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Short communication

New antiulcer quassinoids from *Eurycoma longifolia*

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Summary — A bioassay study of *Pasak bumi (Eurycoma longifolia)* led to the isolation of four quassinoids, pasakbumin-A (**1**), -B (**2**), -C (**4**), and -D (**7**). Both pasakbumin-A (= eurycomanone) **1** and pasakbumin-B **2** exhibited potent antiulcer activity. This prompted us to test some modified quassinoids to reveal that ailanthone (**8**), the main quassinoid of *Ailanthus altissima,* and its α-epoxide (**9**) have potent activity and less toxicity. Thus, quassinoids which are generally recognised as antitumor compounds were revealed to have antiulcer activity.

Résumé — Nouveaux quassinoïdes antiulcéreux à partir d'*Eurycoma longifolia*. *Une étude de* Pasak bumi (Eurycoma longifolia) *a conduit à l'isolement de quatre quassinoïdes, pasakbumine-A (**1**), -B (**2**), -C (**3**), et -D (**4**). Les pasakbumine-A (eurycomanone) **1** et -B, **2**, présentent une forte activité antiulcéreuse. Quelques quassinoïdes modifiés ont montré que l'ailanthone (**8**), le quassinoïde majeur d'*Ailanthus altissima, *et son époxyde α (**9**) ont une activité puissante et une faible toxicité. Ainsi, des quassinoïdes généralement reconnus comme antitumoraux ont manifesté une activité antiulcéreuse.*

quassinoid / pasakbumins / ailanthone / ailanthone α-epoxide / *Pasak bumi* / *Eurycoma longifolia* / *Ailanthus altissima* / Simaroubaceae / antiulcer activity

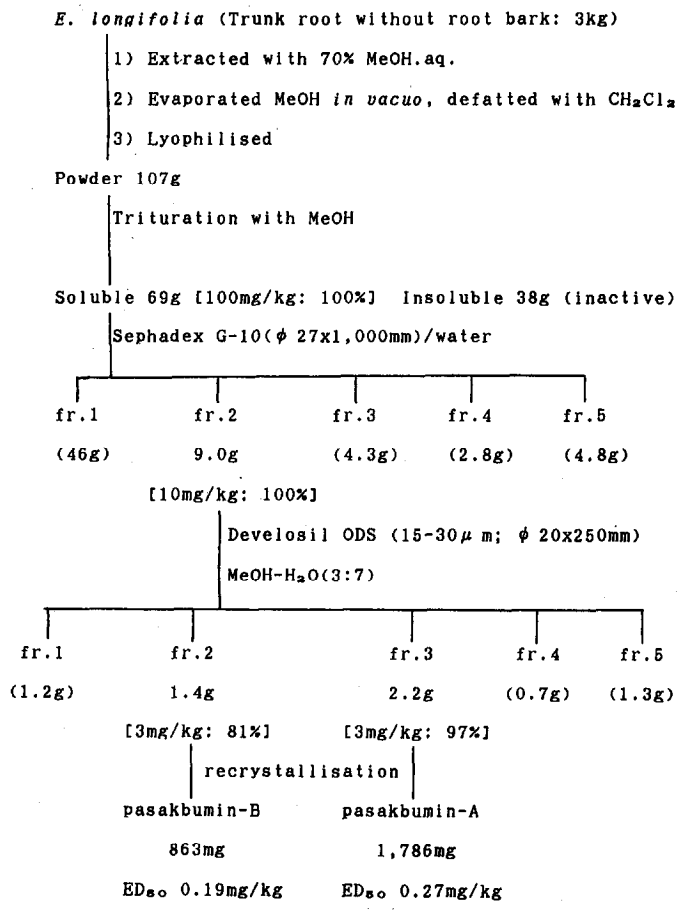
Introduction

Eurycoma longifolia is one of the folk medicines commonly used in South east Asia. Studies on isolating the components of this plant yielded eurycomalactone [1], laurycolactone A and B [2], and eurycomanone and eurycomanol [3]. In some regions of South Kalimantan, the plant is called *Pasak bumi* and the root trunk is used as a tonic. We report here on the isolation and structure elucidation of four quassinoids from the plant originating from South Kalimantan, together with a new finding that some quassinoids have antiulcer activity [4] in addition to the antitumor activity generally recognised to-date [5, 6].

