Pergamon

Phytochemistry, Vol. 35, No. 3, pp. 725–729, 1994

0031-9422/94 $6.00+0.00

MAOECRYSTAL M: A NATURALLY OCCURRING SYMMETRIC *ENT*-KAURANE DIMER FROM *RABDOSIA ERIOCALYX*

XIAOYU SHEN,*† AKIRA ISOGAI,* KAZUO FURIHATA,* HANDONG SUN† and AKINORI SUZUKI*‡

*Department of Agricultural Chemistry, The University of Tokyo, Tokyo 113, Japan; †Kunming Institute of Botany, Academia Sinica, Kunming 650204, Yunnan, China

(*Received* 5 *July* 1993)

Key Word Index—*Rabdosia eriocalyx*; Labiatae; *ent*-kaurane diterpenoid; symmetric dimer; maoecrystal M.

Abstract—A symmetric dimer of an *ent*-kaurane diterpenoid, maoecrystal M, was isolated from the methanol extract of *Rabdosia eriocalyx*. The structure of the dimer was elucidated by means of 2D-COSY and ROESY NMR, and a chemical method. In the genus *Rabdosia*, maoecrystal M is the first example of a naturally occurring symmetric dimer of a diterpenoid.

INTRODUCTION

Rabdosia eriocalyx (Dunn) Hara is widely distributed in southwest China [1] and is used in Chinese folk medicine to reduce swellings. Recently, an analogue of an *ent*-kaurane diterpenoid, named maoecrystal M (**1**), has been isolated from *R. eriocalyx* (Dunn) Hara collected in Yunnan Province, China. This plant has yielded a series of *ent*-kaurane analogues with maoecrystal J (**2**) as the principal component [2, 3]. The structure of **1** has been elucidated as a symmetric dimer of **2** through detailed ^1H and ^{13}C NMR spectroscopy, including 2D NMR experiments (DQF COSY [4], ^1H–^{13}C COSY and ROESY [5]) and saturation transfer [6], as well as by chemical transformations. This is the first report of the isolation and structure elucidation of a symmetric dimer of an *ent*-kauranoid.

RESULTS AND DISCUSSION

Comparison of the ^1H and ^{13}C NMR spectral data of **1** (Tables 1 and 2) with those of **2** showed that both compounds were quite similar. The prominent features distinguishing **1** from **2** were the replacement in **1** of the olefin signals seen in **2** [δ_C153.0 (*s*, C-16) and 116.7 (*t*, C-17); δ_H 5.98 and 5.30 (each 1H, *br s*, H_2-17)] by methylene signals [δ_C25.0 (*t*): δ_H2.35 and 1.92 (*m*)] and a quaternary carbon signal (*s* 65.3). Furthermore, the methylene carbon resonance of C-12 (δ29.4) and the carbonyl carbon of C-15 (δ210.3) in **2** were shifted to higher field (s, 21.7) and lower field (s, 228.4), respectively, in **1**. It was apparent that the substructure encompassing rings A–C with their associated substituents in **1** were identical with those in **2**, indicating that **1** was an analogue of **2**. But it was difficult to assign the signals of the new methylene and the quaternary carbon in **1**. Positive FAB-MS (*m/z* 897, $[M+H]^+$) and negative FAB-MS (*m/z* 895, $[M-H]^-$) analysis indicated that the molecular formula of **1** was $C_{48}H_{64}O_{16}$. Therefore, it was assumed that **1** was a symmetric dimer and only displayed half of the signals in the ^1H and ^{13}C NMR spectra. As a consequence, the formation of a four-membered ring is expected for the 17 degrees of unsaturation required by the formula.

The hypothesis was rationalized through formation of an asymmetric derivative from **1**. Acetylation of **1** under forcing conditions gave a monoacetate (**3**) which was readily verified by FAB-MS: *m/z* 939 $[M+H]^+$. The symmetric nature of **1** was destroyed in **3**, and the ^1H and ^{13}C NMR signals due to the modified half of the molecule were readily observed. This unambiguously confirmed that **1** was a symmetric dimer.

