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Cycloartane type triterpenoids from the rhizomes of *Polygonum bistorta*

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Abstract

Two new compounds, 24(*E*)-ethylidenecycloartanone (**1**) and 24(*E*)-ethylidenecycloartan-3α-ol (**2**) were isolated from the rhizomes of *Polygonum bistorta*, together with seven known compounds viz., cycloartane-3,24-dione (**3**), 24-methylenecycloartanone (**4**), γ-sitosterol (**5**), β-sitosterol (**6**), β-sitosterone (**7**), friedelin (**8**) and 3β-friedelinol (**9**). All the cycloartane type triterpenoids, compounds **7** and **8** are reported for the first time from this plant. A combination of 1D and 2D NMR spectroscopy and MS were mainly used to elucidate the structures of the new compounds **1** and **2**.

Keywords: *Polygonum bistorta*; Cycloartane triterpenoids; β-Sitosterol; β-Sitosterone; Friedelin; 3β-Friedelinol; Cycloartanone

1. Introduction

Polygonum bistorta, commonly known as *Bistort or Snakeroot*, belongs to the *Polygonaceae* family. It is one of the strongest herb astringents. The ethanolic extract showed strong anti-inflammatory effect (Duwiejua et al., 1994); alnusenone and 3β-friedelinol were identified as active constituents for such effect (Duwiejua et al., 1999). It was also reported that the aqueous extract strongly inhibits the mutagenicity of Trp-P-1 (Miki et al., 1995). In the course of our investigations directed to the search for antitumour natural products from terrestrial plants, we have studied the rhizomes of *P. bistorta*. Two new and seven known compounds were isolated and characterised by spectral and other data. The known compounds, β-sitosterol (**6**), friedelin (**8**) and 3β-friedelinol (**9**) were confirmed also through 2D NMR spectroscopy.

2. Results and discussion

2.1. 24(*E*)-ethylidenecycloartanone (*1*)

The molecular formula of compound 1 was deduced as $C_{32}H_{52}O$ by the molecular ion peak at *m*/*z* 452.4019 in the HREIMS and ^{13}C NMR analysis. The IR spectrum of compound **1** showed absorption for ketone functionality (ν_{max} 1712 cm^{-1}). Its ^{13}C NMR spectrum exhibited 32 signals including one carbonyl carbon (δ 216.4 ppm) of cycloartane type (Cantillo-Ciau et al., 2001) and one ethylidene double bond (δ 145.8 and 116.4 ppm). The ^{1}H NMR spectrum of the compound supported the presence of cycloartane type skeleton with typical high-field AB doublets due to the non-equivalent hydrogens at C-19 in the cyclopropane ring (Cantillo-

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Ciau et al., 2001). Protons on methyl group at C-32 gave a clearly defined doublet at δ 1.60 ppm as expected for ethylidene group. The single proton on C-31 was also clearly evident showing the expected quartet at δ 5.13 ppm. Compound **1** and fucosterol have identical side chain structure. The ^{1}H NMR chemical shift values in the latter compound at these positions were δ 1.57 and 5.17 ppm, respectively (Nes et al., 1966). Such correlation also supported the proposed structure as well as used to assign the stereochemistry of compound **1** at C-24, which is similar to that of fucosterol. In otherwords, the methyl group at C-31 has *E*-stereochemistry. Fragment peak at *m*/*z* 313 ($M - C_{10}H_{19}$) was also an evidence of the presence of cycloartane skeleton (Ayatollahi et al., 1992) with C10 side chain. Inspection of its 2D NMR data (^{1}H–^{1}H COSY, HSQC-DEPT, HMBC) allowed us to assemble the structure **1**.

2.2. 24(*E*)-ethylidenecycloarta-3α-ol (**2**)

Compound **2** has molecular ion peak at *m*/*z* 454.4175 in the HREIMS corresponding to the molecular formula $C_{32}H_{54}O$. Fragment peaks at *m*/*z* 315 ($M - C_{10}H_{19}$) is the indicative of cycloartane skeleton with hydroxyl group and C10 side chain. The ^{1}H and ^{13}C chemical shift values at C-3 position were observed as δ 3.45 (*br*, *s*) and δ 77.0 ppm, respectively, indicating that the hydroxyl group is α-oriented at this position, which was confirmed by comparing these values with the related compound (Januario et al., 1992). For a β-orientation, these chemical shift values would be around δ 3.28 (*m*) and δ 78.8 ppm, respectively (Greca et al., 1994; Teres et al., 1987). The spectral correlations regarding its side chain structure and NMR chemical shift values for other positions are comparable to compound **1**.

