CHEMICAL CONSTITUENTS FROM EUPHORBIA WALLICHII

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Abstract Eleven known compounds were isolated from the roots of *Euphorbia wallichii* for the first time. They were elucidated to be three triterpenoids, β-amyrin (1), β-amyrin acetate (2) and 3β-acetoxy-lupenol (3), one nor-triterpene peroxide baccatin (4), two caffeic esters (5**a**, 5**b**), palmitic acid-2,3-dihydroxy-propanenyl ester (6), palmitic acid (7), scopoletin(8), β-sitosterol (9) and daucosterol (10) on the basis of spectral methods. Among them, compound 5**a**, 5**b** were reported firstly in the spurge family. And the NMR assignments of compounds 5a and 5b were given for the first time.

Key words *Euphorbia wallichii*; *Euphorbia*; baccatin; caffeic ester; palmitic acid-2, 3-dihydroxy-propanenyl ester

The genus *Euphorbia* is the largest in the spurge family, comprising about 2000 species. More than 80 of them are distributed in China. Various groups[1~3] had been working on the chemical constituents of this genus, finding many biologically active diterpenes. *Euphorbia wallichii*, a traditional medicinal plant, mainly distributed in Qinghai province, Tibet and Yunnan province of China, has been used by Tibetan of China to cure furuncle, exanthema and cutaneous anthrax. To make full use of this plant, we studied its chemical constituents. From the alcohol extract of the roots of this plant, we have obtained three triterpenoids, β-amyrin (1[4]), β-amyrin acetate (2[4]) and 3β-acetoxy-lupenol (3[5]), one nor-triterpene peroxide baccatin (4[6]), two caffeic esters (5**a**[7], 5**b**[7]), palmitic acid-2,3-dihydroxy-propanenyl ester (6[8]), palmitic acid (7[9]), scopoletin (8), β-sitosterol (9) and daucosterol (10). In many reports[10], 4 was used as material to synthesize drugs. And 5**a** and 5**b** were seldom reported. In this paper, we describe the isolation and structure elucidation of these compounds.

1 Results and discussion

Compound 4 gave a molecular formula $C_{29}H_{46}O_4$ by EIMS and ^{13}C NMR spectrum. The ^1H NMR spectrum showed signals at δ 0.80～1.01 (7-tertiary CH_3), 3.04 (1H, d, 9.4 Hz), 3.78 (1H, m), 6.42 (1H, d, 9.0 Hz), and 6.68 (1H, d, 9.0 Hz), which was very similar to that of Baccatin[6]. Thus 4 was assumed to be Baccatin, supported by the HMQC and HMBC spectra (see table 1). And the ^{13}C NMR data were given (see table 2).

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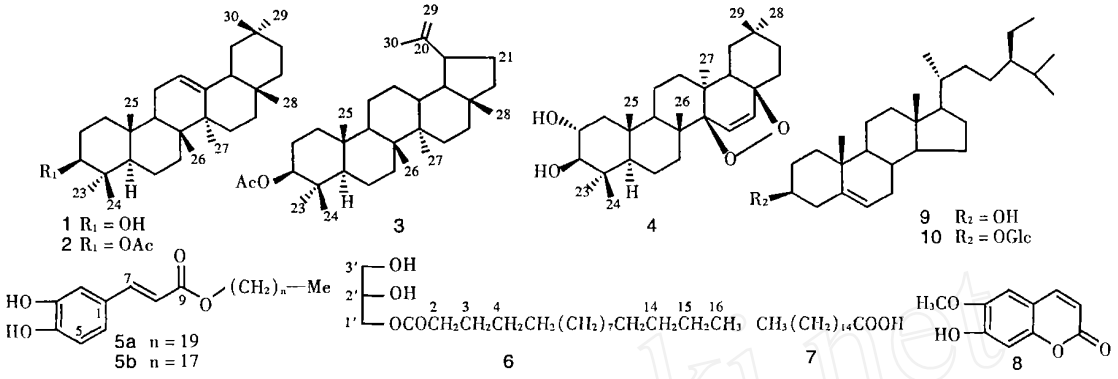

Table 1 The key correlations of HMQC and HMBC for compound 4 (500 MHz, $CDCl_3$)

