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SESQUITERPENELACTONES FROM *BLUMEA BALSAMIFERA*

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Key Word Index—*Blumea balsamifera*, Compositae, sesquiterpene lactone, antitumour activity

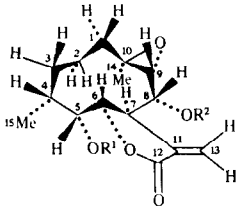
Abstract—Three new sesquiterpene lactones were isolated from dried leaves of *Blumea balsamifera*. The structure of the sesquiterpene lactones were determined by their IR, ^{1}H NMR, ^{13}C NMR and MS spectral data and some chemical reactions. New sesquiterpene lactones exhibited the antitumour activity against Yoshida sarcoma cells in tissue culture

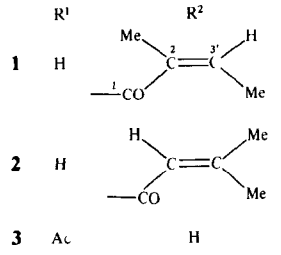
INTRODUCTION

Blumea balsamifera is a medicinal plant which has been used as a carminative, a vermifuge, a diaphoretic and an expectorant in South-East Asia and China The studies on the constituents of *B balsamifera* were carried out from old times and a number of flavones, monoterpenes and triterpenes have been isolated from this plant [1]. We now report here the isolation and structural elucidation of three new antitumour sesquiterpene lactones, named blumealactone A (**1**), B (**2**) and C (**3**) from Indonesian *Blumea balsamifera* D C

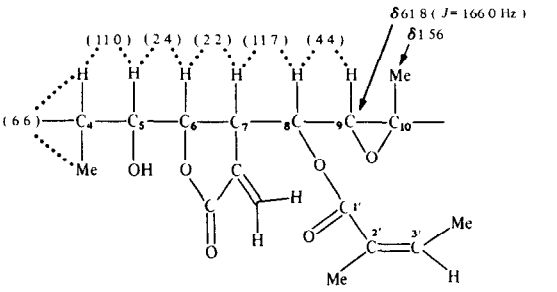
RESULTS AND DISCUSSION

Blumealactone A (**1**), mp 205–208°, $[\alpha]_D$ −45.9° ($CHCl_3$; *c* 0.88), exhibited the molecular ion peak at *m/z* 364, $C_{20}H_{28}O_6$ and the base peak at *m/z* 83 $[MeCH=C(Me)C=O]^+$ Its IR spectrum showed the presence of hydroxyl group (3400 cm^{-1}), γ-lactone (1755 cm^{-1}) and ester group (1720 cm^{-1}) The ^{1}H NMR spectrum of **1**





indicated the presence of four methyl groups at δ1 12, 1.56, 1.91, 2 00, exomethylene protons at δ5.72, 6 23, and a vinyl proton at δ6 13. These spectral data of **1** suggested the presence of α-methylene-γ-lactone and 2-methyl-2-butenoic acid moieties. Furthermore, detailed ^{1}H NMR decoupling experiments on **1** and some chemical reactions revealed the substitution pattern from C-4 to C-9 (Scheme 1)



The numbers in parentheses are *J* values in Hz

Acetylation of **1** with acetic anhydride–pyridine gave a monoacetate (**4**) which exhibited the proton signal due to H-5 (δ5.15) at 1.45 ppm lower field as compared with that (δ3 70) of **1**, thereby suggesting the presence of a hydroxyl group at C-5 The α-methylene-γ-lactone moiety attached to C-6 and C-7 was proved by the chemical shift of the H-6 (δ5 00) and the observation of the allylic coupling ($J = \sim 1.0$ Hz) between H-7 and the exomethylene protons The presence of the 2-methyl-2-butenoic acid moiety at C-8 was confirmed by the following chemical reaction and ^{1}H NMR data of the reaction product Hydrolysis of **1** with 5% MeOH–KOH gave a diol (**4**) which showed the molecular ion peak at *m/z* 282 $[M]^+$ (**1** $-C_5H_6O$) and its ^{1}H NMR spectrum showed the proton signal (δ4 27) due to the H-8 at 1.21 ppm higher field as compared with that (δ5.48) of **1**. Thus the 2-methyl 2-butenoic acid moiety should be connected to C-8 In the ^{13}C NMR spectrum of **1**, the signal with a large coupling constant (J_{C-H} = 166.0 Hz) was observed at δ61 8 which was assigned to C-9 by selective proton decoupling experiment This large coupling constant and the chemical shift of H-9 (δ3.16) indicated the presence of an epoxide ring including C-9 and C-10 Therefore, the tertiary methyl group which appeared at δ1 56 should be attached to the C-10 bearing epoxide oxygen atom. From consideration of the ^{13}C NMR data and the empirical formula, **1** should be a tricyclic compound. Thus, the residual three methylene groups should be inserted between C-4 and C-10 to compose a ten-membered ring

