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CYTOTOXIC TRITERPENES FROM *CLEOME AFRICANA*

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Key Word Index—*Cleome africana*; Capparaceae; cytotoxic; dammarane; triterpene.

Abstract—Eighteen dammarane-type triterpenes were obtained from the whole plant of *Cleome africana* by means of cytotoxic bioassay-directed fractionation. Twelve of them were novel compounds whose structures were elucidated by various spectroscopic methods. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Cleome africana is a native to the Caribbean regions, and is mainly distributed in the tropics and temperate zone. It has been used in folk medicine for the treatment of such things as inflammation and rheumatism. In the course of a cytotoxic screening of ethanolic extracts of higher plants, *C. africana* indicated significant cytotoxic activity against P388 cells. Bioassay-directed fractionation of the extract has led to the isolation and characterization of new cytotoxic principles, which are dammarane-type triterpenes. We report herein on the isolation, structural elucidation and cytotoxic activities.

RESULTS AND DISCUSSION

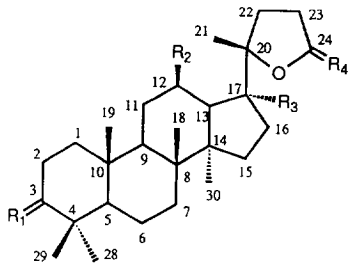
An ethanolic extract of whole plants of *C. africana* was separated by silica gel column chromatography using a *n*-hexane–ethyl acetate gradient solvent system. The fractionation was monitored by cytotoxic bioassay (against the P388 cell line) and afforded 18 compounds (**1**–**18**), which were all dammarane-type triterpenes. The structures of known compounds **2** [1, 2], **5** [3], **9** [4], **11** [5, 6], **13** [7] and **18** [5, 6] were established by comparing their various physical and spectral data with those in the literature.

Compound **1** was assigned the molecular formula $C_{27}H_{42}O_4$ from the $[M]^+$ at m/z 430 on EI mass spectrometry and from the NMR spectral data. Its IR spectrum showed strong peaks at 1760 (γ-lactone) and 1700 cm^{-1} (carbonyl) and the presence of a hydroxyl group at 3600 cm^{-1}. The ^{13}C NMR spectrum in Table 1 was the good agreement with that of cabralealactone (**2**) [1, 2] except for the oxygen-bearing quaternary carbon signal at δ 83.4 and a methine carbon signal (δ 49.3), whose downfield chemical shift was similar to that of a hydroxyl-bearing carbon signal (δ 84.0) at C-17 of **5** [3]. Therefore, the structure of **1** was assumed to be 17α-hydroxycabralealactone.

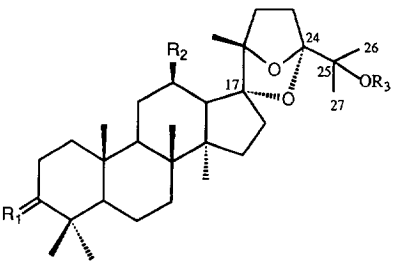
Compound **3** showed IR absorption bands at 1720 (acetyl) and 1760 cm^{-1} (γ-lactone). The ^{13}C NMR spectra estimated by DEPT experiments indicated the presence of eight methyl, eight methylene, five methine and nine quaternary carbons. In comparison with the ^{13}C NMR spectra of **1**, those of **2** showed four additional carbon signals (δ 170.8, 170.1, 22.1 and 21.4) due to two acetoxyl groups. Also, the peaks at δ 217.8 for the C-3 carbonyl carbon and δ 22.9 for the C-12 methylene carbon of **1** were shifted to δ 77.9 and 72.6 for the acetyoxy-bearing carbon signals in **2**. The configurations of the 3- and 12-acetoxyl groups were established to be α and β, respectively, from the J values (δ 4.59, *t*, J = 3.0 Hz and δ 5.24, *td*, J = 5.0 and 10.0 Hz) [8]. Consequently, **3** was adjusted to be 3-*O*-acetyl-12β-acetoxy-17α-hydroxycabraleahydroxylactone.

The molecular formula, $C_{27}H_{44}O_4$, of **4** estimated from the mass spectral and NMR data had two hydrogen atoms more than that of **1**. The ^{13}C NMR spectral data for **4** were similar to those for **1** except for the fact that the signal at δ 217.8 for C-3 was shifted to δ 76.2 for the hydroxyl-bearing carbon signal in **4**. Thus, **4** was assumed to be 17α-hydroxycabraleahydroxylactone.

