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C_{18} AND C_{19} QUASSINOIDS FROM *EURYCOMA LONGIFOLIA*

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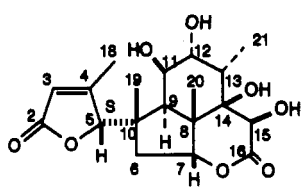
ABSTRACT.—Two new 1,2-seco-1-nor-6(5↦10)*abeo*-picrasan-2,5-olide skeleton quassinoids, eurylactones A [**1**] and B [**2**], and two new C_{19} skeleton quassinoids **3** and **4** were isolated from *Eurycoma longifolia* together with two known C_{18} quassinoids **5** and **6**. Their structures were established by the spectral evidence.

Many quassinoids have already been isolated (1–3) from the roots, wood, and leaves of *Eurycoma longifolia* Jack (Simaroubaceae) and have been used as a folk medicine in southeast Asia. As part of a series of studies, we have further undertaken the chemical investigation of the *n*-BuOH-soluble fraction of the MeOH extract and isolated two new 1,2-seco-1-nor-6(5↦10)*abeo*-picrasane skeleton quassinoids **1** and **2**, which have been known only in shinjulactone B (4,5) and yadanziolide (6), two new C_{19}-type quassinoids **3** and **4**, and two known C_{18}-type quassinoids **5** and **6**. In this paper, the structure elucidation and biogenetic relation of these compounds are described.

RESULTS AND DISCUSSION

The wood of *E. longifolia* collected in Indonesia was extracted with MeOH and then partitioned into CH_2Cl_2, *n*-BuOH, and H_2O. The *n*-BuOH extract was chromatographed on HP-20, and the fractions eluted with 40–80% methanolic H_2O were further separated by Si gel and ODS cc to give compounds **1–6**.

Compound **1** was obtained as colorless needles, mp 148–150° from MeOH, $[\alpha]_D$ +16.7° (*c*=0.11, MeOH). The molecular formula $C_{19}H_{26}O_8$ was established by hrms. The 400 MHz ^{1}H-nmr spectrum of **1** with extensive decoupling experiments allowed the identification of all proton resonances (Table 1). The ir (1730 cm^{-1}) and uv (212 nm, ϵ=9900) spectra indicated the presence of an α,β-unsaturated γ-lactone. In this group, an α proton at δ 5.96 was long-range-coupled with both a lactonic proton at δ 4.80 and a methyl proton at δ 1.92 attached to the β position on the α,β-unsaturated γ-lactone. Furthermore, the methylene protons at δ 2.10 and 2.22 (H-6) were only coupled with



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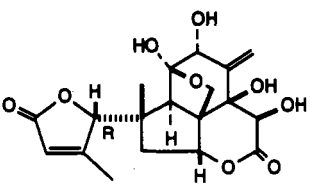

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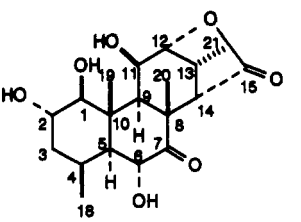

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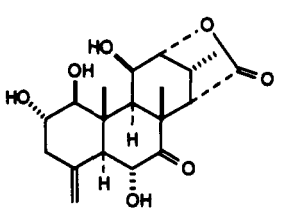

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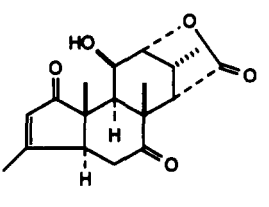

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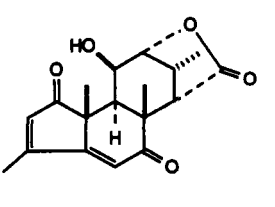

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TABLE 1. ^{1}H-nmr Assignments for Compounds **1**–**4** in Pyridine-d_5.