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Table 1 ^{1}H NMR spectral data for blumealactone A (**1**), B (**2**) and (**3**) [400 MHz, $CDCl_3$, δ]*

H	1	2	3
1β	1 03 *dt* (4 9, 13 4)	1 03 *dt*	1 09 *dt*
1α	2 11 *dt* (3 6, 13 4)	2 12 *dt*	2 16 *dt*
2β	1 86 *m*	1 84 *m*	1 86 *m*
2α	1 42 *m*	1 42 *m*	1 42 *m*
3	1 29 *m*	1 29 *m*	1 32 *m*
4	1 56 *m*	1 60 *m*	1 65 *m*
5	3 70 *br d* (11 0)	3 68 *br d*	5 13 *dd* (2 4, 11 5)
6	5 00 *dd* (2 2, 2 4)	4 94 *dd*	4 87 *dd*
7	3 47 *br dd* (2 2, 11 7)	3 41 *br dd*	3 23 *dd* (2 2, 10 7)
8	5 48 *dd* (4 4, 11 7)	5 42 *dd*	4 26 *dd* (3 7, 10 7)
9	3 16 *d* (4 4)	3 08 *d*	3 04 *d* (3 7)
13	5 72 *br s*	5 76 *br s*	6 11 *br s*
	6 23 *br s*	6 24 *br s*	6 37 *br s*
14	1 56 *s*	1 56 *s*	1 65 *s*
15	1 12 *d* (6 4)	1 12 *d*	0 97 *d*
2′		5 65 *m*	
Me-2′	1 91 *br s*		
3′	6 13 *br q* (7 3)		
Me-3′	2 00 *br d* (7 3)	1 93 *d* (1 2)	
		2 19 *d* (1 2)	
MeCO			1 99 *s*

*Coupling constants (in parentheses) are not repeated if identical with those in preceding column

Finally, the stereostructure of **1** was determined from the magnitude of the coupling constants of each proton signal from H-4 to H-9 and the results of the NOE experiments on **1** (Table 1) These coupling constants and the NOE results are explained satisfactorily by the structure (**1**)

Blumealactone B (**2**), mp 221–222°, $[\alpha]_D$ −34 2° ($CHCl_3$, *c* 0 58), exhibited the molecular ion peak at *m/z* 364, $C_{20}H_{28}O_6$ and the base peak at *m/z* 83 $[C_5H_7O]^+$, and its IR spectrum showed the presence of hydroxyl group (3450 cm^{-1}), γ-lactone (1755 cm^{-1}) and ester (1720 cm^{-1}) These MS, IR and ^{1}H NMR data of **2** indicated that the framework of **2** should be the same as that of **1** and that the structural difference in **2** and **1** must be in the side chain moiety attached to C-8 In the ^{1}H NMR spectrum of **2**, two doublet methyl signals (δ1 93 and 2 19) and a multiplet vinyl proton signal (δ 5 65) due to the side chain moiety were observed Thus, the structure of blumealactone B was represented by the formula **2**

Blumealactone C (**3**), mp 247–250°, $[\alpha]_D$ −4 85 ($CHCl_3$, *c* 1.55), exhibited the molecular ion peak at *m/z* 324, $C_{17}H_{24}O_6$ and the base peak at *m/z* 43 $[C_2H_3O]^+$ and its IR spectrum showed the presence of a hydroxyl group (3450 cm^{-1}), γ-lactone (1755 cm^{-1}) and ester (1720 cm^{-1}) On comparing the MS, IR and ^{1}H NMR (Table 2) data of 3 with those of **1** and **2**, it is clear that **3** should be an acetate with the same framework as those of **1** and **2** The detailed ^{1}H NMR decoupling experiments on blumealactone C indicated that the acetic acid moiety should be attached to C-5 Thus, the structure of blumealactone C was assigned to the structure (**3**) All these blumealactones exhibited growth inhibition against Yoshida sarcoma cells at the concentration of 5 ~ 10 μg/ml, antitumour activities determined according to ref [2] The details on the biological activities of blumealactones will be published elsewhere

Table 2 NOE difference spectral data of **1**

Saturation	Observed NOE (%)
H-6	H-9 (10 0)
H-9	H-6 (8 9), H-8 (16 0)
H-14	H-7 (10 4)
Me-2′	H-3′ (11 6)

EXPERIMENTAL

^{1}H NMR (400 MHz) and ^{13}C NMR(25 MHz) were measured in $CDCl_3$ containing TMS as an int standard The leaves of *Blumea balsamifera* D C were collected in the suburb of Jakarta, Indonesia