2.3. Cycloartane-3,24-dione (**3**)

Compound (**3**) has molecular ion peak at *m*/*z* 440.3655 in the HREIMS, corresponding to the molecular formula $C_{30}H_{48}O_2$. Fragment peaks at *m*/*z* 313 ($M - C_8H_{15}O$) is an indicative of the presence of cycloartane skeleton with C8 side chain. The presence of McLafferty rearrangements product at *m*/*z* 354 indicated the presence of a carbonyl group in the side chain at C-24 (Davies et al., 1992). Although compound **3** had been reported earlier as an inseparable mixture, structure identification was made based on only MS fragmentation pattern of GC-MS analysis (Davies et al., 1992). However the same compound was reported in pure form (Ohtsu et al., 1998) but it had inconsistency with our assignment, in particular at the C-3 position. The MS fragmentation patterns for compound **3** were identical as reported in the literature (Davies et al., 1992; Ohtsu et al., 1998). NMR assignments were made for this compound and were listed.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Buchi Melting point B-540 apparatus. IR spectra were recorded on a Bio Rad, Excalibur Series, 600 Microwatts at 632.8 nm CW, Class II Laser product. ^{1}H and ^{13}C NMR spectra were recorded on Bruker, 300 or 500 MHz spectrometers. Chemical shifts are reported in parts per million (ppm) with TMS as a reference standard and coupling constants (*J*) are expressed in hertz. LREIMS were measured on a Finnigan/MAT MAT 95 XL-T or VG Micromass 7035. HREIMS were measured on Finnigan/MAT MAT 95 XL-T mass spectrometers. HPLC was carried on a Waters associates, μ-Porasil (300 × 5 mm) column with a Shimadzu RID-10A, refractive index detector. Silica gel 60 (Merck, 0.063–0.200 m) was used for column chromatography. Lichroprep RP-18 (Merck, 40–63 μm) was used for separation and/or purification. Precoated silica gel plates (Merck, Kieselgel 60F 254, 0.25 mm or Baker Si250F, 0.25 mm) were used for preparative TLC and/or analytical TLC. Spots were detected using UV light or by spraying with 50% H_2SO_4 and heating at 110 °C for 5 min.

3.2. Plant material

The plant materials were purchased from local market and a voucher specimen (KMano PB 2003) is deposited in the Department of Biological Sciences, National University of Singapore, Republic of Singapore.

3.3. Extraction and isolation

The rhizomes of *P. bistorta* (600 g) were ground into powder and then extracted with chloroform (3 l × 4) at room temperature. The residue dissolved in water/methanol mixture (95:5) and was then extracted successively with *n*-hexane and chloroform. Both extracts were showed significant cytotoxic activity toward the P388 and HL60 cancer cell lines. The IC_{50} values of the hexane extract were less than10 and 20 μg/ml toward P388 and HL60, respectively, while the chloroform extract had less than 20 μg/ml on both cell lines. The chloroform extract was chromatographed over silica gel using *n*-hexane and fractionated with increasing polarity. Further purification of the major fraction by HPLC (hexane/chloroform, 1:9) followed by preparative TLC (chloroform/methanol, 9:1) afforded 24-methylenecycloartanone **4** (7 mg) (Ohtsu et al., 1998; Bohme et al., 1997). We carried out further investigation by purchasing 12 kg of plant material and extracted it as previously. The hexane extract was chromatographed over silica gel using *n*-hexane and eluted in a gradient fashion

with increasing polarity. Purification of eluted fractions afforded friedelin (6 mg) (Klass et al., 1992; Deeb et al., 2003), 3β-friedelinol (112 mg) (Betancor et al., 1980; Salazar et al., 2000), β-sitosterol (1.2 g) (Klass et al., 1992; Nes et al., 1992), γ-sitosterol (4 mg) (Ulubelen, 1969; Biswas and Malik, 1986; Gopalakrishnan et al., 1990), 24(*E*)-ethylidenecycloartanone (580 mg), cycloartane-3,24-dione (ca. 1.5 mg). The chloroform extract was chromatographed over Lichroprep RP-18 and eluted in isocratic fashion with methanol. Purification of eluted fractions afforded β-sitosterol (110 mg), β-sitosterone (ca. 0.5 mg), 24(*E*)-ethylidenecycloartanone (7 mg) and 24(*E*)-ethylidenecycloartan-3α-ol (ca. 1.0 mg) (see Fig. 1).

3.4. 24(*E*)-ethylidenecycloartanone (**1**)

Colorless crystals; m.p. 135.4–137.1 °C; IR (KBr) ν_{max} 3046, 2939, 2864, 1712, 1448, 1376 cm^{-1}; MS (EI, 70 eV), *m/z* (rel. inten. %): 452 $[M]^+$ (72), 437 (42), 354 (86), 340 (48), 313 (72), 271 (44), 216 (78), 175 (51), 95 (100), 55 (54); HREIMS *m/z* 452.4019 (calcd. for $C_{32}H_{52}O$, 452.4018); ^1H and ^{13}C NMR data: see Table 1.