The saturation transfer spectrum of **3** indicated the existence of three hydroxyls: δ_H6.63 (*d*, 10.0 Hz), 6.88 (*d*, 10.0 Hz) and 9.11 (*br s*). The former two doublet signals were revealed to be coupled with protons at δ5.67 (*dd*, 10.0, 6.0 Hz) and 4.37 (*dd*, 10.0, 6.0 Hz), respectively, in the DQF COSY spectrum of **3**. On examination of the ^{13}C–^1H COSY spectrum, these two protons could be assigned to the H-6 and H-6′ on C-6 (δ_C73.3) and C-6′ (δ_C67.3). Thus, the H-6 proton was shifted downfield and the C-6 carbon in **1** was shifted upfield on acetylation. Furthermore, the hemiketal carbon (C-7) shifted from δ_C96.3 in **1** to δ 101.7 in **3**. This assignment was confirmed by the HMBC method.

In the HMBC spectrum of **3**, the hydroxyl signal at δ_H6.63 showed significant long range correlations with the

‡Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for **1–4**

H	**1**		**2**		**3**		**4**	
1	1.75*		1.78*		*		*	
1	1.43*		1.42*		*		*	
2	1.77*		1.78*		*		*	
2	1.05 *br d*	15.0	1.09 *br d*	14.0	*		1.06 *br d*	14.0
3	5.36 *br s*		5.38 *br s*		5.39 *br s*		5.30 *t*	3.0
5	2.23 *d*	6.0	2.22 *d*	6.0	2.14 *d*	6.0	2.10*	
6	4.36 *dd*	10.3, 6.0	4.40 *dd*	10.0, 6.0	5.67 *dd*	10.0, 6.0	5.69 *dd*	10.0, 6.0
9	1.75*		1.78*		*		1.74*	
11	1.39*		1.27*		*		*	
11	1.19 *m*		1.28 *		*		*	
12	1.13 *m*		2.14*		*		*	
12	1.60 *m*		1.62 *m*		*		*	
13	2.83 *dd*	10.5, 4.0	2.91 *dd*	10.5, 4.0	2.82 *dd*	10.0, 4.0	2.90 *dd*	10.0, 4.0
14	2.11 *d*	15.0	2.23 *d*	13.0	2.26 *d*	13.0	2.19 *d*	12.0
14	3.02 *dd*	13.0, 4.0	2.48 *dd*	13.0, 4.0	2.73 *dd*	13.0, 4.0	2.13 *dd*	12.0, 4.0
17	2.35 *m*		5.97 *s*		2.29 *m*		5.99 *s*	
17	1.92 *m*		5.30 *s*		1.94 *m*		5.36 *s*	
18	1.43 *s*		1.46 *s*		1.44 *s*		1.44 *s*	
19	4.62 *d*	11.6	4.65 *d*	11.7	4.67 *d*	12.0	4.68 *d*	11.5
19	4.52 *d*	11.6	4.61 *d*	11.7	4.72 *d*	12.0	4.71 *d*	11.5
20	4.00 *d*	10.8	4.09 *d*	10.5	4.09 *br d*	10.5	4.18 *br d*	10.5
20	3.92 *d*	10.8	4.06 *d*	10.5	4.06 *br d*	10.5	3.98 *br d*	10.5
Ac	1.95 *s*		1.92 *s*		1.89 *s*		1.90 *s*	
Ac	2.15 *s*		2.14 *s*		2.05 *s*		2.12 *s*	
Ac					1.84 *s*		1.98 *s*	
OH-6	6.94	10.3	7.16 *d*	10.0	6.63 *d*	10.0	6.82 *d*	10.0
1′	1.75*				*			
1′	1.43*				*			
2′	1.77*				*			
2′	1.05 *br d*	15.0			1.02 *br d*	14.0		
3′	5.36 *br s*				5.29 *br s*			
5′	2.23 *d*	6.0			2.25 *d*	6.0		
6′	4.36 *dd*	10.3, 6.0			4.37 *dd*	10.0, 6.0		
9′	1.75*				*			
11′	1.39*				*			
11′	1.19 *m*				*			
12′	1.13 *m*				*			
12′	1.60 *m*				*			
13′	2.83 *dd*	10.5, 4.0			2.63 *dd*	10.0, 4.0		
14′	2.13 *d*	13.0			2.10 *d*	13.0		
14′	3.02 *dd*	13.0, 4.0			2.84 *dd*	13.0, 4.0		
17′	2.35 *m*				2.29 *m*			
17′	1.92 *m*				1.89*			
18′	1.43 *s*				1.41 *s*			
19′	4.62 *d*	11.6			4.62 *d*	12.0		
19′	4.52 *d*	11.6			4.56 *d*	12.0		
20′	4.00 *d*	10.8			4.09 *d*	10.5		
20′	3.92 *d*	10.8			3.92 *d*	10.5		
Ac′	1.95 *s*				1.97 *s*			
Ac′	2.15 *s*				2.10 *s*			
OH-6′	6.94 *d*	10.3			6.88 *d*	10.0		
OH-7′					9.11 *br s*			