Chemistry

The aqueous methanol extract was first blindly screened and found to have an activity on ulcers induced by indomethacin as well as by stress. Bioassay-guided isolation of the components from the methanol extract afforded 2 major compounds, pasakbumin-A (0.06%) and pasakbumin-B (0.03%) after successive trituration, gel filtration, preparative RP-HPLC, and recrystallisation, as shown in scheme 1. The *mp*, $[\alpha]_D$, and the spectral data (UV, IR, NMR, and SI-MS) of pasakbumin-A (**1**) showed that it was eurycomanone, previously isolated from leaves of the same plant [3].

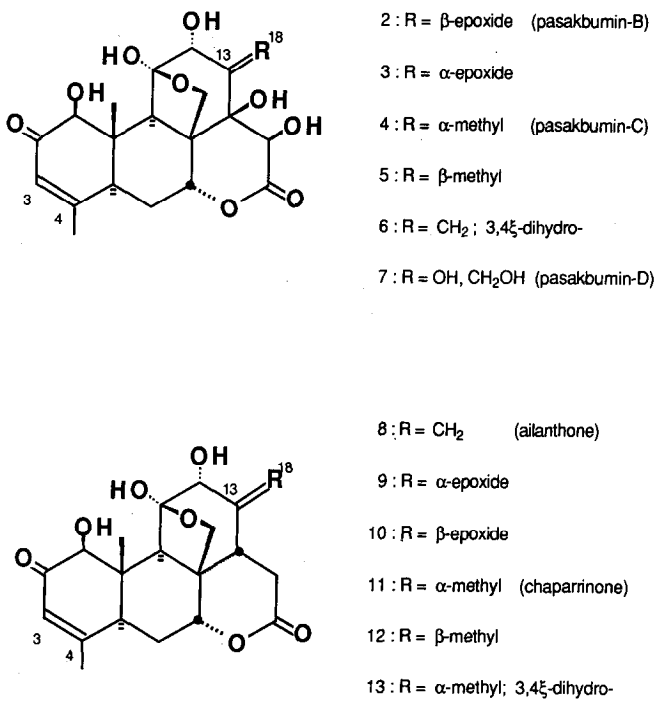
Pasakbumin-B (**2**), *mp* > 300°C, $[\alpha]_D$ + 30.0°, SI-MS (m/z) 425 (M^+ + H), exhibited additional -CH_2O- signals and the absence of the signals for exo-methylene in its ^1H- and ^{13}C- NMR spectra on comparison with those of eurycomanone **1**. This suggests that pasakbumin-B is the epoxide of **1**. Epoxidation of **1** with m-chloroperbenzoic acid followed by separation by preparative RP-HPLC gave epimeric epoxides. The minor epimer proved to be identical with pasakbumin-B **2** whose configuration of epoxide suggested it to be β following mechanistic consideration (reagent attack from the less hindered side, assisted by hydroxy groups, may furnish the α-epoxide). Since a suitable crystal of pasakbumin-B could not be obtained for the X-ray analysis (it gave only long thin needles), a crystal of the major epimeric epoxide was used instead. As shown in figure 1, the major epimer was α-epoxide (**3**), so that the minor epimer as well as pasakbumin-B is the β-epoxide designated as **2**.



Scheme 1.