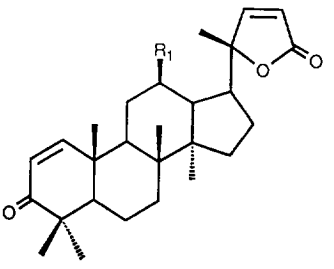
Compound **6**, molecular formula $C_{32}H_{50}O_6$, exhibited one additional acetoxyl signal (δ 170.2 and 21.8) on comparison of its NMR data with those of cleocarpone (**9**) [4]. The position and configuration of the acetoxyl group were established to be 12β from the fact that the peak at δ 23.8 for the C-12 signal in **9** was shifted downfield to δ 73.4 in **6**, whose H-12 signal



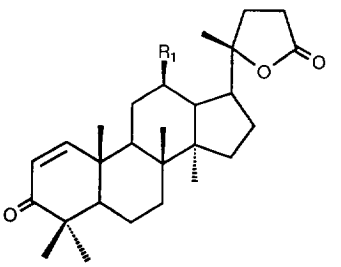
1: R_1=O, R_2=H, R_3=OH, R_4=O
2: R_1=O, R_2=H, R_3=H, R_4=O
3: R_1=α-OAc, β-H, R_2=OAc, R_3=OH, R_4=O
4: R_1=α-OH, β-H, R_2=H, R_3=OH, R_4=O
5: R_1=α-OH, β-H, R_2=H, R_3=OH, R_4=$C(CH_3)_2OH$, H



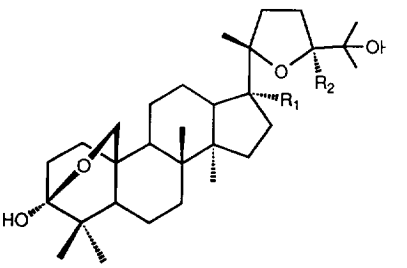
6: R_1=O, R_2=OAc, R_3=H
7: R_1=α-OH, β-H, R_2=OAc, R_3=H
8: R_1=α-OAc, β-H, R_2=OH, R_3=H
9: R_1=O, R_2=H, R_3=H
10: R_1=α-OAc, β-H, R_2=OAc, R_3=CH_2CH_3
11: R_1=α-OH, β-H, R_2=H, R_3=H



12: R_1=H
14: R_1=OAc



13: R_1=H
15: R_1=OAc



16: R_1= -O- =R_2
17: R_1=OH, R_2=H
18: R_1=R_2=H

was present at δ 5.15 (1H, *td*, J = 4.8 and 10.8 Hz). Thus, **6** was assumed to be 12β-acetoxycleocarpone.

Compounds **7** and **8** had the same molecular formula, $C_{32}H_{52}O_6$, and contained two more H atoms than **6**. The NMR spectra of **7** and **8** were similar to those of **6** except for the fact that the peak at δ 218.2 for the C-3 carbonyl carbon signal was replaced by the hydroxyl- and acetoxy-bearing carbon signals at δ 75.8 and 78.2 in **7** and **8**; in addition, the C-12 signal at δ 71.6 in **8** was shifted 1.8 ppm upfield from that of **6**. Therefore, **7** and **8** were identified as 12β-acetoxycleocarpanol and 3-*O*-acetyl-12β-hydroxycleocarpanol, respectively. Compound **10**, possessing the molecular formula $C_{36}H_{58}O_7$, had one additional acetyl group and one ethyl [δ 1.22 (*t*), 3.69 (*q*); δ 17.9, 58.4] group in comparison with the NMR spectra of **7** and **8**. Thus, **10** was assumed to be 3-*O*-acetyl-12β-acetoxy-25-*O*-ethylcleocarpanol.