3.5. 24(*E*)-ethylidenecycloarta-3α-ol (**2**)

Colorless solid; MS (EI, 70 eV), *m/z* (rel. inten. %): 454 $[M]^+$ (72), 437 (42), 354 (86), 340 (48), 315 (72), 271 (44), 216 (78), 175 (51), 95 (100), 55 (54); HREIMS *m/z* 454.4175 (calcd for $C_{32}H_{54}O$, 454.4174); ^1H and ^{13}C NMR data: see Table 1.

3.6. Cycloartane-3,24-dione (**3**)

Colorless crystals; MS (EI, 70 eV), *m/z* (rel. inten. %): 440 $[M]^+$ (84), 425 (61), 407 (21), 379 (19), 354 (78), 342 (30), 313 (100), 302 (59), 271 (20), 203 (66), 175 (84), 135 (87), 127 (77); HREIMS *m/z* 440.3655 (calcd for $C_{32}H_{52}O$, 440.3654); ^1H and ^{13}C NMR data: see Table 1.

3.7. 24-methylenecycloartanone (**4**)

Colorless crystals; m.p. 111–113 °C; IR (KBr) ν_{max} 3040, 2939, 2862, 1712, 1442, 1376, 814 cm^{-1}; MS (EI, 70 eV), *m/z* (rel. inten. %): 438 (100), 423 (47), 395 (31), 355 (28), 340 (40), 313 (75), 300 (39), 219 (34), 175 (54), 147 (49), 95 (68), 69 (28), 55(15); ^1H and ^{13}C NMR data: same as the data reported in Ohtsu et al. (1998).

3.8. γ-Sitosterol (**5**)

Colorless crystals; mp147–148 °C; MS (EI, 70 eV), *m/z* (rel. inten. %): 414 (16), 145 (45), 95 (100), 83 (26), 93 (85), 71 (46), 55 (27).

3.9. β-Sitosterol (**6**)

Colorless flakes; m.p. 136.1–138 °C; IR (KBr) ν_{max} 3429, 2960, 2880, 1662, 1464, 1377, 1330, 1049 cm^{-1}; MS (EI, 70 eV), *m/z* (rel. inten. %): 414 (64), 396 (100), 381 (42), 303 (30), 255 (50), 231 (40), 173 (18), 145 (78), 121 (46), 107 (56), 81 (40), 55 (38); ^1H and ^{13}C NMR data: same as the data reported in Klass et al. (1992).

3.10. β-Sitosterone (**7**)

Amorphous solid; MS (EI, 70 eV), *m/z* (rel. inten. %): 412 (90), 397 (32), 370 (60), 327 (28), 289 (70), 271 (42), 229 (96), 187 (30), 149 (64), 124 (100), 69 (32), 55 (38) 43 (46); ^1H NMR data: same as the data reported in Gopalakrishnan et al. (1990).

3.11. Friedelin (**8**)

Colorless crystals; m.p. 263–265 °C; IR (KBr) ν_{max} 2946, 2882, 2869, 1719, 1464, 1385 cm^{-1}; MS (EI, 70 eV), *m/z* (rel. inten. %): 426 $[M]^+$ (28), 341 (12), 302 (32), 273 (54), 231 (38), 205 (56), 191 (44), 163 (57), 13 (100), 109 (98), 69 (75), 55 (43); ^1H and ^{13}C NMR data: same as the data reported in Deeb et al. (2003).

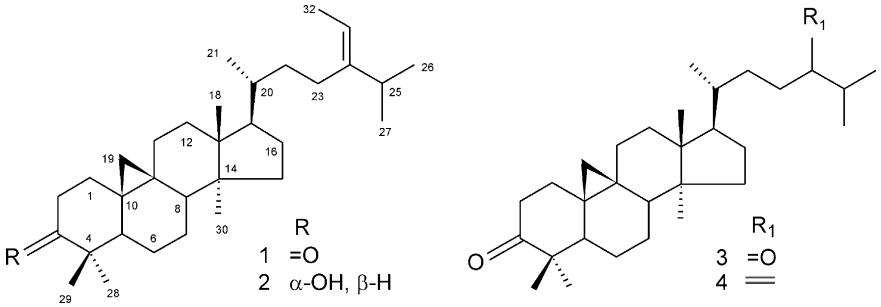

Fig. 1. Structures of compounds isolated from the rhizomes of *P. bistorta*.