The mother liquor of pasakbumin-A **1** was rechromatographed on ODS column then on silica-gel column, giving pasakbumin-C (**4**), *mp* = 256–257°C (dec), SI-MS (m/z) 411 ($M^+ + H$). The ^{1}H-NMR of **4** indicated a signal of the new secondary methyl group instead of the exo-methylene signals on comparison with that of pasakbumin-A **1**. This suggests that **4** is the 13,18-dihydro derivative of **1**. Hydrogenation of **1** catalysed by $(Ph_3P)_3RhCl$ gave 13,18-dihydro epimers **4** and (**5**) along with the 3,4-dihydro compound (**6**). One of the 13,18-dihydro epimers was identified with pasakbumin-C **4** by their spectral data. The configuration of the C-13 methyl group of **4** was finally determined as α by X-ray analysis (fig 2).

The mother liquor of pasakbumin-B **2** was also rechromatographed on ODS column and then on silica-gel column, giving pasakbumin-D (**7**), *mp* = 280–282°C (dec), SI-MS (m/z) 443 ($M^+ + H$). The ^{1}H-NMR of **7** showed no exomethylene signals but the $-CH_2O-$ group as an AB quartet pattern, suggesting **7** to be the 13,18-diol derivative of **1**. The stereochemistry of the diol system is not yet known.



Scheme 2.

The potent antiulcer activity (tables I and II) found for pasakbumin-A **1** (eurycomanone) and pasakbumin-B **2**, led us to convert ailanthone (**8**), the main quassinoid of *Ailanthus altissima*, into several deriva-

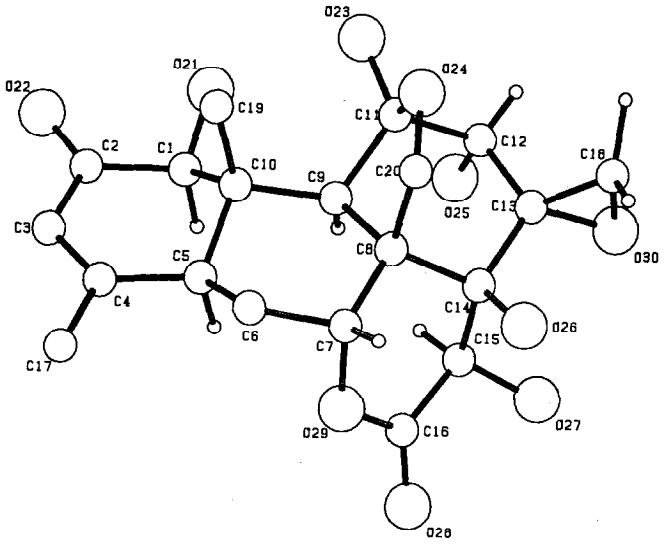


Fig 1. Perspective view of pasakbumin-A α-epoxide.

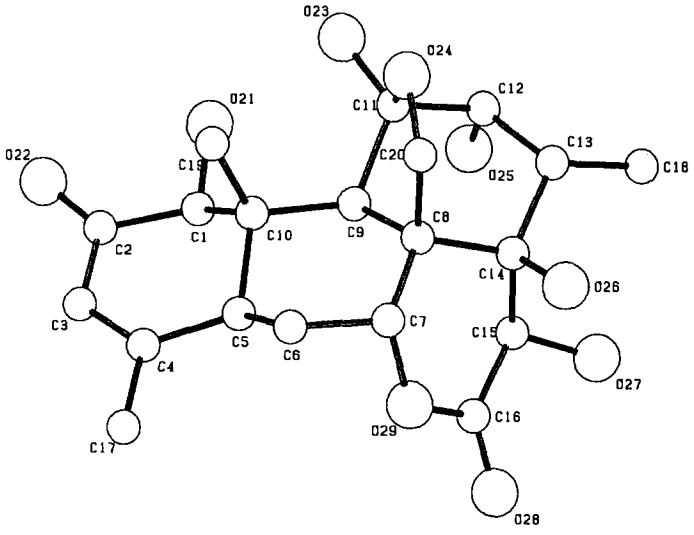


Fig 2. Perspective view of pasakbumin-C.