Position	δ_H	δ_C	HMBC
1	2.08; 0.91	46.7	C-2, C-3, C-5, C-10
3	3.04	83.9	C-1, C-2, C-4, C-23, C-24
9	1.86	51.2	C-1, C-8, C-10, C-26
12	2.04	41.0	C-11, C-27
15	6.68	131.8	C-8, C-13, C-14, C-17
16	6.42	135.2	C-14, C-17, C-18, C-22
19	1.30; 0.65	40.6	C-17, C-18, C-20, C-21, C-29
21	1.43; 1.31	35.1	C-17, C-20, C-22, C-29
22	1.60	27.5	C-16, C-17, C-20, C-21
23	0.99	28.2	C-3, C-4, C-5, C-24
24	0.80	16.0	C-3, C-4, C-5, C-23
25	0.89	18.4	C-1, C-2, C-5, C-9, C-10
26	1.01	18.0	C-8, C-9, C-14
27	0.95	24.8	C-13, C-14, C-18

Table 2 ^{13}C-NMR spectral data for compounds 2～4 (100 MHz, $CDCl_3$)

C	2	3	4
1	38.2 (t)	38.4 (t)	46.7 (t)
2	23.7 (t)	23.7 (t)	69.3 (d)
3	80.9 (d)	81.0 (d)	83.9 (d)
4	37.7 (s)	37.8 (s)	39.2 (s)
5	55.2 (d)	55.4 (d)	55.9 (d)
6	18.2 (t)	18.2 (t)	19.0 (t)
7	32.6 (t)	34.2 (t)	39.0 (t)
8	39.8 (s)	40.9 (s)	43.0 (s)
9	47.5 (d)	50.3 (d)	51.2 (d)
10	36.8 (s)	37.1 (s)	38.9 (s)
11	23.5 (t)	20.9 (t)	19.3 (t)
12	121.6 (d)	25.1 (t)	41.0 (t)
13	145.2 (s)	38.0 (d)	39.7 (s)
14	41.7 (s)	42.8 (s)	87.4 (s)
15	28.4 (t)	27.4 (t)	131.8 (d)
16	26.1 (t)	35.6 (t)	135.2 (d)
17	32.5 (s)	43.0 (s)	77.2 (s)
18	47.2 (d)	48.3 (d)	49.7 (d)
19	46.8 (t)	48.0 (d)	40.6 (t)
20	31.1 (s)	150.9 (s)	30.8 (s)
21	34.7 (t)	29.8 (t)	35.1 (t)
22	37.1 (t)	40.0 (t)	27.5 (t)
23	28.0 (q)	27.9 (q)	28.2 (q)
24	16.7 (q)	16.5 (q)	16.0 (q)
25	15.5 (q)	16.2 (q)	18.4 (q)
26	16.8 (q)	16.0 (q)	18.0 (q)
27	25.9 (q)	14.5 (q)	24.8 (q)
28	26.9 (q)	18.0 (q)	32.9 (q)
29	33.3 (q)	109.3 (t)	23.7 (q)
30	23.6 (q)	19.3 (q)	
31	171.0 (s)	171.0 (s)	
32	21.3 (q)	21.3 (q)	

Compound 5**a**,5**b** were isolated as a mixture. The ^{1}H NMR of 5 showed signals for a typical ABX spin system at δ 6.61 (1H, d, 8.2 Hz), 6.72 (1H, dd, 8.2 Hz, 2.1 Hz) and 6.93 (1H, d, 2.1 Hz), indicating the presence of three protons with *ortho*, *ortho*/*meta* and meta coupling, respectively. A trans double bond was presented by signals at δ 6.05 and 7.34 ppm with a coupling constant of J = 15.9 Hz. It also showed signals for a methyl (δ 0.68, t, 7.0 Hz), a methylene (δ 1.47, m) and numbers of methylenes at δ 1.05 ppm. The above ^{1}H NMR spectra were identical to those of caffeic ester in reference. So it was established as a caffeic ester, which was confirmed by HMQC and HMBC spectra. Furthermore, the EIMS spectrum showed two molecular weight at m/z 460 (28) and 432 (68). Hence 5 was a mixure of Caffeic acid eicosyl ester (5**a**, $C_{29}H_{48}O_4$) and Caffeic acid octadecyl ester (5**b**, $C_{27}H_{44}O_4$). According to the relative intensitites of the molecular ions, it was estimated by the method of ref.[11] that 5**a** and 5**b** were present in the approximate ratio 1∶2.4.