Table 1
^{1}H NMR and ^{13}C spectral data of compounds **1**, **2** and **3** in $CDCl_3$

Position	1		2		3	
	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)
1α	33.3	1.87 *tdd* (13.8, 4.4, 1.5)	27.5	1.56 *m*	33.3	1.85 *dddd* (13.5, 13.0, 4.0, 1.4)
1β		1.56 *ddd* (13.5, 6.4, 2.7)		1.24 *m*		1.54 *ddd* (13. 5, 4.4, 2.7)
2α	37.4	2.72 *ddd* (13.5, 4.4, 2.7)	28.5	1.75 *m*	37.4	2.72 *ddd* (13.4, 4.4, 2.7)
2β		2.32 *ddd* (13.5, 4.5, 6.4)		1.56 *m*		2.32 *ddd* (13.5, 13.4, 6.5)
3	216.4		77.0	3.45 *br*, *s*	216.4	
4	50.2		39.5		50.2	
5	48.4	1.73 *dd* (12.5, 4.5)	41.0	1.28 *m*	48.4	1.70 *dd* (12.5, 4.5)
6α,6β	21.5	1.57 *m*, 0.96 *m*	21.1	1.58 *m*, 0.80 *m*	21.5	1.56 *m*, 0.93 *m*
7α	25.8	1.39 *m*	28.2	1.87 *m*	25.8	1.37 *dddd* (12.5, 12.5, 3.0, 12.5)
7β		1.16 *m*		1.28 *m*		1.12 *dddd* (12.5, 2.5, 4.9, 4.9)
8	47.8	1.61 *dd* (12.5, 4.9)	48.0	1.50 *m*	47.9	1.58 *dd* (12.5, 4.9)
9	21.0		19.8		21.0	
10	25.9		26.4		25.9	
11α,11β	26.7	2.06 *m*, 1.19 *m*	26.2	1.96 *m*, 1.10 *m*	26.7	2.05 *m*, 1.16 *m*
12	32.7	1.68 *t* (7.5), (2H)	32.8	1.60 *m*	32.8	1.64 *t* (7.5), (2H)
13	45.3		45.3		48.7	
14	48.7		48.9		45.3	
15	35.5	1.33 *m* (2H)	35.4	1.28 *m*	35.5	1.32 *m* (2H)
16α,16β	28.1	1.95 *m*, 1.33 *m*	25.9	1.33 *m*, 1.10 *m*	28.0	1.94 *m*, 1.32 *m*
17	52.2	1.63 *m*	52.0	1.56 *m*	52.2	1.59 *m*
18	18.0	1.01 *s*	19.3	0.94 *s*	18.3	1.09 *s*
19α	29.4	0.80 *d* (4.5)	29.7	0.49 *d* (4.0)	29.5	0.78 *d* (4.3)
19β		0.59 *d* (4.3)		0.33 *d* (4.0)		0.57 *d* (4.3)
20	36.4	1.41 *m*	35.9	1.36 *m*	35.6	1.39 *m*
21	18.3	0.92 *d* (6.5)	18.1	0.92 *d* (5.2)	20.8	1.09d (6.5)
22α,22β	36.2	1.55 *m*, 1.12 *m*	34.7	1.34 *m*, 0.92 *m*	30.0	1.77 *m*, 1.24 *m*
23α	28.2	2.03 *m*	26.8	1.33 *m*	37.4	2.14 *ddd* (16.6, 9.7, 6.0)
23β		1.78 *m*		1.10 *m*		2.14 *ddd* (16.6, 10.0, 5.2)
24	145.8		149.5		215.4	
25	28.5	2.84 *m*	29.5	2.80 *m*	40.8	2.61 *m*
26	21.0	1.00 *d* (6.4)	21.9	0.92 *d* (6.0)	18.6	0.86 *d* (6.5)
27	21.0	1.00 *d* (6.4)	21.9	0.81 *d* (6.0)	18.0	0.98 *d* (6.5)
28	22.1	1.06 *s*	22.2	0.88 *s*	22.1	1.04 *s*
29	20.7	1.11 *s*	20.8	0.92 *s*	20.7	1.09 *s*
30	19.2	0.92 *s*	19.3	0.85 *s*	19.3	0.90 *s*
31	116.4	5.13 *q* (6.5) (3H)	116.4	5.17 *q* (6.7) (3H)	–	–
32	12.7	1.60 *d* (6.0) (1H)	12.8	1.58 *d* (6.1) (1H)	–	–

3.12. 3β-Friedelinol (**9**)

Colorless crystals; m.p. 280–282 °C; IR (KBr) ν_{max} 3500, 1380, 1370, 1265, 1175, 1020, 800 cm^{-1}; *m*/*z* (rel. inten. %): 428 $[M]^+$ (215), 413 (27), 275 (46) 248 (26), 231 (52), 220 (42), 205 (53), 177 (57), 165 (82), 125 (98), 95 (100), 69 (76), 55(34); ^{1}H and ^{13}C NMR data: same as the data reported in Salazar et al. (2000).

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