Compound **12** was assigned the molecular formula $C_{27}H_{38}O_3$ from the mass spectral and NMR data, which indicated the presence of six methyls, six methylenes, eight methines including two disubstituted olefins and seven quaternary carbons, which included one conjugated and one lactonized carbonyl carbon. The spectral data for **12** and **2** were similar except for the olefinic signals [δ 7.23 and 5.80 (*d*, J = 10.2 Hz, respectively); δ 159.6 and 125.2, δ 7.40 and 6.07 (*d*, J = 5.7 Hz, respectively); δ 159.2 and 121.4]. It is evident that two double bonds, respectively, were conjugated with the C-3 and C-24 carbonyl groups from the NMR chemical shift values. Therefore, **12** was assumed to be 1(12), 22(23)-tetradehydrocabralealactone. Compound **13** possessing a molecular formula $C_{27}H_{40}O_3$ estimated from the mass spectral and NMR data had two more protons than **12**, and was assumed to be a $\Delta^{1,2}$-dehydrocabralealactone, which was reported by Witz *et al.* (however, its detailed spectral data were not described) [7].

Compound **14** was ascribed the molecular formula $C_{29}H_{40}O_5$ from the mass spectral (*m/z* 468 $[M]^+$) and NMR data, which were quite similar to those for **12** except for the spectral data due to one additional acetoxyl group (δ 2.04; *d* 21.7 and 169.6). The proton (δ 5.20), by shifting downfield the acetoxyl group, located at C-12 (δ 73.1), was deduced to be α-oriented from the coupling constants (1H, *td*, J = 11.1 and 4.9 Hz). Thus, **14** could be deduced to be 12β-acetoxy-1(2),22(23)-tetradehydrocabralea-lactone. By the same logic, **15** was deduced to be 12β-acetoxy-$\Delta^{1,2}$-dehydrocabralealactone.

Compound **16** possessing the molecular formula $C_{30}H_{48}O_5$ (*m/z* 488 $[M]^+$) showed six oxygen-containing carbon signals [δ 68.0 (*t*), 70.4 (*s*), 89.3 (*s*), 91.3 (*s*), 98.2 (*s*) and 112.8 (*s*)] in the ^{13}C NMR spectrum. By comparison with the spectral data for the dammarane triterpene **18** [5, 6], two hemiketals (δ 98.2 and 112.8), formed between the primary alcohol at C-19 and keto

group at C-3 and between C-17–C-24 and C-20–C24 ether linkages, were suggested. Therefore, the structure was deduced to be **16**.

Compound **17**, possessing the molecular formula $C_{30}H_{50}O_5$ (*m/z* 490 $[M]^+$), gave rise to a ^{13}C NMR spectrum which was quite similar to that of **18**, except for a downfield signal at δ 83.5. By comparison with the ^{13}C spectral data for **16**, the C-24 carbon signal (δ 112.8) was shifted upfield to δ 83.5.

These dammarane triterpenes have significant cytotoxic activity against P388 leukaemia cells as shown in Table 2. Compounds **2**, **4**, **6**, **10**, **12** and particulary **13** exhibited potent activity; however, the relationship between structure and cytotoxic activity could not be deduced.

EXPERIMENTAL

General. Mps: uncorr.; MS: VG AutoSpec; ^1H- and ^{13}C-NMR: Bruker AM 400 and 500 MHz at 303 K. NOESY experiments were performed with a mixing time of 0.6 s and processed on a Bruker data station with an Aspect 3000 computer. CC: Merck Kieselgel 60 (70–230 mesh) in amounts equivalent to 100 times that of the sample; MPLC column (22 mm i.d.x 300 mm) packed with 40 mm silica gel or 20 mm ODS; HPLC: Hibar RT RP-18 column (20 mm i.d.x 250 mm) packed with 7 mm ODS.

Plant material. The whole plant of *C. africana* was collected in January 1993 at Gabal Elba (southeastern corner of Egypt). The plant was identified by Prof. N. El-Hadidy, Department of Botany, Faculty of Science, Cairo University, Egypt, and a voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Egypt.

Extraction and isolation. Air-dried whole plants of *C. africana* (300 g) were cut into pieces and extracted (×3) with EtOH. The concd extract (11 g) was subjected to silica gel CC using a *n*-hexane–EtOAc (4:1–1:4) gradient system to give 10 frs (A–J). Fr D (0.17 g), E (0.63 g) and F (1.59 g) were each further chromatographed on silica gel columns eluted with *n*-hexane–EtOAc gradient system and purified by ODS HPLC with MeOH–H_2O (9:1 or 19:1) to give **1** (5.7 mg) from fr. D, **2** (18.6 mg), **3** (6.4 mg), **4** (7.1 mg), **6** (2.3 mg), **9** (4 mg), **10** (14.1 mg), **11** (21.6 mg), **12** (13.1 mg), **13** (13.1 mg) and **18** (7.2 mg) from fr. E, and **5** (20.6 mg), **7** (10 mg), **8** (40.3 mg), **14** (2.5 mg), **15** (48.3 mg), **16** (14.2 mg) and **17** (8.4 mg) from fr. F. The structures of known compounds **2**, **5**, **9**, **11**, **13** and **18** were established by comparing their various physical and spectral data with the lit. values [1–7].

