd* signal instead of a *dd* for the proton under it, therefore the C-6, C-11 and C-12 positions were unlikely, and so the group had to be at C-7. Its configuration was established by measuring the *J* values and studying a Dreiding model. The ^{13}C NMR spectrum of **7** showed the presence of the third acetyl group at δ76.8. The fact that the signal at C-8 was shifted to δ42.8 and that for C-6 to δ32.9 confirmed the C-7 substitution of the third acetyl group (Table 1).

EXPERIMENTAL

General. IR: $CHCl_3$; ^1H NMR 200 MHz; ^{13}C NMR: 50.34 MHz; HRMS: VG Zabspec; Prep. TLC: Kieselgel $60F_{254}$ (E.Merck); CC: silica gel and Sephadex LH-20 (Fluka).

Plant material. The aerial parts of *Cleome amblyocarpa* were collected from Qassim province, Saudi Arabia in May 1993, and identified by the Botany Dept. College of Science, King Saud University. A voucher specimen is deposited in the Herbarium of the College of Agriculture and Veterinary Medicine of the same University.

Extraction and fractionation. Powdered aerial parts of the plant (800 g) were exhaustively extracted with 95% EtOH at room temp. Upon evapn under red. press. a dark green residue (75 g) was obtained. The residue was dissolved in EtOH, the waxes were filtered off and 20% water was added. After extraction with petrol (31 g), $CHCl_3$ (11.5 g), EtOAc (3.5 g) the remaining aq. layer was discarded. The $CHCl_3$-soluble fr. was fractionated on a silica gel column (4 × 65 cm), eluted with a petrol–$CHCl_3$ (0 to 100%) gradient. Compounds **1** and **4**–**7** were eluted from the column in the following order: **1** (55 mg), **4** (12 mg), **5** (23 mg), **6** (15 mg) and **7** (20 mg).

The EtOAc fr. was fractionated on a Sephadex LH-20 column eluted with MeOH. Two compounds were obtained, luteolin 3′-methyl ether (6 mg) and luteolin 3′-methyl ether 7-glucoside (8 mg).

Amblyone (**4**). $[\alpha]_D = +91°$ ($CHCl_3$; *c* 0.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2960, 2870, 1765, 1460, 1380, 1250, 1190, 1070, 1030, 940, 760; UV λ_{max}^{MeOH} nm: 222 (logε 4.0); ^1H NMR ($CDCl_3$): see text; ^{13}C NMR: Table 1; HRMS *m/z* (rel. int.): 430.3097 $[M]^+$ ($C_{27}H_{42}O_4$) (72), 412 $[M - H_2O]^+$ (18), 383 (15), 357 (20), 329 (16), 121 (44), 109 (53), 99 (100), 81 (57), 69 (66).

Cleomblynol A (**5**). $[\alpha]_D = +41°$ ($CHCl_3$; *c* 0.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 2980, 2880, 1760, 1730, 1450, 1370, 1250, 1160, 1150, 1110, 1020, 820, 730; UV λ_{max}^{MeOH} nm: 225 (logε 4.1); ^1H NMR: see text; ^{13}C NMR: Table 1; HRMS *m/z* (rel. int.): 530.3267 $[M]^+$ ($C_{31}H_{46}O_7$) (68), 503 $[M - CO + H]^+$ (100), 427 $[M - OAc - Ac + 2H]^+$ (12), 409 $[427 - H_2O]^+$ (8), 383 (20), 353 (38), 97 (95).

Isocleomblynol A (**6**). $[\alpha]_D = +71°$ ($CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 2980, 2880, 1765, 1735, 1460, 1370, 1250, 1160, 1120, 1020, 950, 820; UV λ_{max}^{MeOH} nm: 225 (logε 4.0); ^1H NMR: see text; ^{13}C NMR: Table 1; HRMS *m/z* (rel. int.): 530.3255 $[M]^+$ ($C_{31}H_{46}O_7$) (98), 502 $[M - CO]^+$ (40), 428 $[M - OAc - Ac + H]^+$ (15), 385 (20), 355 (50), 97 (55).

Cleomblynol B (**7**). $[\alpha]_D = +28°$ ($CHCl_3$; *c* 0.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 2980, 2860, 1760, 1735, 1720 (sh), 1455, 1375, 1250, 1165, 1150, 1110, 1020, 820, 750; UV λ_{max}^{MeOH} nm: 222 (logε 4.1); ^1H NMR: see text; ^{13}C NMR: Table 1; HRMS *m/z* (rel. int.): 588.3290 $[M]^+$ ($C_{33}H_{48}O_9$) (1), 546 $[M - Ac]^+$, 502 $[M - 2 \times Ac]^+$ (60), 486 $[M - OAc - Ac]^+$ (38), 468 $[M - 2 \times OAc]^+$ (45), 440 $[M - 2 \times OAc - CO]^+$ (35), 426 $[M - 2 \times OAc - Ac]^+$ (42), 283 (7), 127 (10), 99 (15), 97 (100), 81 (65), 69 (50).

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