Euphane Triterpenes from the Bark *of Broussonetia papyrifera*

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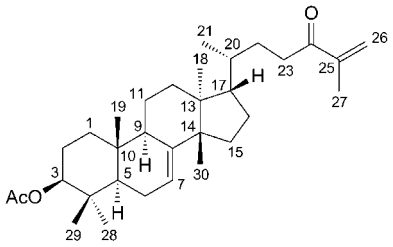
Four new euphane triterpenes, (3*β*)-3-(acetyloxy)eupha-7,25-dien-24-one (**1**), (3*β*,24*R*)-3-(acetyloxy)eupha-7,25-dien-24-ol (**2**), (3*β*,24*S*)-eupha-7,25-diene-3,24-diol (**3**), and (3*β*,24*R*)-eupha-7,25-diene-3,24-diol (**4**) were isolated from the barks of *Broussonetia papyrifera*, a tree whose roots, barks, and fruits were used as herbal drugs in China. The structures of **1**–**4** were elucidated on the basis of spectroscopic evidence and chemical methods.

Introduction. – *Broussonetia papyrifera* (LINN.) VENT., belonging to the family Moraceae, has a wide distribution in China. The roots, barks, and fruits are all used in Chinese traditional and herbal drugs [1]. Previous phytochemical investigations of this plant have resulted in the isolation of flavonols, coumarins, and 1,3-diphenylpropanes [2–4]. In a previous study, we isolated three alkaloids from this plant with *α*-glycosidase-inhibiting activities as a part of our search for bioactive compounds from natural resources [5]. In this article, we describe the isolation and structural elucidation of four new euphane triterpenoids.

Results and Discussion. – The 90% EtOH extract of the dried barks of *B. papyrifera* was suspended in H_2O and extracted with $CHCl_3$. After evaporation, the $CHCl_3$ extract was dissolved in MeOH/H_2O 1:1 and extracted with petroleum ether. The petroleum ether extract was subjected to repeated column chromatography (silica gel) to afford compounds **1**–**4** (*Fig. 1*).

Compound **1** was isolated as a white powder. The molecular formula was determined as $C_{32}H_{50}O_3$ by the HR-ESI-MS (*m*/*z* 505.3649 ($[M+Na]^+$)). The IR spectrum of **1** exhibited absorptions at 1732 and 1672 cm^{-1}, indicating the presence of C=O and C=C groups. The presence of an AcO function was shown by a signal in the ^{13}C-NMR spectrum (*Table*) at *δ*(C) 170.6, an additional Me *s* in the ^{1}H-NMR spectrum at *δ*(H) 1.95, and a cross-peak in the HMBC plot between *δ*(H) 1.95 and *δ*(C) 170.6 (*Fig. 2*). The ^{13}C-NMR (DEPT) spectra showed signals for eight quaternary C-atoms and six CH and ten CH_2 groups, including two characteristic C-atoms (*δ*(C) 117.6 and 145.7), indicating for **1** an euphane or tirucallane triterpene skeleton (differing from the latter in configuration at C(20)) with a C=C bond between C(7) and C(8) [6]. The presence of a NOESY correlation of Me(21) with CH_2(16) and the positive optical



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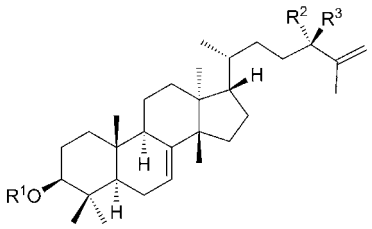