Compound **1**. Needles, mp 211–216°, $[\alpha]_D = +64$ ($CHCl_3$; *c* 0.094);IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1780 (γ-lactone CO), 1700 (CO), 1470, 1400; UV $\lambda_{max}^{CHCl_3}$ nm (ε): 245 (688), 278 (602); HRMS *m/z* (rel. int.): 430.307 $[M]^+$($C_{27}H_{42}O_4$)(15), 413 (0.4), 397 (3), 331 (80), 314 (84), 107 (100); ^1H NMR ($CDCl_3$): *s* 0.94, 1.00, 1.04, 1.08, 1.16, 1.44 (each 3H, *s*).

Compound **3**. Needles, mp 104°, $[\alpha]D = +10$ ($CHCl_3$; *c* 0.082); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780 (γ-lactone CO), 1720 (CO), 1380, 980; UV $\lambda_{max}^{CHCl_3}$ nm (ε): 245 (180), 277 (138); HRMS *m/z* (rel. int.): 532.340 $[M]^+$ ($C_{31}H_{48}O_3$) (0.3), 472 (0.6), 397 (0.7), 373 (7), 313 (100), 295 (6); ^1H NMR ($CDCl_3$): *s* 0.86, 0.90, 1.00, 1.03, 1.27, 1.42 (each 3H, *s*), 1.98, 2.11 (each 3H, *s*, Ac), 4.59 (1H, *br td*, *J* = 11, 3 Hz), 5.24 (1H, *br t*, *J* = 2 Hz).

Compound **4**. Powder, HRMS *m/z* (rel. int.): 432.321 $[M]^+$ ($C_{27}H_{44}O_4$) (2), 399 (1), 333 (11), 315 (57), 297 (58), 207 (41), 107 (100); ^1H NMR ($CDCl_3$): *s* 0.84, 0.86, 0.94, 0.97, 1.17, 1.44 (each 3H, *s*), 2.55–2.73 (*m*, 2H), 3.40 (1H, *br t*, *J* = 2 Hz).

Compound **6**. Powder, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1730, 1710 (CO); HRMS *m/z* (rel. int.): 530.361 $[M]^+$ ($C_{32}H_{50}O_6$) (0.2), 470 (2), 437 (3), 384 (4), 366 (100); ^1H NMR ($CDCl_3$): *s* 0.94, 1.04, 1.06, 1.11, 1.11, 1.27, 1.28, 1.42 (each 3H, 2s), 2.03 (3H, *s*, Ac), 2.34 (1H, *m*), 2.57 (1H, *m*), 5.15 (1H, *td*, *J* = 10.8, 4.8 Hz).

Compound **7**. Needles, mp 94°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3600 (OH), 1720 (CO), 1380, 1260, 1160, 1120, 1080; HRMS *m/z* (rel. int.): 532.376 $[M]^+$ ($C_{32}H_{52}O_6$) (0.1), 368 (6), 159 (3), 142 (100); ^1H NMR ($CDCl_3$): *s* 0.86, 0.91, 0.95, 1.00, 1.10, 1.27, 1.28, 1.40 (each 3H, *s*), 2.01 (3H, *s*, Ac), 3.38 (1H, *br t*, *J* = 2 Hz), 5.21 (1H, *br. td*, *J* = 10, 5 Hz).

Compound **8**. Needles, mp 210°, $[\alpha]_D = -14$ ($CHCl_3$; *c* 0.688); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1720 (CO), 1380, 1260, 1160, 1120, 1080; HRMS *m/z* (rel. int.): 532.376 $[M]^+$ ($C_{32}H_{52}O_6$) (0.2), 514 (3), 481 (2), 428 (22), 411 (61), 178 (100); ^1H NMR ($CDCl_3$): *s* 0.84, 0.91, 0.95, 1.07, 1.10, 1.26, 1.27, 1.40 (each 3H, *s*), 2.07 (3H, *s*, Ac), 3.35 (1H, *br td*, *J* = 10, 5 Hz), 4.59 (1H, *br t*, *J* = 2 Hz).

