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PICRASANE QUASSINOIDS FROM *PICRASMA JAVANICA*

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Key Word Index—*Picrasma javanica*; Simaroubaceae; quassinoid; javanicin Z; dihydrojavanicin Z; hemiacetaljavanicin Z.

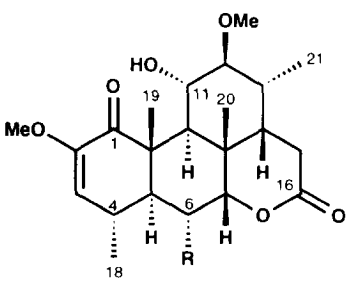
Abstract—Three new picrasane quassinoids were isolated from the bark of *Picrasma javanica*. Their structures were established using spectroscopic data and X-ray analysis. The absolute stereochemistry of their structure was determined by a modified form of Mosher's method and CD spectra.

INTRODUCTION

Picrasma javanica Bl is a medium-sized tree found in New Guinea, Southeast Asia, and Indonesia. Decoctions of its bark are used in folk medicine as a febrifuge and as a substitute for quinine [1, 2]. A number of quassinoids and alkaloids have been obtained from *P. javanica* [3, 4]. We now report on the isolation and structures of three additional picrasane-type quassinoids from the bark of this plant collected on Java Island, Indonesia.

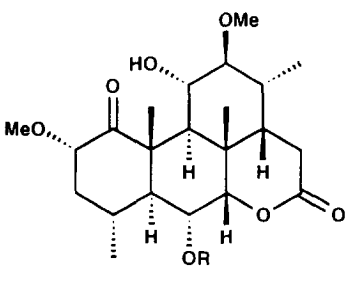
RESULTS AND DISCUSSION

Javanicin Z (**1**) was obtained as needles. Its molecular formula, $C_{22}H_{32}O_7$, was determined by HR-mass spectrometry and required seven degrees of unsaturation. The IR spectrum of **1** indicated the presence of hydroxyl (3520 and 3450 cm^{-1}), δ-lactone (1720 cm^{-1}), α,β-unsaturated ketone (1680 cm^{-1}) and double bond (1650 cm^{-1}) groups. The resonances in the ^{13}C NMR spectrum at δ205.0 (*s*), 170.0 (*s*), 147.8 (*s*) and 120.4 (*d*) and the UV absorption at 272 nm confirmed the presence of the α,β-unsaturated ketone and δ-lactone groups. The ^{13}C NMR and DEPT spectra contained no evidence of additional unsaturated functionalities, and thus we concluded that **1** was tetracyclic. The ^1H and ^{13}C NMR spectra showed the characteristic resonances of quassinoids of the picrasane type [5, 6]. The ^1H NMR data established the presence of two tertiary methyls (δ1.21 and 1.60, each 3H, *s*), two secondary methyls (δ0.99 and 1.56, each 3H, *d*, J = 7 Hz), and two methoxyls (δ3.47 and 3.69, each 3H, *s*). Thus javanicin Z (**1**) was structurally related to nigakilactone B (**4**) [7, 8], except for the presence of a H-6 methine and a new hydroxyl group. In addition, a ^1H–^1H COSY experiment revealed a spin system that proceeded from the olefinic proton at δ5.36 (1H, *d*, J = 3 Hz), the bridge head methine proton at δ2.89 (1H, *m*), an oxygenated methine proton at δ4.51 (1H, *br d*, J = 8 Hz), and a lactone terminal proton at δ4.40 (1H, *d*, J = 2 Hz), which were easily attributed at H-3, H-4, H-5, H-6 and H-7. The presence of a hydroxyl group at C-6 for **1** was suggested by the downfield shift ($\Delta\delta$3.8 ppm) of the H-6 signal corresponding with an axial H-6 of **4**. The coupling constants, $J_{5,6}$ = 8 Hz and