*Ambiguous due to signal overlapping.

Table 2. ^{13}C NMR spectral data for **1**–**4**

C	1	2	3	4	mult.
1	22.5	22.1	22.7	22.5	*t*
2	22.5	22.6	22.5	23.3	*t*
3	72.6	72.7	72.6	72.2	*d*
4	41.3	41.3	41.5	41.4	*s*
5	57.6	57.4	56.9	56.4	*d*
6	73.1	73.2	67.3	67.6	*d*
7	96.3	96.0	101.7	101.3	*s*
8	61.6	60.2	61.9	60.3	*s*
9	50.8	49.9	51.0	50.1	*d*
10	35.9	36.3	36.0	36.1	*s*
11	16.1	16.7	15.9	16.5	*t*
12	21.7	29.4	21.9	29.3	*t*
13	33.5	35.0	33.6	34.6	*d*
14	27.0	26.7	26.7	26.1	*t*
15	228.4	210.3	226.9	208.5	*s*
16	65.3	153.7	65.3	152.8	*s*
17	25.0	116.7	24.9	118.3	*t*
18	21.4	21.7	21.4	21.9	*q*
19	66.6	66.5	66.3	66.8	*t*
20	66.5	66.4	66.5	67.3	*t*
COMe	170.8	170.8	170.7	170.6	*s*
COMe	170.4	170.3	170.2	170.2	*s*
COMe	—	—	168.1	168.2	*s*
COMe	20.9	21.0	20.9	20.9	*q*
COMe	20.4	20.6	20.4	20.6	*q*
COMe	—	—	20.5	21.9	*q*
1′	22.5		22.7		*t*
2′	22.5		22.5		*t*
3′	72.6		72.6		*d*
4′	41.2		41.3		*s*
5′	57.6		57.6		*d*
6′	73.1		73.3		*d*
7′	96.3		96.4		*s*
8′	61.6		61.8		*s*
9′	50.8		51.0		*d*
10′	35.9		35.9		*s*
11′	16.1		16.2		*t*
12′	21.7		21.9		*t*
13′	33.5		33.1		*d*
14′	27.0		27.1		*t*
15′	228.4		227.7		*s*
16′	65.3		65.3		*s*
17′	25.0		24.9		*t*
18′	21.4		21.4		*q*
19′	66.6		66.9		*t*
20′	66.5		66.9		*t*
COMe′	170.8		170.7		*s*
COMe′	170.4		170.3		*s*
COMe′	20.9		20.9		*q*
COMe′	20.4		20.4		*q*

^{13}C signals at δ101.7 (*s*, C-7) and 67.3 (*d*, C-6), the latter correlating with the ^{1}H signal at δ5.67 (H-6) and 4.09 (H_a-20), as well as δ_H2.14 (*d*, 6.0 Hz, H-5), respectively.