2 Experimental

2.1 Apparatus and Plant materials

MS spectra were obtained with a VG Auto Spec-3000 spectrometer, at 70 eV for EI. 1D and 2D-NMR spectra were recorded on a Bruker AM-400 and a DRX-500 MHz spectrometer with TMS as internal standard. And Silica gel (200～300 mesh) for CC and GF254 for analytical TLC were from the Qingdao Marine Chemical Factory, P. R. China. *Euphorbia*

wallichii was collected from Xinghai county, Qinghai province, China, in July 2001. It was identified by Prof. ZHANG Xiao-feng, Northwest Plateau Institute of Biology, Academia Sinica, Xining, Qinghai, P. R. China, where a voucher specimen (No. 1002) was deposited.

2.2 **Extraction and Isolation**

Air-dried roots (10 kg) were extracted with EtOH (95 %) for four times. After removal of the solvent by evaporation, the residues were suspended in H_2O and extracted with $CHCl_3$ for three times. The $CHCl_3$ fraction was concentrated in vacuo to give 100 g of the residue. The residue was separated repeatedly by chromatography on silica gel column and RP-18 to afford 1(46 mg), 2(3691 mg), 3(16 mg), 4(30 mg), 5(20 mg), 6(10 mg), 7(20 mg), 8(90 mg), 9(500 mg), and 10(102 mg).

3 Indentification

β-Amyrin (1) $C_{30}H_{50}O$, colorless needles; EIMS *m/z* 426 $[M]^+$ (49), 411 (15), 257 (12), 247 (11), 229 (11), 218 (100), 203 (68), 189 (51), 176 (29), 161 (28), 147 (32), 135 (57), 121 (49), 109 (58), 95 (68), 81 (57), 69 (64), 55 (50). These data were identical with those of β-amyrin[4], and its TLC was identical with an authentic sample.

β-Amyrin acetate (2) $C_{32}H_{52}O_2$, colorless needles; ^{1}H-NMR ($CDCl_3$): δ 5.15 (1H, t, 3.4 Hz, H-12), 4.47 (1H, m, H-3), 2.01 (3H, s, H-32); ^{13}C-NMR ($CDCl_3$) see table 2; EIMS *m/z* 468 $[M]^+$ (22), 453 (6), 257 (6), 229 (3), 218 (100), 203 (55), 189 (25), 135 (27), 121 (28), 107 (39), 95 (38), 81 (36), 69 (54), 55 (36). These data were identical with those of β-amyrin acetate[4].

3β-Acetoxy-lupenol (3) $C_{32}H_{52}O_2$, colorless needles; ^{1}H-NMR ($CDCl_3$): δ 4.66 (1H, d, 2.0 Hz, H-29′), 4.55 (1H, d, 2.0 Hz, H-29), 4.50 (1H, dd, 7.2, 13.0 Hz, H-3), 2.02 (3H, s, H-32), 1.66 (3H, s, H-30); ^{13}C-NMR ($CDCl_3$) see table 2; EIMS *m/z* 466 $[M-2H]^+$ (100), 451 (29), 406 (24), 391 (18), 355 (32), 296 (18), 274 (20), 247 (32), 227 (18), 217 (53), 202 (50), 188 (82), 174 (38), 160 (39), 146 (42), 135 (56), 120 (57), 108 (55), 94 (56), 80 (52), 68 (51), 54 (47). These data were identical with those of 3β-acetoxy-lupenol[5].

Baccatin (4) $C_{29}H_{46}O_4$, colorless needles; ^{1}H-NMR ($CDCl_3$): δ 0.91, 2.08 (2H, m, H-1), 3.78 (1H, m, H-2), 3.04 (1H, d, 9.4 Hz, H-3), 0.92 (1H, m, H-5), 1.61 (2H, m, H-6), 1.92 (2H, m, H-7), 1.86 (1H, m, H-9), 1.70 (2H, m, H-11), 2.04 (2H, m, H-12), 6.68 (1H, d, 9.0 Hz, H-15), 6.42 (1H, d, 9.0 Hz, H-16), 1.91 (1H, m, H-18), 0.65, 1.30 (2H, m, H-19), 1.31, 1.43 (2H, m, H-21), 1.60 (2H, m, H-22), 0.99 (3H, s, H-23), 0.80 (3H, s, H-24), 0.89 (3H, s, H-25), 1.01 (3H, s, H-26), 0.95 (3H, s, H-27), 0.90 (3H, s, H-28), 0.93 (3H, s, H-29); ^{13}C-NMR ($CDCl_3$) see table 2; EIMS *m/z* 458 $[M]^+$ (19), 443 (17), 426 (36), 308 (37), 291 (28), 221 (20), 208 (94), 191 (70), 177 (86), 163 (46), 151 (51), 135 (46), 121 (81), 109 (96), 95 (75), 81 (71), 69 (97), 55 (100). These data were identical with those of baccatin[6].