1 R=OH

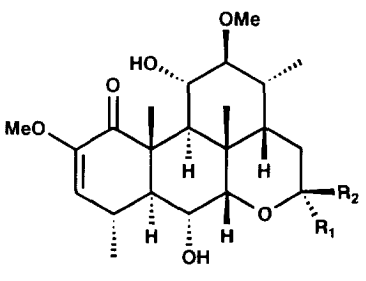
4 R=H



2 R=H

5 R=(*S*)-MTPA

6 R=(*R*)-MTPA



3a R_1=OH R_2=H

3b R_1=H R_2=OH

$J_{6,7}$ = 2 Hz, suggested a *trans*-relationship between H-6 and an α-axial proton on C-5, indicating that the hydroxyl group at C-6 had an α-equatorial configuration. A NOE experiment was employed to confirm this assignment (Fig. 1). The strong NOE correlations between H-6 and Me-8 and Me-10 also suggested that the configuration of the hydroxyl group attached at C-6 was α-equatorial. Other NOESY correlations supported the proposed stereochemistry in Fig. 1. The absolute configuration of **1** was determined to be same as **4** by the similarity of its CD cotton curve to that of **4**.

Dihydrojavanicin Z (**2**) was obtained as needles. Its molecular formula, $C_{22}H_{34}O_7$, was determined by HR-MS and required six degrees of unsaturation. The IR and ^{13}C NMR spectra of **2** indicated the presence of hydroxyl (3360 cm^{-1}), δ-lactone (1710 cm^{-1} and δ_C170.1) and ketone (1695 cm^{-1} and δ_C213.7) groups. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of javanicin Z (**1**); however, the spectra of **2** contained no olefinic signals at C-2 and C-3. As the saturation number of **2** was one unit lower than that of **1** this suggested that the α,β-unsaturated ketone group in **1** was replaced by a saturated ketone group in **2**. The ^1H–^1H COSY further suggested the presence of a partial structure, from H2 to H7. These data indicated that the structure of **2** is that of 2,3-dihydrojavanicin Z. The α configuration of the methoxyl group at C-2 was determined from the ^1H NMR coupling constants (J = 11, 7 Hz) of H-2 (δ4.66 *dd*) and the NOE correlations between H-2 and H-4 and Me-10 (Fig. 1). The absolute configuration of **2** was established by using the modified Mosher's method [9]; the $\Delta\delta(\delta_S - \delta_R$ (ppm)) values of the MTPA esters (**5** and **6**, respectively) obtained for the respective protons located around the chiral center at C-6 are shown in Fig. 2. The systematic arrangement of the positive and negative Δδ's confirmed the *R* configuration at C-6 in **2**.

Hemiacetaljavanicin Z (**3**) was obtained as prisms. Its molecular formula, $C_{22}H_{34}O_7$, was determined by HR-MS. The IR and UV spectrum of **3** indicated the presence of hydroxyl (3560 and 3485 cm^{-1}), α,β-unsaturated

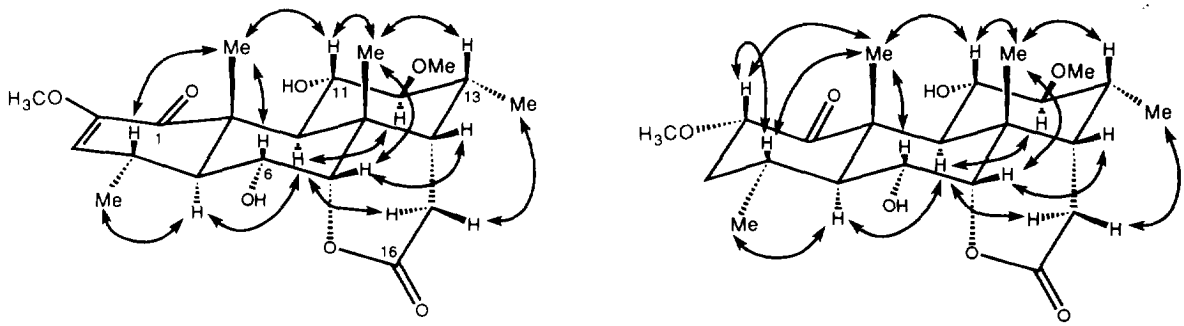


Fig. 1. NOESY correlations of **1** and **2**. Arrows show NOE relationships.