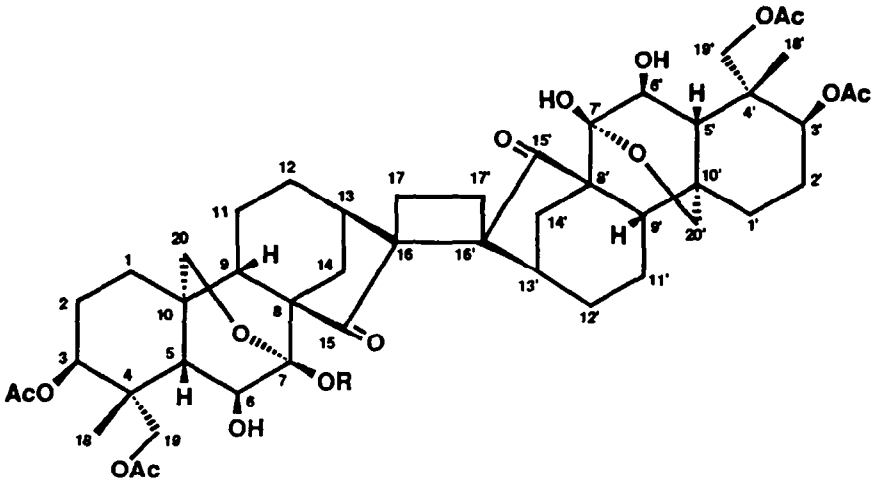
These data suggested that the acetylation of **1** afforded a monoacetate in which the C-7 hemiketal hydroxyl was acetylated, while the C-6 and C-6′ hydroxyls remained free. Thus, acetylation occurred at a rather unusual position so maoecrystal J (**2**) was acetylated in the same way to confirm the reaction. Compound **2** gave the acetate **4**. The ^{1}H NMR signal of H-6 shifted from δ_H4.40 in **2** to δ5.69 in **4**, which coupled with a free hydroxyl at δ_H6.82. Simultaneously, the ^{13}C NMR signal of C-7 shifted from δ_C96.0 in **2** to δ101.3 in **4**, and the ^{13}C signal of C-6 shifted from δ73.2 in **2** to δ67.6 in **4**. These data clearly indicated that acetylation occurred at the C-7 hemiketal hydroxyl in **2**. The change in the chemical shifts of the protons and carbons as mentioned above were the same as in **3** and **4**. If the C-6 (and C-6′) hydroxyl is protected by the C-15 (and C-15′) ketone carbonyl through the formation of a hydrogen bond this would explain the unusual acetylation patterns of **1** and **2**.

In the DQF COSY and ^{1}H–^{13}C COSY spectral data of **3** a spin system due to an AA′ BB′ system in a four-membered ring (Fig. 1A) was readily discernible, because no spin corresponding to an AB system in a four-membered ring (Fig. 1B) appeared in the DQF COSY spectrum of **3**, indicating that the four-membered ring possessing the A form was present in **1**.

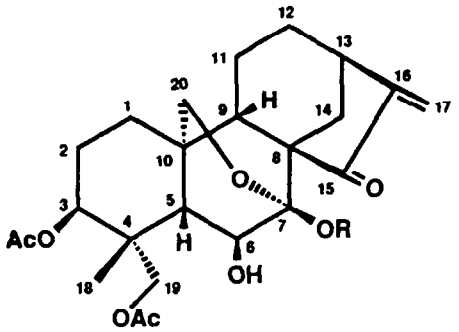
Elucidation of the stereostructure of C-16 and C-16′ in **3** now became the pivotal step for determining the structure of **1**. The stereostructures of the skeleton and the substituents in **1**, except for those of C-16 and C-16′, must be identical with those of **2** [2]. Theoretically, four kinds of possible configurations of C-16 and C-16′ could be present in **1**: (16*S*,16′*R*), (16*S*,16′*S*), (16*R*,16′*S*) and (16*R*,16′*R*). In fact, the configurations of (16*S*,16′*R*) and (16*R*,16′*S*) can be eliminated, because **1** is a symmetric dimer in which a C_2 symmetric axis exists. This leaves the two configurations (16*S*,16′*S*) and (16*R*,16′*R*) (Fig. 2).