Compound **10**. Needles, mp 88—89°, $[\alpha]_D = -16$ ($CHCl_{13}$, *c* 0.204); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (CO), 1380, 1260; HRMS *m/z* (rel. int.): 602.416 $[M]^+$ ($C_{36}H_{58}O_7$) (0.1), 515 (2), 481 (1), 439 (0.9), 410 (37), 142 (100); ^1H NMR ($CDCl_3$): *s* 0.85, 0.89, 0.99, 1.00, 1.12, 1.26, 1.27, 1.40 (each 3H, *s*), 1.26 (3H, *t*, *J* = 7 Hz), 2.00, 2.09 (each 3H, *s*, Ac), 3.69 (2H, *q*, *J* = 7 Hz), 4.58 (1H, *br t*, *J* = 2 Hz).

Compound **12**. Needles, mp 140–142°, $[\alpha]_D = +21$ ($CHCl_3$; *c* 0.114);,IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone CO), 1680 (CO), 1460 1400, 1120; UV $\lambda_{max}^{CHCl_3}$ nm (ε): 245 (11 700); HRMS *m/z* (rel. int.): 410.284 $[M]^+$ ($C_{27}H_{38}O_3$) (100), 395 (4), 299 (8), 281 (5), 163 (20), 97 (56); ^1H NMR ($CDCl_3$): *s* 0.88, 0.97, 1.06, 1.08, 1.29, 1.47 (each 3H, *s*), 2.09 (1H, *m*, H-17), 5.80 (1H, *d*, *J* = 10.2 Hz), 6.07 (1H, *d J* = 5.7 Hz), 7.23 (1H, *d*, *J* = 10.2 Hz), 7.40 (1H, *d*, *J* = 5.7 Hz).

Compound **13**. Needles, mp 151–152°, $[\alpha]_D = +58$ ($CHCl_3$; *c* 0.33); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone CO), 1660 (CO), 1460, 1380, 1100, 1080; UV $\lambda_{max}^{CHCl_3}$ nm (ε): 245 (3280); HRMS *m/z* (rel. int.): 412.295 $[M]^+$ ($C_{27}H_{40}O_3$) (100), 397 (13), 301 (34), 275 (18), 219 (92), 99 (75); ^1H NMR ($CDCl_3$): *s* 0.89, 1.03, 1.07, 1.08, 1.13, 1.37, (each 3H, *s*), 2.64 (1H, *m*, H-17), 5.80 (1H, *d*, *J* = 10.2 Hz), 7.12 (1H, *d*, *J* = 10.2 Hz).