	R^1	R^2	R^3
2	Ac	H	OH
3	H	OH	H
4	H	H	OH

Fig. 1. *Compounds* **1**–**4**, *isolated from* Broussonetia papyrifera

rotation strongly suggested that **1** belonged to the euphane rather than tirucallane series [7]. The ^{13}C-NMR data of C(24), C(25), and C(26) were in good agreement with those of methyl (3α)-3-(acetyloxy)-24-oxotirucalla-8,25-dien-21-oate [8], suggesting the presence of a conjugated ketone in the side chain. The structure of the side chain of **1** was further confirmed by the HMBCs δ(H) 1.12–1.19 (1 H of CH_2(22))/δ(C) 202.1 (C(24)), δ(H) 2.53 and 2.61 (CH_2(23))/δ(C) 35.6 (C(20)), δ(H) 5.66 and 5.86 (CH_2(26))/δ(C) 202.1 (C(24)), δ(H) 1.78 (Me(27))/δ(C) 202.1 (C(24)), δ(H) 1.78 (Me(27))/δ(C) 124.0 (C(26)), and δ(H) 0.76 (Me(21))/δ(C) 29.7 (C(22)). The other part of the structure could be confirmed on the basis of the HMBCs (*Fig. 2*). Thus, compound **1** was determined to be (3β)-3-(acetyloxy)eupha-7,25-dien-24-one.

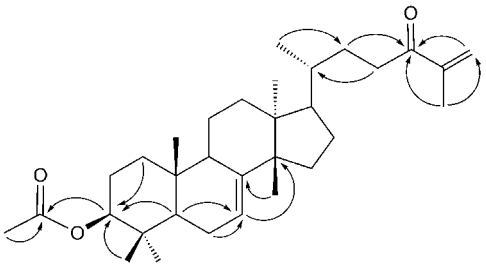


Fig. 2. *Important HMBC features of compound* **1**

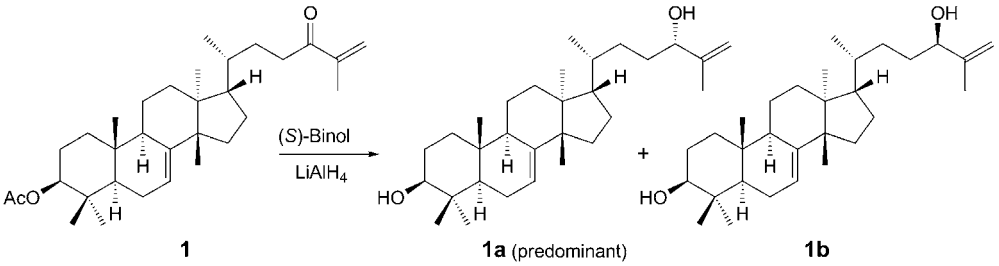
Compound **2** was obtained as a white powder. The HR-ESI-MS (*m*/*z* 507.3823 ([M + Na]$^+$)) and ^{13}C-NMR (DEPT) data indicated the molecular formula $C_{32}H_{52}O_3$ for **2**. In the IR spectrum of **2**, the absorption bands at 3514 and 1714 cm^{-1} revealed the presence of OH and ester groups. The NMR features of **2** were similar to those of **1**. The main difference between **1** and **2** were the molecular mass and the chemical shifts of the H- and C-atoms associated with C(23), C(24), C(25), and C(26). The molecular mass of **2** was 2 mass units higher than that of **1**. In the ^{13}C-NMR spectrum, the chemical shift of C(24) and C(26) was upshifted to δ(C) 76.2 and 111.0 with respect to δ(C) 202.1 and 124.0 for **1**. Based on the above data, an OH group was deduced to be at C(24) in **2**. The absolute configuration of C(24) was determined by *Noyori*'s asymmetric reduction of compound **1** with $LiAlH_4$ as reducing reagent and (*S*)-

Table. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; $CDCl_3$) *of Compounds* **1**–**4**. δ in ppm, J in Hz.