In order to establish which configuration is present in **1**, a ROESY experiment was performed on **3**. The significant NOE correlation cross-peaks between H-14 (δ_H2.73, *dd*, 13.0, 4.0 Hz) and H-14′ (δ_H2.84, *dd*, 13.0, 4.0 Hz) could only be satisfied with a (16*R*,16′*R*) configuration, because the (16*S*,16′*S*) configuration would not allow a NOE between H-14 and H-14′. Thus, the stereostructure of **3** was deduced to be that shown in Fig. 3. The fact that the H-6 signal resonated at lower field (δ_H5.67) in **3** than in **1** (δ_H4.36) was most plausibly interpreted as the result of anisotropy of the C-7 acetate to H-6. On the other hand, the interactions between H-17, H-17′ and H-12, H-12′ in **1** resulted in a striking γ-gauche effect shifting the resonances of C-12 and C-12′ to higher field (Δ_δ −7.7 ppm). Thus, the structure of **1** was determined to be a symmetric dimer of **2** conjugating at (16*R*,16′*R*) through a four-membered ring. The four-membered ring of **1** should be formed by condensation between the olefin group in the α,β-unsaturated ketone group of the monomer **2**, the mechanism being probably through a [2+2] cycloaddition [7].

In order to dispel the possibility that **1** might be an artifact produced during the extraction and isolation procedures, **2** was dissolved in MeOH and *n*-hexane–EtOAc (2:1) with a little silica gel and left for three weeks at room temperature. HPTLC of the solutions showed only the presence of **2**. The result suggested that **1** was a metabolic product existing in the plant itself.



	R
1	H
3	Ac



	R
2	H
4	Ac

Scheme 1.

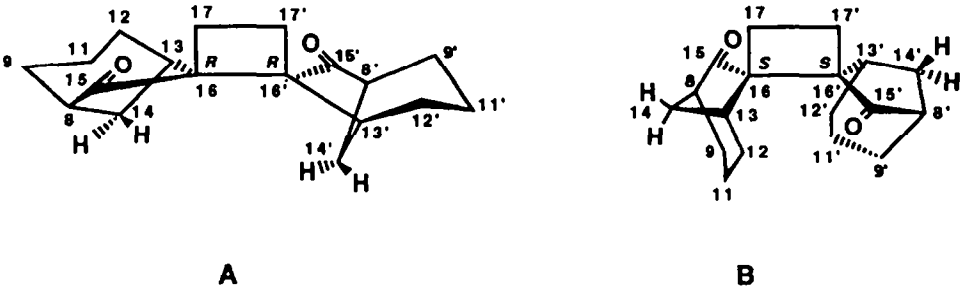
Fig. 1. Two possible forms of the four-membered rings of 1.

EXPERIMENTAL

Mp: uncorr: ^1H and ^{13}C NMR: 500 MHz.

Plant material. Rabdosia eriocalyx (Dunn) Hara was collected in Oct. 1985 at Yanzhonghai, Yunnan, China. Voucher specimens are deposited in the Kunming Institute of Botany, Academia Sinica.

Extraction and isolation of maoecrystal M (1). Dried and finely powdered leaves of *R. eriocalyx* (Dunn) Hara (3 kg) were extracted with MeOH (3 × 3 l) at room temp. for 20 days. Filtration and evapn of the solvent yielded 110 g of residue which was dissolved in MeOH–H_2O (1:9) and shaken with 3 × 2 l of Et_2O. The Et_2O phase was evapd *in vacuo* to yield 75 g residue. This was treated



A B

Fig. 2. Two possible configurations for C-16 and C-16' of 1.

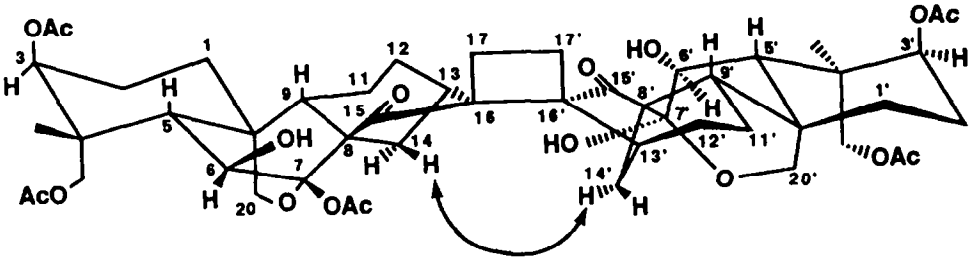


Fig. 3. NOEs observed in 3.