Proton	Compound			
	1	**2**	**3**	**4**
H-1			3.49 d (8.6)	3.71 d (8.3)
H-2			4.32 m	4.15 m
H-3	5.96 brs	5.97 brs	1.72 m	2.34 t (12.0)
			2.14 ddd (1.9, 2.7, 12.9)	2.89 dd (5.8, 12.0)
H-4			2.70 m	
H-5	4.80 brs	5.16 brs	1.76 m	2.47 d (11.8)
H-6	2.22 d (15.4)	3.04 d (16.2)	5.10 dd (3.4, 12.9)	5.20 dd (3.7, 11.8)
	2.10 dd (15.4, 4.9)	2.40 dd (16.2, 4.8)		
H-7	4.78 d (4.9)	5.11 d (4.7)		
H-9	3.21 d (2.2)	3.90 s	1.89 d (3.4)	2.11 d (3.4)
H-11	5.14 ddd (2.2, 2.5, 5.1)		5.22 dd (3.6, 8.5)	5.36 dd (3.5, 8.6)
H-12	4.33 ddd (2.5, 3.0, 6.5)	4.80 s	4.47 dd (1.0, 4.0)	4.51 dd (1.1, 3.7)
H-13	3.01 dq (3.0, 7.3)		3.16 q (6.9)	3.18 q (6.9)
H-14			3.25 d (1.0)	3.27 d (1.1)
H-15	6.23 s	5.67 s		
H-18	1.92 brs	2.56 brs	1.25 d (7.6)	5.31 brs
				5.39 brs
H-19	1.34 s	1.59 s	1.84 s	1.77 s
H-20	1.88 s	3.90 d (8.7)	1.73 s	1.68 s
		4.58 d (8.7)		
H-21	1.81 d (7.3)	5.58 d (1.2)	1.05 d (6.9)	1.06 d (6.9)
		6.06 d (1.2)		

a proton at δ 4.78 (H-7). The complete assignments of ^{1}H- and ^{13}C-nmr resonances were made by a combination of 2D techniques, such as ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, and COLOC spectra (Tables 1 and 2). These data suggested that compound **1** possesses 1,2-seco-1-nor-6(5↦10)*abeo*-picrasan-2,5-olide skeleton. The presence of a δ-lactone was supported by a ^{13}C-nmr resonance at δ 175.46, and the configurations of the hydroxyl groups at C-11 and C-12 were determined by ^{1}H coupling constants to be similar to those of 14,15β-dihydroxyklaineanone (1) except for the A and B rings. From these data, the structure **1**, named eurylactone A, was determined as shown in Figure 1. The nOe experiment was employed to assign the stereochemistry (Figure 1). The nOe enhancement between H-9 and H-15 suggested that the configuration of a hydroxyl group attached at C-15 was β. Furthermore, as can be seen in Figure 1, the nOe enhancements

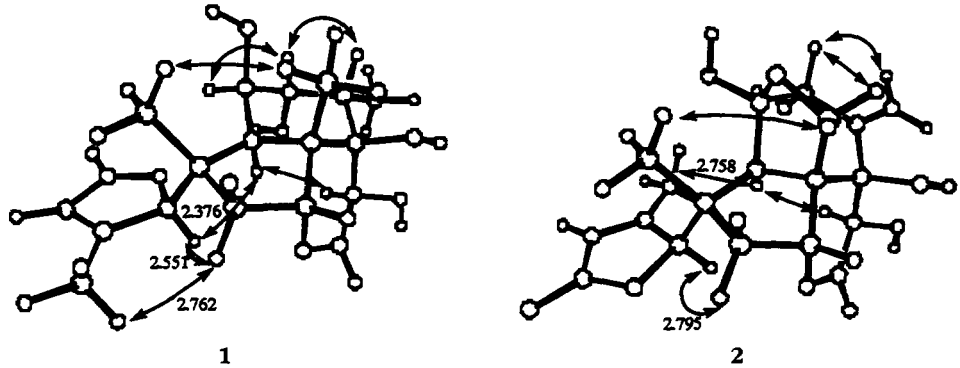

FIGURE 1. Low energy conformers of **1** and **2** calculated by MNDO method (**1**, 273.3835; **2**, 291.7129 kcal/mol) and fractional nOe enhancements. Arrows show nOe relationship. The values (Å) indicate the distances, which were obtained by energy calculations, between the protons (the carbons for methyl groups) related to nOe enhancements.

TABLE 2. ^{13}C-nmr Assignments for Compounds **1–4** in Pyridine-d_5.