Caffeic acid eicosyl ester(5a) ($C_{29}H_{48}O_4$) **and caffeic acid octadecyl ester (5b)** ($C_{27}H_{44}O_4$) White powders; ^{1}H-NMR (Me_3OD): δ 7.34 (1H, d, 15.9 Hz, H-7), 6.93 (1H, d, 2.1 Hz, H-2), 6.72 (1H, dd, 8.2, 2.1 Hz, H-6), 6.61 (1H, d, 8.2 Hz, H-5), 6.05 (1H, d, 15.9 Hz, H-8), 3.94 (2H, t, 6.7 Hz, H-1′), 1.47 (2H, m, H-2′), 1.05 (br s, H-3′～H-n′), 0.68 (3H, t, 7.0 Hz, Me); ^{13}C-NMR (Me_3OD): δ 127.6 (C-1), 115.2 (C-2), 146.9 (C-3), 146.9 (C-4), 116.5 (C-5), 122.9 (C-6), 146.7 (C-7), 115.2 (C-8), 169.2 (C-9), 65.5 (C-1′), 29.8 (C-2′), 27.1 (C-3′), 30.4～30.7 (C-4′～C-(n-2)′), 33.1 (C-(n-1)′), 23.7 (C-n′), 14.5 (Me); EIMS *m/z* 460 ($[M]^+$ of 5**a**) (28), 432 ($[M]^+$ of 5**b**) (68), 180 (100), 163 (71), 136 (38), 123 (20), 97 (12), 89 (13), 83 (18), 69 (26), 57 (41), 55 (39). These data were identical with those of caffeic esters[7].

Palmitic acid-2,3-dihydroxy-propanenyl ester (6) $C_{19}H_{38}O_4$, white powders; ^{1}H-NMR ($CDCl_3$): δ 4.19 (2H, m, H-1′), 3.93 (1H, m, H-2′), 3.64

(2H, m, H-3′), 2.33 (2H, t, 7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.24 (24H, br s, H-4～H-15), 0.85 (3H, t, 6.7 Hz, H-16); ^{13}C-NMR (CDCl3): δ 65.1 (C-1′), 70.2 (C-2′), 63.3 (C-3′), 174.4 (C-1), 34.1 (C-2), 24.9 (C-3), 29.1～29.7 (C-4～C-13), 31.9 (C-14), 22.7 (C-15), 14.1 (C-16); EIMS *m/z* 299 $[M-H]^+$ (39), 270 (21), 257 (48), 239 (100), 134 (70), 112 (38), 98 (94), 84 (58), 74 (60), 57 (49). These data were identical with those of reference[8].

Palmitic acid (7) $C_{16}H_{32}O_2$, white powders; EIMS *m/z* 256 $[M]^+$ (53), 239 (4), 221 (4), 213 (9), 196 (14), 185 (22), 171 (13), 165 (5), 157 (10), 140 (7), 129 (100), 115 (23), 99 (24), 85 (21), 73 (57), 60 (33), 57 (50). These data were identical with those of reference[9].

Scopoletin (8), β-sitosterol (9) and daucosterol (10) were respectively indentified by TLC with authentic samples.

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大果大戟的化学成分

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摘 要 从大果大戟的根部首次分离得到11个化合物。利用波谱方法鉴定为β-香树素(1) β-香树素乙酸酯(2), 3β-乙酰化羽扇豆烯醇(3), baccatin(4), 2个caffeic esters(5**a**, 5**b**), 棕榈酸-1-甘油酯(6), 棕榈酸(7), 东莨菪内酯(8) β-谷甾醇(9)和胡萝卜甙(10)。其中5**a**, 5**b**是第一次在大戟属中得到; 并对5**a**, 5**b**的碳谱和氢谱数据进行了全归属。

关键词 大果大戟; 大戟属; baccatin; caffeic ester; 棕榈酸-1-甘油酯