(2 × 50 g) with activated C in MeOH (1.5 l). The soln was filtered and the solvent evapd to yield 44 g of a yellow gum which was subjected to CC over silica gel (700 g). The column was eluted successively with *n*-hexane–EtOAc (9:1, 4:1, 3:1, 13:9, 1:1), EtOAc, and EtOAc–MeOH (4:1). Frs 33–35 (1.02 g) were bulked and subjected to CC on Kieselgel 60 (25 g) to give 0.18 g white residue from frs 12–14 eluted by $CHCl_3$–MeOH (25:1). The residue was purified by crystallization ($CHCl_3$) to give 95 mg of **1** as a powder.

Maoecrystal M (**1**) $C_{48}H_{64}O_{16}$: mp >300°; $[\alpha]_D^{22}$ +44.0° ($CHCl_3$: *c* 0.2); IR ν_{max}^{KBr} cm^{-1} 3560–3250, 2940, 1740–1700, 1440, 1380, 1280–1200, 1030; FAB-MS (pos.) *m/z*; 897 $[M+H]^+$, 801, 741, FAB-MS (neg.) *m/z*: 895 $[M-H]^-$, 448, 427 and 367; ^1H and ^{13}C NMR Tables 1 and 2.

Acetylation of maoecrystal M (**1**). Ac_2O (1 ml) was added to a soln of **1** (10 mg) in pyridine (1 ml). After being stirred for 10 hr at 80°, the reaction mixt. was poured into ice–H_2O and absorbed on to SEP-PAK C_{18}. The column was eluted with $CHCl_3$, and the reaction product was purified by a wet-column flash chromatography. Kieselgel 60H (Merck) (10 g) was packed under red. pres. in a glass column (3.5 cm × 15 cm) and the reaction product was eluted with Et_2O to give a crude residue of **3**, which was purified by crystallization ($CHCl_3$) to provide 3 mg of **3**. FAB-MS (pos.): *m/z* 939 $[M+H]^+$; ^1H and ^{13}C NMR: Tables 1 and 2.

Acetylation of maoecrystal J (**2**). Ac_2O (1.5 ml) was added to a soln of **2** (20 mg) in pyridine (1.5 ml). After being treated under the same conditions as **1**, the reaction product (21 mg) was purified by ODS-H-2501 column (1.2 × 16 cm), which was eluted with MeCN–H_2O (1:1) to give 11.5 mg monoacetate of maoecrystal J (**4**).

Compound **4**: FAB-MS *m/z*: 491 $[M+H]^+$, 431 $[MH-AcOH]^+$, 371 $[MH-2\times AcOH]^+$ and 311 $[MH-3\times AcOH]^+$; ^1H and ^{13}C NMR: Tables 1 and 2.

Compound **2** (2 mg) was dissolved in MeOH and 1-hexane–EtOAc (2:1) with a little silica gel, and stood for 3 weeks at room temp. On HPTLC (2 × 10 cm) developed with Et_2O (×2), only one spot, corresponding to **2** (identified by means of an authentic sample), was visible.

Acknowledgements—This work was supported in part by the Kampou Science Foundation (Japan). We would like to thank the Central Research Laboratory of Yamanouchi Pharmaceutical for running the FAB-mass spectra of **1**.

REFERENCES

1. Yunnan Inst. of Bot. (1977) in *Flora Yunnanica Tomus*, Vol. 1, p. 449. Science Press, Peking.
2. Shen, X., Isogai, A., Furihata, K., Kaniwa, H., Sun, H. and Suzuki, A (1989) *Phytochemistry* **28**, 855.
3. Isogai, A., Shen, X., Furihata, K., Kaniwa, H., Sun, H. and Suzuki, A (1989) *Phytochemistry* **28**, 2427.
4. Shaka, J. J. and Freeman, R. (1983) *J. Magn. Reson.* **51**, 169.
5. Bax, A. and Davis, D. G. (1985) *J. Magn. Reson.* **63**, 207.
6. Freeman, R. and Hill, H. D. W. (1971) *J. Chem Phys.* **54**, 3367.
7. Streitwieser, Jr A. and Heathcock, C. H. (1985) in *Introduction to Org. Chem.*, 3rd Edn., p. 1108. Macmillan, New York.