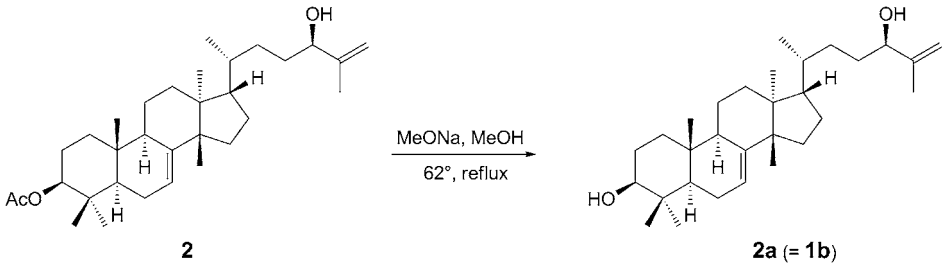
	1		**2**		**3/4**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	36.7	1.12–1.19, 1.52–1.61 (2*m*)	36.8	1.20–1.31, 1.59–1.66 (2*m*)	37.2	1.21–1.36, 1.59–1.68 (2*m*)
CH_2(2)	24.1	1.52–1.61 (*m*)	24.2	1.59–1.66 (*m*)	27.7	1.59–1.68 (*m*)
H–C(3)	80.9	4.42 (*d*, *J* = 9.8)	81.1	4.49 (*d*, *J* = 9.6)	79.2	3.23 (*d*, *J* = 11.4)
C(4)	37.7		37.8		38.9	
H–C(5)	50.7	1.33 (*m*)	50.8	1.40–1.43 (*m*)	50.6	1.36–1.57 (*m*)
CH_2(6)	23.7	1.84–1.89, 2.02–2.07 (2*m*)	23.8	1.85–1.96, 2.09–2.12 (2*m*)	23.9	1.87–1.98, 2.12–2.20 (2*m*)
H–C(7)	117.6	5.15 (*s*)	117.6	5.23 (*s*)	117.8	5.25 (*s*)
C(8)	145.7		145.9		145.8	
H–C(9)	48.7	2.13 (*d*, *J* = 9.5)	48.8	2.20 (br. *s*)	48.9	2.12–2.20 (*m*)
C(10)	34.7		34.8		34.9	
CH_2(11)	18.0	1.40 (*m*)	18.2	1.47–1.56 (*m*)	18.2	1.36–1.57 (*m*)
CH_2(12)	33.6	1.52–1.61 (*m*)	33.7	1.59–1.66 (*m*)	33.7	1.59–1.68 (*m*)
C(13)	43.4		43.5		43.5	
C(14)	51.2		51.2		51.2	
CH_2(15)	33.8	1.72 (*t*, *J* = 9.8), 1.84–1.89 (*m*)	33.9	1.59–1.66 (*m*), 1.85–1.96 (2*m*)	34.0	1.59–1.68 (*m*), 1.87–1.98 (2*m*)
CH_2(16)	28.3	1.12–1.19 (*m*)	28.9	1.20–1.31 (*m*)	28.4	1.21–1.36 (*m*)
H–C(17)	53.2	1.40 (*m*)	53.2	1.47–1.56 (*m*)	53.3	1.36–1.57 (*m*)
18)	21.9	0.74 (*s*)	22.1	0.79 (*s*)	22.1	0.80 (*s*)
19)	13.1	0.68 (*s*)	13.1	0.74 (*s*)	13.1	0.74 (*s*)
H–C(20)	35.6	1.33 (*m*)	35.9	1.40–1.43 (*m*)	36.1	1.36–1.57 (*m*)
21)	18.5	0.76 (br. *s*)	18.7	0.83 (br. *s*)	18.5	0.85 (*d*, *J* = 6.8)
CH_2(22)	29.7	1.12–1.19, 1.84–1.89 (2*m*)	30.8	1.20–1.31, 1.85–1.96 (2*m*)	30.8	1.21–1.36, 1.87–1.98 (2*m*)
CH_2(23)	34.9	2.53, 2.61 (2*m*)	32.2	0.98–1.04 (*m*), 1.79 (*t*, *J* = 9.6)	32.2	1.77–1.85, 1.77–1.85 (2*m*)
C(24) or H–C(24)	202.1		76.2	4.00 (*t*, *J* = 6.2)	76.5/76.2	4.01 (*t*, *J* = 5.9)
C(25)	144.5		147.8		147.6/147.8	
CH_2(26)	124.0	5.66, 5.86 (2*s*)	111.0	4.82, 4.91 (2*s*)	111.2/111.0	4.83, 4.92 (2*s*)
Me(27)	17.6	1.78 (*s*)	17.5	1.71 (*s*)	17.4	1.72 (*s*)
Me(28)	27.5	0.76 (br. *s*)	27.6	0.83 (br. *s*)	27.6	0.81 (*s*)
Me(29)	15.8	0.84 (*s*)	15.8	0.91 (*s*)	14.7	0.97 (*s*)
Me(30)	27.2	0.89 (*s*)	27.3		27.3	0.97 (*s*)
Me*C*O	170.6		171.0			
*Me*CO	21.2	1.95 (*s*)	21.3	2.03 (*s*)		