Table I. Effects of quassinoids on indomethacin-induced gastric erosion and gastric secretion in pylorus-ligated rat.

Compd	*Dose (mg/kg)*	*Indomethacin ulcer* *Inhibition (%)*	ED_{50} *(mg/kg)*	*Gastric acid output* *Inhibition (%)*	ED_{50} *(mg/kg)*
1	0.1	12.5	0.27	23.2	0.35
	0.3	58.5	–	48.8	–
	1.0	94.7	–	69.9	–
	3.0	100.0	–	82.3	–
2	0.1	26.4	0.19	26.5	0.39
	0.3	71.6	–	52.3	–
	1.0	92.6	–	61.4	–
	3.0	100.0	–	92.0	–
3	3.0	4.9	–	–	–
4	3.0	47.1	–	–	–
5	3.0	0.0	–	–	–
6	3.0	0.0	–	–	–
8	0.1	2.1	0.36	61.9	0.04
	0.3	44.3	–	80.5	–
	1.0	95.8	–	96.6	–
	3.0	98.0	–	–	–
9	0.3	36.8	0.45	7.4	0.47
	1.0	77.1	–	48.1	–
	3.0	100.0	–	64.8	–
10	3.0	74.4	–	–	–
11	3.0	67.8	–	–	–
13	3.0	13.7	–	–	–

Table II. Effects of quassinoids on water-immersion stress-induced erosion in rats.

Compound	*Dose (mg/kg)*	n	*Inhibition (%)*
1	1.0 (ip)	5	80.7
2	1.0 (ip)	5	91.8
8	1.0 (*po*)	5	64.0
	6.0 (*po*)	5	89.7
9	3.0 (*po*)	5	39.3
	10.0 (*po*)	5	53.2
	0.3 (ip)	5	49.8
	1.0 (ip)	5	92.4

tives to test for antiulcer activity. Epoxidation of ailanthone **8** with *m*-chloro-perbenzoic acid gave an epoxide mixture, which was separated by RP-HPLC into (**9**) and (**10**). X-ray analysis of the major epimer **9** revealed it to be the α-epoxide, as shown in figure 3.

Catalytic hydrogenation of ailanthone **8** with $(Ph_3P)_3RhCl$ followed by RP-HPLC gave chaparrinone (**11**) and β-isomer **12** along with 3,4-dihydrochaparrinone (**13**). Among these, ailanthone **8** and its α-epoxide **9** were found to have potent activity (tables I and II).

Pharmacology

The results of the examination of antiulcer activity and acute toxicity are summarised in tables I to III.

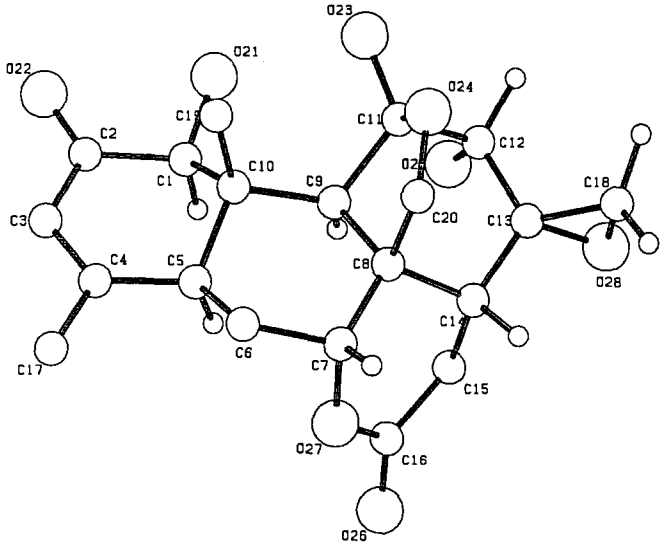


Fig 3. Perspective view of ailanthone α-epoxide.