Carbon	Compound			
	1	**2**	**3**	**4**
C-1			83.12	82.61
C-2	172.84	172.58	67.87	73.03
C-3	119.88	119.09	39.63	44.37
C-4	168.81	170.03	28.59	141.51
C-5	94.60	92.14	56.66	59.49
C-6	43.29	46.14	70.98	70.12
C-7	87.35	82.75	209.69	209.76
C-8	44.60	63.04	44.00	43.95
C-9	47.92	47.98	51.87	50.62
C-10	50.42	47.43	51.11	50.99
C-11	74.10	110.20	70.01	69.90
C-12	79.25	82.75	83.69	83.87
C-13	37.83	149.22	32.35	32.44
C-14	77.00	77.92	53.74	54.06
C-15	70.08	71.94	176.32	176.44
C-16	175.46	173.58		
C-18	16.20	18.38	14.56	111.43
C-19	19.80	16.19	23.41	23.56
C-20	17.78	68.62	16.77	14.17
C-21	13.39	119.28	16.50	16.63

between H-5 and H-9, between H-5 and H-6α, and between H-6α and H-18 were observed. From these facts, the configuration at C-5 can be interpreted to be *S*, considering the biogenesis of quassinoids.

Compound **2**, named eurylactone B, was obtained as colorless needles, mp 210–212° from EtOAc, $[\alpha]_D$ +62.4° (c=0.17, MeOH), with the molecular formula $C_{19}H_{22}O_9$, which was reported in our earlier communication (7). The structure of **2** was considered to be closely related to compound **1** by the spectral data. The ir (1742 cm^{-1}) and uv (213 nm, ϵ=12700) spectra indicated the presence of an α,β-unsaturated γ-lactone. In the ^{1}H- and ^{13}C-nmr spectra, the chemical shifts of the A and B rings are similar to those of compound **1** (Tables 1 and 2), and the rest of the molecule is similar to that of eurycomanone (1). The presence of the A ring in **2** was inferred from the mass spectrum, in which a peak was observed at *m/z* 97. The appearance of this peak, which was also observed in **1**, implies the presence of the $\{C_5H_5O_2\}^+$ grouping, which is susceptible to fragmentation on electron bombardment. The structure can therefore be determined to be as shown in Figure 1. The stereochemistry at C-5 was determined by a NOESY spectrum, which showed cross peaks between H-9 and H-18, and between H-5 and H-6α, suggesting that C-5 had an *R* configuration (Figure 1).

Compounds **1** and **2** possess structures similar to 14,15β-dihydroxyklaineanone and eurycomanone, which were isolated from the same plant. One of the most interesting features in **1** is that the configuration at C-5 is opposite to that in **2**. Ishibashi *et al.* (8) proposed a biogenetic pathway from C_{20}-type quassinoids to the 1,2-seco-1-nor-6(5↦10)*abeo*-picrasan-2,5-olide skeleton, the configuration of C-5 being affected by the presence of an ether linkage between C-20 and C-11. Compounds **1** and **2** were also considered to be derived by a similar pathway from 14,15β-dihydroxyklaineanone and eurycomanone, respectively, and each configuration at C-5 was identical with the proposed biogenetic pathway (Figure 2).

Furthermore, to obtain the stable rotamers of **1** and **2** and to verify the configurations of C-5, molecular mechanics calculations using TRIPOS force field (9) were

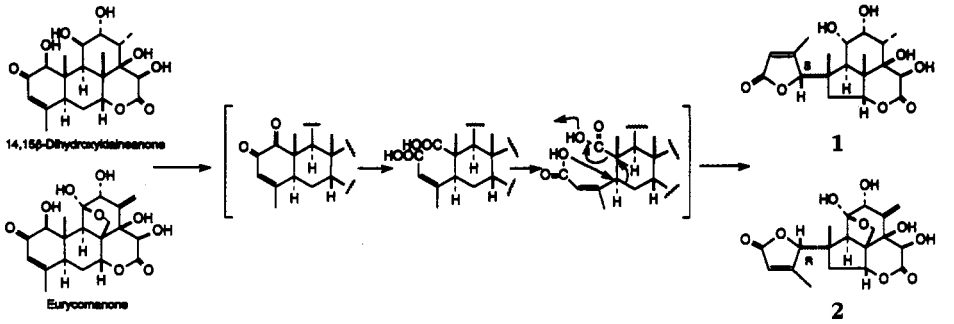

FIGURE 2. Proposed biogenetic pathway from C_{20}-type quassinoids to 1,2-seco-1-nor-6(5↦10)*abeo*-picrasan-2,5-olide skeleton quassinoids, eurylactones A [**1**] and B [**2**].