binaphthol as asymmetric ligand [9] (*Scheme 1*). Two diastereoisomers, **1a** and **1b**, were obtained, and the (24*S*)-isomer **1a** was predominant. As the ^{13}C-NMR signals of **1a** and **1b** were very close, the configuration of **2** could not be determined by just comparing the ^{13}C-NMR spectra. To clarify the small spectral differences, **2** was hydrolyzed to remove the Ac group to afford **2a** (*Scheme 2*). In the ^{13}C-NMR spectra of the mixture **2a**/**1a**/**1b**, the signals of **1b** at δ(C) 111.0 and 147.8 were increased with respect to those

Scheme 1. *Stereoselective Reduction of Compound* **1**



Scheme 2. *Hydrolysis of Compound* **2**



of **1a**, indicating that the absolute configuration at C(24) of **2** is (24*R*). Therefore, **2** was identified as (3*β*,24*R*)-3-(acetyloxy)eupha-7,25-dien-24-ol.

Compounds **3** and **4** were obtained as an inseparable stereoisomer mixture. Its molecular formula was established as $C_{30}H_{50}O_2$ by the HR-ESI-MS (*m*/*z* 443.3906 ($[M+H]^+$)), in combination with the ^{13}C-NMR data. The ^{13}C-NMR data of **3**/**4** were identical to those of the mixture **1a**/**1b** in equal quantity. As a result, compounds **3** and **4** were identified as (3*β*,24*S*)- and (3*β*,24*R*)-eupha-7,25-diene-3,24-diol, respectively.

The presence of euphane triterpenes in *Broussonetia papyrifera* was reported for the first time in this article. Assignment of the absolute configuration of **2** at C(24) was established by a stereoselective reduction. Compounds **3** and **4** were isolated as a mixture due to their strong structural similarity. It is noteworthy that lanostane derivatives with the same substitution pattern have also been obtained as 24-epimer mixtures [10].

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Experimental Part

General. Column Chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*). TLC: SiO_2 *G* precoated plates (*Qingdao Haiyang Chemical Co.*), with AcOEt/petroleum ether 1:4; visualization by spraying with 5% H_2SO_4 soln., followed by heating. Optical rotations: *Perkin–Elmer 341* polarimeter. IR Spectra: *Perkin–Elmer 1725X-FT* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker Avance-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Bruker Apex-III* spectrometer; in *m*/*z*.

Plant Material. The dried bark of *B. papyrifera* was collected in Kaixian County, Chongqing, P. R. China, in November 2007, and identified by Prof. *Wei-Kai Bao*, Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (CIB 2007–11–15) was deposited in our laboratory.