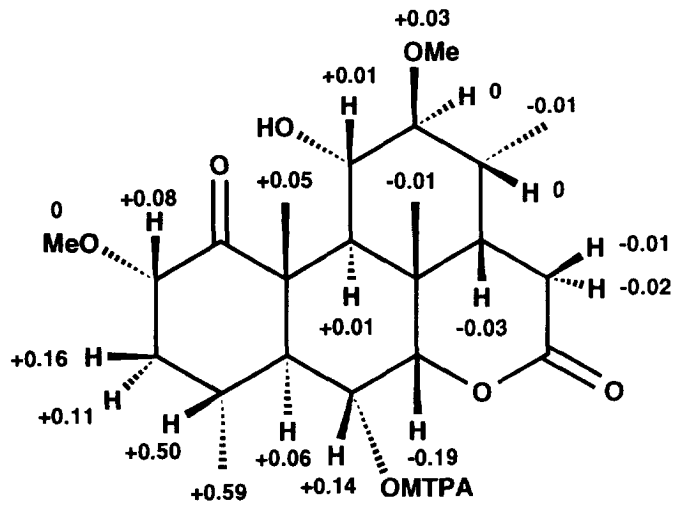


Fig. 2. ^1H NMR chemical shift differences for MTPA esters:
$\Delta\delta$ (ppm) = δ{(S) – (MTPA ester **S**)} – δ{(R) – (MTPA ester **6**)}.

ketone and double bond (1670, 1645 cm^{-1} and 272 nm) groups. However, no absorption was observed for a lactone group. The ^1H NMR spectrum of **3** exhibited a characteristic difference in the H-16 values and J_{HH} coupling constants [δ5.01 *dd* (J = 9, 3 Hz) and 5.75 *br s*)]. According to the ^1H NMR spectrum, **3** existed as two isomers in solution. Javanicin Z (**3**) showed two peaks (ratio 67:33) on HPLC (ODS column, solvent system MeOH–H_2O). Hemiacetals of this type exist as a mixture of isomers at C-16 [10, 11]. When each component was isolated by preparative HPLC and subjected to analytical HPLC, the formation of the same mixture occurred. Compound **3** was crystallized from methanol–water as a prism of orthorhombic crystals of space group $P2_12_12_1$ with lattice parameters: a = 11.603(1), b = 16.145(2), c = 10.846(1) Å and Z = 4. The final R value was 0.033 for 1689 reflections observed. The solid state conformation of the molecule is shown in Fig. 3. It can be seen from Fig. 3 that the hydroxyl group at C-16 was in an α-equatorial configuration. Molecular dynamics (MD) calculations were performed starting with the X-ray structure by means of the Discover 2.9 program package [11]. These established that the geometry of the most stable conformer of **3a** is the chair conformation of the D-ring, where the hydroxyl group at C-16 adopts an α-equatorial position. This conformer is more stable than the same molecule in the β-axial position (**3b**) by 1.95 kcal mol^{-1}. This geometry is shown in Scheme 1. Upon oxidation with Ag_2O,

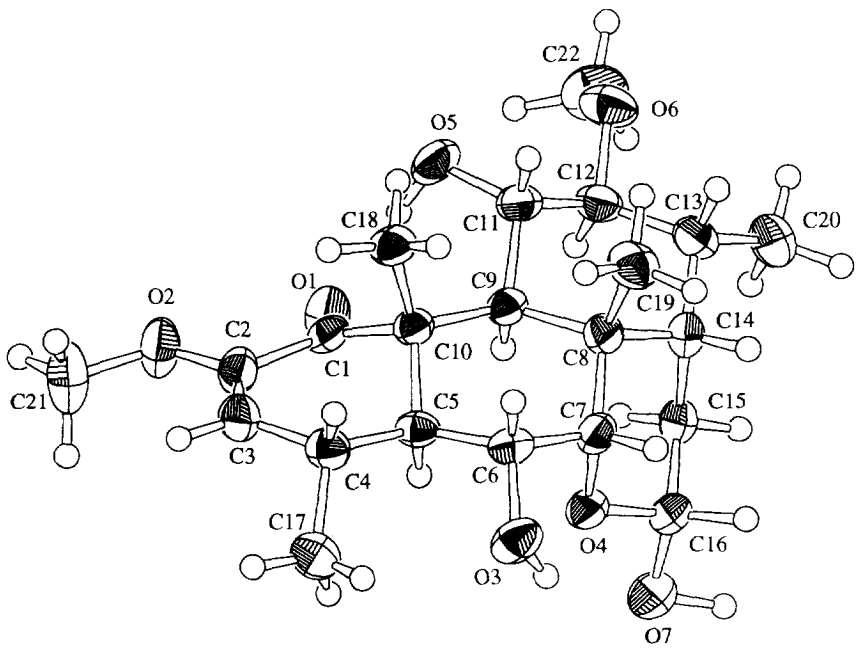
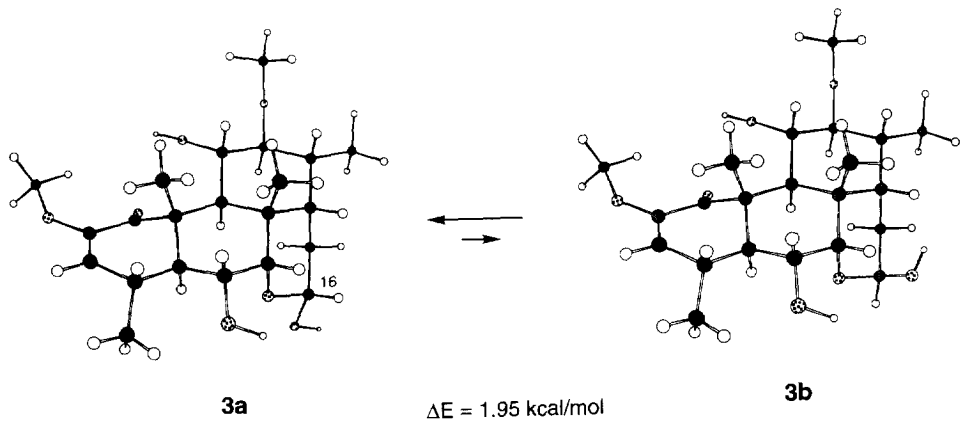