conducted by axis rotations per 5 degrees around the bond between the A and B rings. For each compound the most energetically stable conformer was further refined by MNDO in MOPAC version 5.0 (10). In these conformers as shown in Figure 1, the torsion angle of H-5–C-5–C-10–C-19 in **1** was −169.3 degrees and that in **2** was 177.8 degrees. Distances between some protons depicted in Figure 1 reflected the nOe relationship as described above.

Compound **3**, colorless needles, mp 205–207° from MeOH, $[\alpha]_D$ +29.3° (c=0.27, MeOH), molecular formula $C_{19}H_{28}O_7$, was shown to have an eurycomalactone-type C_{19} skeleton. However, the spectroscopic data for the A ring were different from those of eurycomalactone; they show no uv absorption band ascribable to an α,β-unsaturated ketone. In the ^{1}H-^{1}H COSY spectrum, the H-1 proton (δ 3.49) attached to a hydroxyl-bearing carbon was coupled with the H-2 proton (δ 4.32) also attached to a hydroxyl-bearing carbon, which showed further coupling with the C-3 methylene protons (δ 1.72 and 2.14). Except for the A ring, the spectroscopic data of the B, C, and D rings were almost identical with those of 6α-hydroxyeurycomalactone (2). The stereostructure of ring A was assigned by NOESY experiments, in which H-1 (δ 3.49) exhibited an nOe correlation with H-9 and H-3α, H-2 (δ 4.32) with H-18, H-19, and H-3β, and H-18 (δ 1.25) with H-2, H-6, and H-19. The stereochemistry about ring A indicated the stereostructure of **3** as shown in Figure 3.

Compound **4**, colorless needles, mp 208–210° from MeOH, $[\alpha]_D$ +48.6° (c=0.14, MeOH), $C_{19}H_{26}O_7$, was shown to also have an eurycomalactone-type C_{19} skeleton. In the ^{1}H- and ^{13}C-nmr spectra, the coupling sequence of B, C, and D rings was similar to that of compound **3**, but compound **4** showed the presence of an exomethylene group (^{1}H δ 5.31 and 5.39; ^{13}C δ 141.51 and 111.43). In the ^{1}H-^{1}H COSY spectrum, the exomethylene protons were coupled with methylene protons of H-3 (δ 2.34 and 2.89)

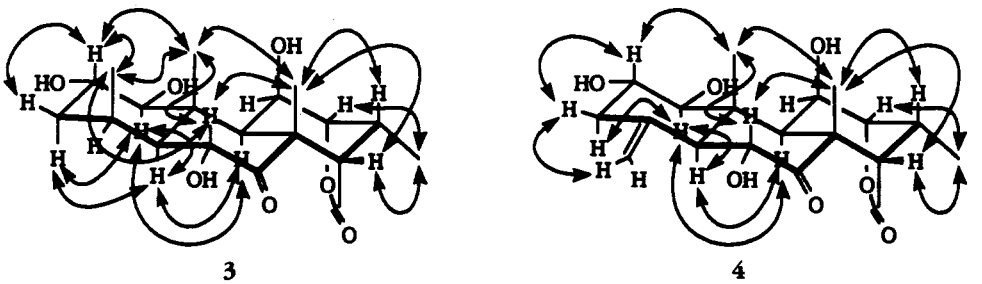

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FIGURE 3. Fractional nOe enhancements of compounds **3** and **4**. Arrows show nOe relationship.

and H-5 (δ 2.47). These couplings and nOe relationships indicated the structure as in Figure 3.

Compounds **5** and **6** were identical with laurycolactone A (11) and laurycolactone B (11), respectively, which possess a C_{18} skeleton, by comparison of various spectral data.