Extraction and Isolation. The dried barks of *B. papyrifera* (16.0 kg) was exhaustively extracted 3 × with 90% EtOH (3 × 20 l) for a week at r.t. to give an extract (*ca.* 1.3 kg), which was suspended in H_2O (2.0 l) and then partitioned with $CHCl_3$ (4 × 1.0 l). After evaporation of the solvent, the $CHCl_3$ extract (680 g) was dissolved in MeOH/H_2O 1:1 and then extracted with petroleum ether (4 × 0.5 l). The petroleum ether extract (500 g) was subjected to CC (SiO_2, petroleum ether/AcOEt 100:0 → 0:100): *Fractions A–K*. Repeated CC (SiO_2, petroleum ether/AcOEt 100:0 → 30:70, and petroleum ether/acetone 0:100 → 30:70) of *Fr. D* (24.3 g) yielded **1** (130 mg) and **2** (1.2 g). *Fr. F* (38.9 g) was subjected to CC (SiO_2, petroleum ether/AcOEt 90:10 → 40:60): **3**/**4** (41 mg).

(3β)-3-(Acetyloxy)eupha-7,25-dien-24-one (=*(3β,13α,14β,17α)-3-(Acetyloxy)lanosta-7,25-dien-24-one*; **1**): White powder. $[\alpha]_D^{20} = +11.4$ ($c = 0.22$, $CHCl_3$). IR (KBr): 3107, 2965, 2950, 2874, 2852, 1732, 1672, 1247. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 505.3649 ($[M+Na]^+$, $C_{32}H_{50}NaO_3^+$; calc. 505.3658).

*(3β,24*R*)-3-(Acetyloxy)eupha-7,25-dien-24-ol* (=*(3β,13α,14β,17α,24*R*)-Lanosta-7,25-diene-3,24-diol 3-Acetate*; **2**): White powder. $[\alpha]_D^{20} = +6.7$ ($c = 0.27$, $CHCl_3$). IR (KBr): 3514, 2946, 1714, 1260. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 507.3823 ($[M+Na]^+$, $C_{32}H_{52}NaO_3^+$; 507.3814).

(3β,24S)/(3β,24R)-Eupha-7,25-diene-3,24-diol (=*(3β,13α,14β,17α,24*S*)/(3β,13α,14β,17α,24*R*)-Lanosta-7,25-diene-3,24-diol*; (**3**/**4**): Colorless gum. $[\alpha]_D^{20} = +1.7$ ($c = 0.66$, $CHCl_3$). IR (KBr): 3452, 2962, 1061, 1029. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 443.3906 ($[M+H]^+$, $C_{30}H_{51}O_2^+$; 443.3889).

Stereoselective Reduction of Compound **1**. To $LiAlH_4$ (0.583 g, 0.015 mmol) under N_2 anh. THF (10 ml) was added by syringe. The mixture was stirred for 25 h and then filtered through dry *Celite* under N_2. To the resulting soln., 1.0M MeOH in THF (5.6 ml) was added through an injector at r.t. within 10 min under stirring. Subsequently, a THF soln. of optically pure (*S*)-binaphthol (5.6 ml; 1.0M) was added dropwise, and the resulting mixture was stirred for an additional 30 min at r.t. The thus prepared reducing agent was cooled to −100° in a liq. N_2/MeOH bath. A soln. of **1** (35.0 mg, 0.073 mmol) in THF (0.5 ml) was then added dropwise within 12 min at −100°. The mixture was stirred at −100° for 2.5 h and then at −78° for additional 23 h. After addition of MeOH (1 ml) at −78°, the mixture was warmed to r.t., and 2N HCl (20 ml) was added. The mixture was extracted with CH_2Cl_2, the org. extract dried ($MgSO_4$) and concentrated, and the residue subjected to CC (SiO_2 (5 g), petroleum ether/AcOEt 2:8): **1a**/**1b** (11.3 mg, 32%), with **1a** as the predominant component.

Hydrolysis of Compound **2**. To the soln. of **2** (50.0 mg, 0.10 mmol) in MeOH (1 ml), MeONa (14.0 mg, 0.26 mmol) was added. The mixture was maintained at 62° for 24 h with stirring and then concentrated. The residue was subjected to CC (SiO_2 (5 g), petroleum ether/AcOEt 3:7): **2a** (10.4 mg, 23%).

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