Isolation of blumealactone A (**1**), B (**2**), and C (**3**) The dried leaves (500 g) of *Blumea balsamifera* were extracted with 90% EtOH (1 l × 3) After concen of the EtOH soln, the crude extracts were chromatographed on HP-20 resin (Nippon Rensui) (eluted successively with 1 5 l each of 40% MeOH, 60% MeOH, 80% MeOH, MeOH and Me_2CO) The growth inhibition of each fraction against Yoshida sarcoma cells was tested and only the 80% MeOH fraction was found to be active Thus, this fraction was further purified by HPLC (Nucleosil 50–5, 20 × 300 mm, *n*-hexane–EtOAc (1 1), flow rate, 9 ml/min) to give 200 mg of **1** (R_t 30 min), 28 mg of **2** (R_t 34 min) and 200 mg of **3** (R_t 49 min) 1, EIMS 70 eV, *m/z* (rel int), 364 $[M]^+$ (3 8), 344 (8 8), 330 (3 8), 264 (5 3), 125 (11 3), 97 (20), 83 (100), IR (KBr) ν_{max} cm^{-1} 3400 (*br*), 1755, 1720 ^{13}C NMR 15 7 (*q*, C-19), 16 3 (*q*, C-14), 17 6 (*q*,

C-15), 20 3 (*q*, C-20), 22.6 (*t*, C-2), 30.0 (*t*, C-3), 36 2 (*d*, C-4), 39 7 (*d*, C-7), 40 7 (*t*, C-1), 58 8 (*s*, C-10), 61 8 (*d*, C-9), 68 8 (*d*, C-8), 74 8 (*d*, C-5), 78 0 (*d*, C-6), 124 3 (*t*, C-13), 126 4 (*s*, C-17), 136 2 (*s*, C-11), 139 8 (*d*, C-18), 165 7 (*s*, C-12), 169 8 (*s*, C-16), 2, EIMS 70 eV, *m/z* (rel int) 364 $[M]^+$ (2.5), 355 (5 0), 232 (7 8), 189 (7 5), 180 (8 8), 123 (17 5), 83 (100), IR (KBr) ν_{max} cm^{-1} 3450 (*br*), 1755, 1720, 3, EIMS 70 eV, *m/z* (rel int) 324 $[M]^+$ (1 0), 282 (2 5), 264 (11 2), 232 (20 0), 189 (27 0), 123 (71 2), 109 (72 5), 97 (45 0), 85 (43 7), 69 (28 7), 43 (100), IR (KBr) ν_{max} cm^{-1} 3450 (*br*), 1755–1730 (*br*).

Acetyl blumealactone A (**4**) Acetyl blumealactone A (**4**) was prepared by usual means using acetic anhydride–pyridine **4**, mp 71–72°, ^{1}H NMR; 9 75 (1H, *d*, J = 6 6 Hz, H-15), 1 60 (3H, *s*, H-14), 1 96 (3H, *br s*, H-20), 1 97 (3H, *s*, MeCO), 2 02 (3H, *dq*, J = 1 5, 7 3 Hz, H-19), 3 13 (1H, *d*, J = 4 2 Hz, H-9), 3 47 (1H, *br dd*, J = 2 2, 11 7 Hz, H-7), 5 00 (1H, *dd*, J = 2 2, 2 4 Hz, H-6), 5 15 (1H, *dd*, J = 2 4, 11 0 Hz, H-5), 5 47 (1H, *dd*, J = 4 2, 11 7 Hz, H-8), 5 77 and 6 31 (1H each, *br s*, H-13), 6 15 (1H, *br q*, J = 7 3 Hz, H-18)

Hydrolysis of blumealactone A (**1**) Blumealactone A (**1**) was hydrolysed with 5% MeOH–KOH for 3 hr at room temp The mixture was diluted with H_2O and then acidified with 5% HCl Usual treatment of this mixture gave an oil which was purified by HPLC (Nucleosil 50-5, *n*-hexane–EtOAc (2 1), 8 × 300 mm) to give a diol (**5**), EIMS 70 eV, *m/z* (rel int) 282 $[M]^+$ (3 1), 264 (6 2), 222 (13 8), 179 (15 0), 164 (28.1), 138 (30 0), 127 (53 8), 109 (100), 95 (45 0), 81 (33 7), 71 (30), ^{1}H NMR, 1 12 (3H, *d*, J = 6 4 Hz, H-15), 1 62 (3H, *s*, H-14), 3 02 (1H, *d*, J = 3.9 Hz, H-9), 3 24 (1H, *dq*, J = 2 6, 10 7 Hz, H-7), 3 65 (1H, *dd*, J = 1 9, 11 0 Hz, H-5), 4 27 (1H, *dd*, J = 3 7, 10 7 Hz, H-8), 4 83 (1H, *dd*, J = 22 2, 3 2 Hz, H-5), 6 10 and 6 35 (1H, each, *dd*, J = 1 0, 2 0 Hz, H-13)

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