Compounds **1–6** show no cytotoxic activities. The presence of an α,β-unsaturated ketone and a six-membered A ring may be structural requirements for antileukemic activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were obtained with a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The optical rotations were measured on a JASCO DIP-4 polarimeter. Uv spectra were taken in MeOH on a Hitachi 557 spectrophotometer. Ir spectra were obtained on KBr plates on a Perkin-Elmer 1710 spectrophotometer. ^{1}H- and ^{13}C nmr were run in pyridine-d_5 using Bruker AM 400 and with shifts (δ) reported in ppm. Mass spectra were recorded on a Hitachi M-80 and VG Autospec instrument. Hplc was performed on an Inertsil PREP-ODS packed with 10 μm ODS. Detection: spraying with 10% H_2SO_4 followed by heating for 10 min at 200°.

PLANT MATERIAL.—The wood of *E. longifolia* was collected in Indonesia in May 1990. The botanical identification was made by Dr. Kosashi Padmawinata, Natural Sciences, Bandung Institute of Technology, Indonesia. A voucher specimen has been deposited in the herbarium of the Tokyo College of Pharmacy.

EXTRACTION AND ISOLATION.—The wood (4.5 kg) of *E. longifolia* was extracted with MeOH and concentrated; the extract was successively partitioned into CH_2Cl_2, *n*-BuOH, and H_2O; and the *n*-BuOH phase was evaporated to dryness to give a brown residue (53.02 g). The *n*-BuOH extract was chromatographed on Diaion HP-20, eluting with H_2O followed by H_2O with increasing amounts of MeOH. The fraction (26.24 g) eluted with 40–80% MeOH was subjected to Si gel cc using a CH_2Cl_2/MeOH gradient system. Fraction 2 was further separated on an ODS column eluted with 15% MeOH to give compound **2** (0.035%), and fraction 5 eluted with 40% MeOH to give compound **1** (0.021%) and a 1:1 mixture of compounds **3** and **4**, followed by purification by hplc with 20% MeOH to give **3** (0.008%) and **4** (0.004%).

Fractions eluted with 80% MeOH and 100% MeOH on Diaion HP-20 were further purified using an ODS column eluted with 53% and 55% MeOH, respectively, to yield compounds **5** (0.004%) and **6** (0.004%).

Compound **1**.—Mp 148–150°; [α]D +16.7° (*c*=0.11, MeOH); uv (MeOH) λ max 212 nm (ε=9900); ir (KBr) 3436, 1730, 1635, 897 cm^{-1}; eims *m/z* (%) [M]$^+$ 382 (8), 363 (58), 325 (24), 249 (20), 203 (35), 123 (65), 97 (base peak), 69 (88); cims *m/z* [MH]$^+$ 383, 363, 329, 255, 187, 99, 69; ^{1}H nmr see Table 1; ^{13}C-nmr see Table 2; hrms found 382.1622 (calcd 382.1627).

Compound **2**.—Mp 210–212°; [α]D +62.4° (*c*=0.17, MeOH); uv (MeOH) λ max 213 (12700); ir (KBr) 3402, 1742, 1642, 888 cm^{-1}; eims *m/z* (%) [M]$^+$ 394 (10), 376 (20), 279 (16), 205 (base peak), 159 (40), 97 (62), 69 (48); cims *m/z* [MH]$^+$ 395, 377, 302, 279, 205, 177, 97, 69; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hrms found 394.1264 (calcd 394.1263).

Compound **3**.—Mp 205–207°; [α]D +29.3° (*c*=0.27, MeOH); ir (KBr) 3471, 2965, 1765, 1707 cm^{-1}; eims *m/z* (%) [M]$^+$ 368 (38), 350 (38), 332 (15), 267 (15), 205 (22), 175 (27), 135 (40), 107 (48), 69 (base peak); cims *m/z* [MH]$^+$ 369, 351, 333, 287, 187, 147, 123, 91, 69; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hrms found 368.1865 (calcd 368.1835).

Compound **4**.—Mp 208–210°; [α]D +48.6° (*c*=0.14, MeOH); ir (KBr) 3503, 3378, 2927, 1782, 1715, 910 cm^{-1}; eims *m/z* (%) [M]$^+$ 366 (20), 348 (68), 330 (22), 221 (37), 175 (34), 147 (50), 129 (35), 109 (36), 91 (61), 69 (base peak); cims *m/z* [MH]$^+$ 367, 349, 331, 257, 147, 129, 91, 69; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hrms found 366.1687 (calcd 366.1678).