Table 1. ^{13}C NMR chemical shifts of compounds **1–18**

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1	39.9	39.9	35.5	33.7	33.7	41.0	30.9	36.1	39.9	30.9	33.7	159.6	159.6	162.4	162.4	35.7	37.0	36.0
2	34.0	34.5	30.2	25.4	25.4	34.3	35.2	36.0	34.1	35.7	25.4	125.2	125.2	124.6	124.6	29.6	29.6	29.6
3	217.8	217.8	77.9	76.2	76.2	218.2	75.8	78.2	217.9	78.0	76.3	205.3	205.3	205.0	204.9	98.2	98.0	98.2
4	47.4	47.4	37.3	37.3	37.3	47.7	37.9	37.0	47.4	36.0	37.3	44.8	44.7	45.3	45.3	40.5	40.5	40.4
5	55.3	55.4	50.2	49.5	49.6	54.4	49.0	50.7	55.3	50.2	49.6	47.3	49.2	46.7	48.9	45.5	49.5	49.4
6	19.7	19.6	17.9	18.2	18.3	19.6	18.1	17.9	19.7	17.9	18.2	19.0	19.0	18.8	18.8	19.8	19.9	19.8
7	34.0	34.1	34.9	34.6	34.6	34.1	35.0	35.3	34.1	35.1	34.7	34.6	34.6	33.9	34.0	32.7	33.1	33.4
8	40.9	40.3	41.7	41.2	41.2	42.1	41.4	41.2	40.3	41.4	40.7	41.2	41.2	41.4	41.2	39.3	39.8	39.3
9	50.0	49.9	52.5	50.5	50.7	52.7	52.9	56.2	50.4	52.9	50.8	53.9	53.9	53.1	53.1	49.9	45.9	45.3
10	36.9	36.8	38.9	37.7	37.6	37.9	39.1	39.2	36.9	38.9	37.7	39.5	39.5	40.0	39.9	35.6	35.6	35.6
11	22.0	21.9	23.0	21.3	21.4	31.0	25.6	22.9	22.2	23.0	21.6	21.6	21.4	25.0	24.9	22.8	22.7	22.7
12	22.9	25.0	72.6	22.8	23.3	73.4	73.1	71.6	23.8	73.1	23.7	25.2	25.0	73.1	73.2	24.0	26.5	27.5
13	46.1	43.3	43.3	46.0	45.6	42.4	41.9	42.5	44.5	42.0	44.4	44.7	44.7	46.5	46.4	44.6	45.4	43.2
14	49.9	50.2	49.8	50.0	49.9	49.4	49.5	49.6	49.6	49.5	49.7	50.3	50.3	50.0	49.8	49.4	50.2	49.6
15	32.5	31.2	32.2	32.5	32.6	31.6	31.5	31.7	32.2	31.6	31.8	31.0	31.0	30.4	30.4	31.8	32.6	31.4
16	36.3	26.9	37.0	36.3	37.0	38.0	37.9	37.9	37.7	37.9	37.7	26.9	26.7	35.1	35.1	37.7	23.5	25.6
17	83.5	49.3	82.9	83.5	84.0	89.2	89.1	89.1	89.3	89.1	89.3	43.6	43.3	41.9	41.4	89.3	83.9	50.1
18	16.1	16.0	17.0	16.1	16.1	17.6	16.2	16.2	16.1	16.2	15.6	15.8	15.8	15.9	15.9	15.1	15.3	15.2
19	15.4	15.2	16.8	15.8	15.7	16.2	16.9	16.7	15.2	17.0	16.2	16.1	16.1	16.8	16.7	68.0	68.1	68.1
20	92.8	90.0	91.9	92.8	90.1	90.8	91.0	90.9	91.4	90.9	91.4	92.2	89.9	91.3	89.1	91.3	90.0	86.3
21	22.9	25.5	22.6	23.0	22.1	22.1	17.1	16.9	17.9	16.9	16.2	23.9	25.4	23.1	24.4	16.3	22.2	23.4
22	29.2	29.2	29.1	29.2	33.0	32.2	32.2	32.1	31.8	32.2	32.2	159.2	29.1	159.4	28.9	32.1	35.6	35.6
23	29.2	31.1	29.2	29.2	26.4	32.1	32.0	32.0	32.0	32.0	32.0	121.4	31.0	121.1	32.0	32.0	32.8	26.2
24	176.5	176.7	176.5	176.6	83.6	112.9	112.8	112.8	112.8	112.8	112.7	172.4	176.6	172.2	176.3	112.8	83.5	83.3
25					71.9	70.4	70.4	70.3	70.4	70.3	70.4					70.4	71.9	71.4
26					27.5	25.5	25.5	25.4	25.5	25.4	25.5					25.5	26.8	26.8
27					25.0	24.2	24.2	24.1	24.1	24.1	24.1					24.1	25.1	24.3
28	26.7	26.7	28.3	28.3	28.3	27.7	28.7	28.4	26.8	28.3	28.3	27.8	27.8	28.7	28.7	26.8	27.5	24.3
29	21.0	21.0	21.8	22.1	22.1	20.3	22.2	21.9	21.0	22.1	22.1	21.4	21.6	21.3	21.3	18.5	18.5	18.5
30	16.9	16.2	17.0	17.1	17.2	17.8	17.7	18.1	16.2	18.4	18.1	19.2	19.2	20.1	20.1	17.6	16.7	16.0
Ethyl										58.4								
										17.9								
3Ac-CO			170.8					170.8		170.8								
-Me			22.1					21.4		21.8								
12Ac-CO			170.1			170.2	170.5			170.3				169.6	169.6			
-Me			21.4			21.8	22.2			21.4				21.7	21.6			

Table 2. Cytotoxic activity against P388 leukaemic cell line

Compound	IC_{50} ($\mu g\ ml^{-1}$)	Compound	IC_{50} ($\mu g\ ml^{-1}$)
1	18.5	**10**	3.9
2	3.8	**11**	12.0
3	15.0	**12**	4.1
4	3.1	**13**	1.9
5	14.0	**14**	13.5
6	8.9	**15**	15.0
7	14.0	**16**	15.0
8	15.0	**17**	13.5
9	46.0	**18**	15.0