Fig. 3. ORTEP drawing of **3a** with numbering scheme used in the X-ray analysis.



Scheme 1. ΔE = 1.95 kcal/mol.

Table 1. ^{1}H NMR spectral data for compounds **1**–**4** in pyridine-d_5

H	1	2	3a	3b	4
2		4.66 *dd* (11, 7)			
3	5.36 *d* (3)	1.38 *m* 2.38 *m* (3)	5.36 *d* (3)	5.39 *d* (3)	5.33 *d* (3)
4	2.89 *m*	2.42 *m*	2.83 *m*	2.83 *m*	2.32 *m*
5	2.29 *dd* (9, 8)	1.93 *t* (11)	2.43 *dd* (11, 9)	2.35 *dd* (11, 9)	1.91 *m*
6	4.51 *br t* (8)	4.35 *br d* (11)	4.37 *dd* (11, 2)	4.37 *dd* (11, 2)	1.74 *ddd* (14, 13, 2) 1.89 *dt* (14, 3)
7	4.40 *d* (2)	4.37 *d* (2)	3.52 *d* (2)	4.35 *d* (2)	4.14 *dd* (3, 2)
9	2.63 *d* (8)	3.13 *d* (11)	3.06 *d* (11)	3.06 *d* (11)	2.49 *d* (11)
11	3.97 *q* (8)	3.97 *m*	3.93 *br t* (11)	3.97 *t* (11)	3.98 *br t* (11)
12	3.13 *dd* (8, 7)	3.22 *dd* (11, 9)	3.02 *dd* (11, 9)	3.01 *dd* (11, 9)	3.11 *dd* (11, 9)
13	2.14 *m*	2.12 *m*	2.16 *m*	2.20 *m*	2.12 *m*
14	1.73 *m*	1.70 *m*	1.40 *m*	2.08 *m*	1.67 *m*
15	2.72 *dd* (15, 6) 2.83 *dd* (15, 10)	2.66 *dd* (19, 8) 2.78 *dd* (20, 12)	1.91 *dd* (13, 9) 1.97 *m*	1.83 *m* 1.97 *m*	2.67 *dd* (19, 8) 2.79 *dd* (19, 12)
16			5.01 *dd* (9, 3)	5.75 *br s*	
18	1.56 *d* (7)	1.38 *d* (7)	1.54 *d* (7)	1.57 *d* (7)	0.87 *d* (7)
19	1.60 *s*	1.55 *s*	1.58 *s*	1.59 *s*	1.43 *s*
20	1.21 *s*	1.20 *s*	1.10 *s*	1.17 *s*	1.09 *s*
21	0.99 *d* (7)	0.97 *d* (7)	1.02 *d* (7)	1.02 *d* (7)	1.00 *d* (7)
2-OCH_3	3.47 *s*	3.36 *s*	3.47 *s*	3.48 *s*	3.47 *s*
12-OCH_3	3.69 *s*	3.62 *s*	3.63 *s*	3.69 *s*	3.71 *s*

the hemiacetaljavanicin Z gave javanicin Z (**1**). Thus, the absolute configuration of **3** was determined to be the same as that of **1**.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H and ^{13}C NMR: 400 MHz and 100 MHz, respectively, using TMS as an int. standard; CC: silica gel 60 (Merck), Diaion HP-20 (Mitsubishi Kasei); MPLC: ODS (LiChroprep RP-18, 25 × 310 mm, Merck); HPLC: ODS (PEGASIL, 10 × 250 mm, Senshu).