Compound **5**.—Mp 265–267°; [α]D +112.9° (*c*=0.14, $CHCl_3$); ir ($CHCl_3$) 3620, 3020, 2927, 1780, 1720, 1700 cm^{-1}; ^{1}H nmr (pyridine-d_5) δ 5.86 (1H, brs, H-2), 5.70 (1H, br dd, *J*=5.7 and 3.3 Hz, H-11), 4.48 (1H, dd, *J*=4.1 and 1 Hz, H-12), 3.32 (1H, q, *J*=6.9 Hz, H-13), 3.12 (1H, d, *J*=1 Hz, H-14), 2.85 (1H, dd, *J*=16.2 and 13.1 Hz, H-6β), 2.75 (1H, dd, *J*=13.1 and 4.9 Hz, H-5), 2.20 (dd, *J*=16.2 and 4.9 Hz, H-6α), 2.43 (d, *J*=3.4 Hz, H-9), 1.88 (3H, s, H-18), 1.72 (3H, s, H-19), 1.64 (3H, s, H-20), 1.02 (3H, d, *J*=7.0, H-21); ^{13}C nmr (pyridine-d_5) δ 212.3 (C-1), 127.1 (C-2), 177.5 (C-4), 52.1 (C-5), 42.5 (C-6), 210.1 (C-7), 48.5 (C-8), 37.7 (C-9), 49.4 (C-10), 67.5 (C-11), 85.1 (C-12), 31.9 (C-13), 54.5 (C-14), 177.2 (C-15), 17.2 (C-18), 24.6 (C-19), 21.3 (C-20), 16.7 (C-21); eims *m/z* (%) [M]$^+$ 318 (10), 312 (13), 274 (40), 259 (40), 191 (9), 152 (15), 123 (base peak), 91 (25), 69 (35); cims *m/z* [MH]$^+$ 319, 301, 274, 255, 179, 152, 123, 91, 69.

Compound **6**.—Mp 215–218°; [α]D +65.7° (*c*=0.15, $CHCl_3$); ir ($CHCl_3$) 3620, 3030, 1780, 1715, 1670 cm^{-1}; uv λ max (MeOH) 285 nm (ε 13300); ^1H nmr ($CDCl_3$) δ 6.13 (1H, br d, *J*=1.3, H-2), 5.91 (1H, s, H-6), 5.03 (1H, dd, *J*=5.0 and 1.3, H-11), 4.28 (1H, dd, *J*=4.9 and 1.1, H-12), 3.03 (1H, q, *J*=7.0, H-13), 2.99 (1H, d, *J*=1.2, H-14), 2.21 (3H, brs, H-18), 2.13 (1H, d, *J*=3.5, H-9), 1.68 (3H, s, H-19), 1.59 (3H, s, H-20), 1.16 (3H, d, *J*=7.0, H-21); ^{13}C nmr ($CDCl_3$) δ 205.2 (C-1), 132.9 (C-2), 166.3 (C-4), 164.8 (C-5), 116.2 (C-6), 198.0 (C-7), 47.6 (C-8), 53.1 (C-9), 48.6 (C-10), 67.9 (C-11), 83.5 (C-12), 32.1 (C-13), 40.7 (C-14), 176.3 (C-15), 21.5 (C-18), 13.8 (C-19), 23.1 (C-20), 16.8 (C-21); eims *m/z* (%) $[M]^+$ 316 (100), 288 (9), 272 (22), 189 (73), 150 (80), 121 (52), 91 (92), 69 (94).

MOLECULAR ENERGY CALCULATIONS.—Computer modeling and all calculations were performed using the molecular-modeling software SYBYL (Tripos Associates, Inc.) on an IRIS 4-D workstation. Conformational search calculation was employed by the standard SYBYL force field (9). The coordinates required were made from X-ray data of eurylactone B (7). The structures obtained by molecular mechanics calculations were further minimized with the MNDO program (version 5.0) and the convergence criterion PRECISE option.

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