Compound **14**. Needles, mp 63–65°, $[\alpha]_D = +22$ ($CHCl_3$; *c* 0.246); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone CO), 1720, 1660 (CO), 1380, 1100, 1080; UV $\lambda_{max}^{CHCl_3}$ nm (ε): 246 (18 000); HRMS *m/z* (rel. int.): 468.290 $[M]^+$ ($C_{29}H_{40}O_5$) (4), 444 (13), 409 (32), 311 (22), 121 (100); ^1H NMR ($CDCl_3$): *s* 0.95, 1.07, 1.08, 1.10, 1.12, 1.44, (each 3H, 2s), 2.04 (3H, *s*, Ac), 5.20 (1H, *td*, $J = 11.1$, 4.9 Hz), 5.74 (1H, *d*, $J = 10.5$ Hz), 6.05 (1H, *d*, $J = 5.7$ Hz), 7.36 (1H, *d*, $J = 5.7$ Hz), 7.79 (1H, *d*, $J = 10.5$ Hz).

Compound **15**. Needles, mp 162°, $[\alpha]_D = +40$ ($CHCl_3$; *c* 112); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone CO), 1720, 1660 (CO), 1380, 1100, 1080; UV $\lambda_{max}^{CHCl_3}$ nm (ε): 245 (3480); HRMS *m/z* (rel. int.): 470.299 $[M]^+$ ($C_{29}H_{42}O_5$) (6), 428 (10), 411 (52), 313 (52), 121 (100); ^1H NMR ($CDCl_3$): *s* 0.94, 1.07, 1.09, 1.09, 1.11, 1.34 (each 3H, *s*), 2.02 (3H, *s*, Ac), 5.21 (1H, *td*, $J = 11.1$, 4.9 Hz), 5.74 (1H, *d*, $J = 10.5$ Hz), 7.79 (1H, *d*, $J = 10.5$ Hz).

Compound **16**. Needles, mp 75–77°, $[\alpha]_D = +56$ ($CHCl_3$; *c* 0.284); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1480, 1380, 1120, 1080; HRMS *m/z* (rel. int.): 488.350 $[M]^+$ ($C_{30}H_{48}O_5$) (0.1), 470 (2), 383 (18), 142 (50), 84 (100); ^1H NMR ($CDCl_3$): *s* 0.86, 0.99, 0.99, 1.03, 1.26, 1.28 (each 3H, *s*), 3.72 (1H, *br d*, $J = 9$ Hz, H-19), 4.25 (1H, *br d*, $J = 9$ Hz, H-19′).

Compound **17**. Needles, mp 63–65°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 3400 (OH), 1460, 1380, 1080; HRMS *m/z* (rel. int.): 490.366 $[M]^+$ ($C_{30}H_{50}O_5$) (0.1), 472 (0.7), 457 (3), 430 (9), 405 (20), 347 (44), 329 (24), 126 (100); ^1H NMR ($CDCl_3$): *s* 0.88, 0.98, 1.03, 1.13, 1.20, 1.23 (each 3H, *s*), 3.73 (1H, *br d*, $J = 9$ Hz, H-19), 4.24 (1H, *br d*, $J =$ Hz, H-19′).

Cytotoxicity. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay was performed in a 96-well plate [9, 10]. The assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Mouse P388 leukaemia cells (2×10^4 cells ml^{-1}) were inoculated in each well with 100 ml ml^{-1} of RPMI-1640 medium (Nissui Pharmaceutical Co.) supplemented with 5% foetal calf serum (Mitsubishi Chemical Industry Co.) and kanamycin (100 $\mu g\ ml^{-1}$) at 37° in a humidified atmosphere of 5% CO_2. Various drug concns (in 10μl) were added to the cultures at day 1 after transplantation. At day 3, 20 μl of MTT sol (5 mg ml^{-1}) per well was added to each cultured medium. After a further 4 hr of incubation, 100 μl 10% SDS–0.01 N HCl sol was added to each well, and formazan crystals in each well were dissolved by stirring. The measurements were performed using a microplate reader (Tohso MPR-A4*i*) with a two-wavelength system (550 and 700 nm). In all these experiments, 3 replicate wells were used to determine each point.

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