Extraction and isolation. The dried bark (1.8 kg) was collected in Indonesia in July 1986 and extracted with MeOH (16 l). The extract was concd under red. pres. to give a residue (263 g), to which an equal vol. of H_2O was added. The aq. soln was extracted with $CHCl_3$ (4 l) and then *n*-BuOH (4 l). The *n*-BuOH fr. (38 g) was applied to a column of HP-20 which was then eluted stepwise with H_2O containing increasing amounts of MeOH (40, 50, 60, 70 and 100%). The 60% MeOH eluate was applied to a silica gel column (CH_2Cl_2–MeOH with an increasing amount of MeOH). The 10% MeOH eluate was further applied to a MPLC column (ODS, 60% MeOH, flow rate 1 ml/min, detect UV 254 nm) to give compound **1** (40 mg) and a mixture of compounds **2** and **3** (75 mg). The mixture was purified by HPLC (ODS, 60% MeOH, flow rate 1 ml/min, detect UV 254 nm) to give compounds **2** (28 mg) and **3** (45 mg).

Javanicin Z (**1**). Needles (MeOH), mp 287–288°, $[\alpha]_D^{22}$ + 35.3° (MeOH; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3520, 3450, 2930, 1720, 1680, 1650, 1255, 1095, 1055; UV λ_{max}^{MeOH} (log ε) nm: 272 (3.67); CD (MeOH; *c* 9.56 × 10^{-5}) $[\theta]^{25}$ (nm): − 27500 (266), 5600 (311); EIMS *m/z* (rel. int.): 408 (4), 376 (16), 361 (8), 342 (12), 317 (7), 299 (5), 257 (4), 233 (25), 171 (11), 98 (100); HR-MS *m/z*: 408.2169 $[M]^+$, calcd for $C_{22}H_{32}O_7$; 408.2139.

Dihydrojavanicin Z (**2**). Needles (MeOH), mp 231–232°, $[\alpha]_D^{22}$ − 21.2° (MeOH; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3360, 2925, 1710, 1695, 1265, 1135, 1095, 1025; CD (MeOH; *c* 7.07 × 10^{-5}) $[\theta]^{25}$ (nm): − 27500 (295), 5600

Table 2. ^{13}C NMR spectral data for compounds **1–4** in pyridine-d_5

C	1	2	3a	4
1	205.0	213.7	206.2	205.4
2	147.8	79.5	148.0	148.5
3	120.4	46.5	120.5	118.5
4	32.9	30.5	33.3	32.0
5	49.7	53.8	50.8	43.7
6	68.5	68.6	68.9	25.6
7	87.8	88.1	83.9	82.2
8	36.8	35.8	38.2	36.1
9	37.0	36.3	37.8	37.6
10	49.9	51.6	50.4	48.1
11	74.1	72.6	74.7	74.2
12	88.5	88.1	89.6	88.7
13	34.9	34.9	35.1	35.0
14	44.6	45.6	48.2	44.7
15	28.7	28.7	31.3	28.6
16	170.0	170.1	98.2	170.2
18	23.7	22.5	23.8	19.1
19	14.3	14.4	14.5	12.6
20	23.7	21.5	21.2	21.3
21	14.5	14.6	15.4	14.6
2-OCH_3	55.0	57.0	55.1	55.0
12-OCH_3	61.2	60.7	60.9	61.4

(254), − 2000 (232); EIMS *m/z* (rel. int.): 410 (7), 392 (3), 378 (40), 360 (17), 344 (37), 329 (23), 311 (20), 284 (29), 249 (19), 233 (34), 40 (100); HR-MS *m/z*: 410.2335 $[M]^+$, calcd for $C_{22}H_{34}O_7$; 410.2295.

Preparation of MTPA ester. A soln of **2** (2 mg) in dry pyridine (0.1 ml) was treated with (+)-(*S*)-MTPA chloride (20 mg), and the soln allowed to stand at room temp for 20 h. After removing the solvent under reduced pressure, the residue was subjected to prep TLC (hexane–EtOAc 9:1) to give the 6-(*S*)-MTPA ester of **2** (**5**) (1.2 mg). The 6-(*R*)-MTPA ester of **2** (**6**) was obtained in the same manner.

6-(*S*)-MTPA ester (**5**): ^1H NMR (400 MHz, $CDCl_3$): δ4.44 (1H, *dd*, *J* = 12, 8 Hz, H-2), 1.31 (1H, *m*, H-3α), 2.33 (1H, *m*, H-3β), 2.30 (1H, *m*, H-4), 1.99 (1H, *t*, *J* = 11 Hz, H-5), 5.50 (1H, *dd*, *J* = 11, 2 Hz, H-6), 4.21 (1H, *d*, *J* = 2 Hz, H-7), 2.61 (1H, *d*, *J* = 11 Hz, H-9), 3.67 (1H, *dd*, *J* = 11, 9 Hz, H-11), 2.88 (1H, *dd*, *J* = 10, 9 Hz, H-12), 2.08 (1H, *m*, H-13), 1.77 (1H, *m*, H-14), 2.63 (1H, *dd*, *J* = 19, 8 Hz, H-15α), 2.51 (1H, *dd*, *J* = 19, 12, H-15β), 0.90 (3H, *d*, *J* = 6 Hz, Me-4), 1.37 (3H, *s*, Me-8), 1.53 (3H, *s*, Me-10), 1.02 (3H, *d*, *J* = 7 Hz, Me-13), 3.58 (3H, *s*, OMe-2), 3.34 (3H, *s*, OMe-12).

6-(R)-MTPA ester (**6**): ^1H NMR (400 MHz, $CDCl_3$): δ4.36 (1H, *dd*, *J* = 12, 8 Hz, H-2), 1.20 (1H, *m*, H-3α), 2.17 (1H, *m*, H-3β), 1.80 (1H, *m*, H-4), 1.93 (1H, *t*, *J* = 11 Hz, H-5), 5.36 (1H, *dd*, *J* = 11, 2 Hz, H-6), 4.40 (1H, *d*, *J* = 2 Hz, H-7), 2.60 (1H, *d*, *J* = 11 Hz, H-9), 3.66 (1H, *dd*, *J* = 11, 9 Hz, H-11), 2.88 (1H, *dd*, *J* = 10, 9 Hz, H-12), 2.08 (1H, *m*, H-13), 1.80 (1H, *m*, H-14), 2.65 (1H, *dd*, *J* = 19, 8 Hz, H-15α), 2.52 (1H, *dd*, *J* = 19, 12, H-15β), 0.31 (3H, *d*, *J* = 6 Hz, Me-4), 1.38 (3H, *s*, Me-8), 1.48 (3H, *s*, Me-10), 1.03 (3H, *d*, *J* = 7 Hz, Me-13), 3.58 (3H, *s*, OMe-2), 3.31 (3H, *s*, OMe-12).

Hemiacetaljavanicin Z (**3**). Prisms (MeOH), mp 277–278°, $[\alpha]_D^{22}$ + 40.8° (MeOH; *c* 0.8). IR ν_{max}^{KBr} cm^{-1} : 3560, 3485, 2930, 1670, 1645, 1240, 1205, 1155, 1100, 1060; CD (MeOH; *c* 4.63×10^{-5} $[\theta]^{25}$ (nm): − 27500 (295), 5600 (254), − 2000 (232); EIMS *m/z* (rel. int.): 410 (29), 393 (46), 378 (93), 360 (38), 344 (77), 326 (49), 311 (44), 301 (20), 284 (46), 267 (37), 249 (29), 233 (44), 203 (21), 153 (85), 41 (100); HR-MS *m/z*: 410.2315 $[M]^+$, calcd for $C_{22}H_{34}O_7$; 410.2295.

Oxidation of compound **3**. Compound **3** (15 mg) was dissolved in EtOH (10 ml) and H_2O (8 ml) and treated with Ag_2O for 15 hr under reflux. The reaction mixture was filtered through Celite; the filtrate diluted with H_2O and extracted with $CHCl_3$. The solvent was evaporated under reduced pressure and the residue was applied to a silica gel column (CH_2Cl_2–MeOH) to afford the lactone of **3**, which was shown to be identical with javanicin Z (**1**) by ^1H NMR, IR, $[\alpha]_D$, TLC and mmp.

X-ray analysis of compound **3**. Crystals of **3**, crystallized from MeOH–H_2O (7:3), belong to the orthorhombic space group $P2_1P2_1P2_1$. The lattice constants and itensity data were measured on a Rigaku RASA–7R diffractometer with graphite monochromated CuKα radiation and a 18 kW rotating anode generator. The crystal data was as follows: $C_{22}H_{34}O_7$, $M = 410.51$, $a = 11.603(1)$, $b = 16.145(2)$, $c = 10.846$ (1) Å, $V = 2031.8(3)$ Å^3, $Z = 4$, $D_c = 1.342$ g cm^{-3}, μ(CuKα) = 8.15 cm^{-1}. The crystal dimension was $0.2 \times 0.2 \times 0.3$ mm. A total of 1689 independent reflections with $I > 3\sigma(I_0)$ were used for the structure analysis. The structure was determined using a direct method (SAPI91) [13] and expanded using Fourier techniques (DIRDIF92) [14]. The structure was then refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen and isotropic atoms for hydrogen atoms to the '*R* factor of 0.033 ($R_W = 0.029$). The final Fourier difference synthesis showed a maximum and minimum of + 0.18 and − 0.13 e Å^{-3}, respectively. The atomic coordinates, bond lengths, bond angles, thermal parameters and structure factors are available at the Cambridge Crystallographic Centre.

Molecular modelling. Molecular modelling studies were performed using the Discover 2.8 program on a personal IRIS Elan R–4000 computer. Molecular dynamics simulations involved a heating period of 1.0 ps, followed by 1.0 ps equilibration period and then 100 ps of dynamic simulation. The time step of integration was 1 fs. The coordinates produced by simulation were saved every 0.1 ps, giving 1000 structures. Each of them was subjected to energy minimization using the conjugated gradient protocol. All energies are relative to the lowest conformers ($E = 218.860$ kcal/mol for **3a** and $E = 220.807$ kcal/mol for **3b**).

REFERENCES

1. White, T. C. (1973) *Tree Flora of Malaya* Vol. II, p. 351, Longman.

2. Ridely, N. H. (1976) *The Flora of the Malay Peninsula*, Vol I, p. 361, A. Sher, Amsterdam.
3. Yoshikawa, M., Harada, E., Uchida, S., Aoki, S., Yamahara, J., Murakami, N., Shibuya, H. and Kitagawa, I. (1993) *Chem. Pharm. Bull.* **41**, 201.
4. Koike, K., Ohmoto, T., Uchida, A. and Oonishi, I. (1994) *Heterocycles* **38**, 1413.
5. Polonsky, J. (1973) *Fortshr. Chem. Org. Naturst.* **30**, 101.
6. Polonsky, J. (1986) *Fortshr. Chem. Org. Naturst.* **47**, 221.
7. Murae, T., Tsuyuki, T., Nishihama, T., Masuda, S. and Takahashi, T. (1969) *Tetrahedron Letters* 3013.
8. Murae, T., Ikeda, T., Tsuyuki, T., Nishihana, T. and Takahashi, T. (1970) *Bull. Chem. Soc. Jpn.* **43**, 969.
9. Ohtani, I., Kusumi, T., Kashman, Y. and Kakisawa, H. (1991) *J. Am. Chem. Soc.* **113**, 4092.
10. Okano, M., Fujita, T., Fukamiya, N. and Aratani, T. (1985) *Bull. Chem. Soc. Jpn.* **58**, 1793.
11. Koike, K. and Ohmoto, T. (1990) *Phytochemistry* **29**, 2617.
12. *Discover 2.9 program*, Biosym Technol. Inc., San Diego, CA, USA.
13. Fan, H.-F. (1991) *Structure Analysis Programs with Intelligent Control*, Rigaku Co., Tokyo, Japan.
14. Burskens, P. T., Admiraal, G., Beurskens, G., Bosman, W. P., Garcia-Granada, S., Gould, R. O., Smits, J. M. M. and Smylalla, C. (1992) *The DIRDIF Program System Technical Report of the Crystallography Laboratory*, University of Nijimegen, Netherlands.