Table III. Acute toxicity.

Compound	*LD_{50} (mg/kg)*
1	18.9
2	5.1
8	31.4
9	> 100.0
10	> 30.0
11	> 30.0
13	> 30.0

Conclusion

Bioassay study of *Pasak bumi* (*E longifolia*) has led to the discovery of antiulcer activity for quassinoids which were hitherto known as a group of cytotoxic compounds.

The relationship between cytotoxicity and antiulcer activities, as well as the structure-activity relationships are future interesting problems.

Experimental protocols

Chemistry

Unless otherwise specified, the specific rotations were measured in 1% pyridine solution, using a Perkin Elmer 241 Polarimeter. The IR spectra were mesured using JASCO A-700 spectrometer. ^{1}H FT NMR spectra were recorded at 200 MHz using 3-5 mg/0.5 ml of sample solution, and ^{13}C FT NMR spectra were determined at 50 MHz using 20 mg/0.6 ml of sample solution, in pyridine-d_5 containing TMS as internal reference on Varian XL-200 NMR spectrometer. Develosil ODS 15–30 μm (Nomura Chemical Co) was packed (home-made, N = 4000) in a heavy-wall glass column GCH-20 (20 x 250 mm, Umetani Precision Co, Ltd). Preparative HPLC system was built up with Altex 110A (pump), 6-way valve connected with 10 ml sample loop (injection), and JASCO UVIDEC-100 (UV detector).

Isolation of pasakbumins

The pulverised root trunk without root bark (3 kg) was extracted twice with 70% MeOH aq at room temperature. Evaporation of MeOH and extraction of residual aqueous solution with CH_2Cl_2 followed by lyophilisation of the aqueous solution gave 107 g of light brown powder, which, upon trituration with MeOH, gave 69 g of a MeOH soluble material with 38 g of insoluble material. The former was applied to Sephadex G-10 column and eluted with H_2O, being monitored by UV and RI detectors simultaneously (repeated injection of 10 g sample on 27 x 1000 mm column). The active eluate (fr 2, 9.0 g) was further chromatographed on ODS column [repeated injection of 0.2 g sample on Develosil ODS, MeOH-H_2O (3/7)]. The active eluates fr 2 (1.4 g) and fr 3 (2.2 g) were recrystallised from AcOEt-MeOH to give pasakbumin-B **2** (863 mg, 0.03%) and pasakbumin-A (= eurycomanone) **1** (1786 mg, 0.06%), respectively.

Pasakbumin-A 1. $C_{20}H_{24}O_9 \cdot 2H_2O \cdot$MeOH (after X-ray analysis) *mp* = 273–275°C; $[\alpha]_D$ + 33.7°; IR (KBr) 3400, 1735, 1670, 1625 cm^{-1}; SI-MS m/z 409 (M^+ + H); ^{1}H-NMR δ 6.16 (br-s, H-3), 6.10, 5.65 (each d, $=CH_2$), 5.66 (s, H-15), 5.26 (t-like, H-7), 4.80 (s, H-12), 4.53 (s, H-1), 4.56, 4.03 (ABq, H-20), 3.83 (s, H-9), 3.27 (br-d, H-5), 2.34 (br-d, H-6α), 2.02 (br-t, H-6β), 1.79 (s, Me-17), 1.63 (s, Me-19); ^{13}C-NMR δ 197.5 (C-2), 173.8 (C-16), 162.6 (C-4), 147.9 (C-13), 126.1 (C-3), 119.4 (C-18), 109.5 (C-11), 84.5 (C-1), 81.0 (C-12), 79.4 (C-14), 75.9 (C-7), 71.8 (C-15), 67.7 (C-20), 52.6 (C-8), 47.7 (C-9), 45.9 (C-10), 42.2 (C-5), 25.7 (C-6), 22.4 (C-17), 10.4 (C-19).

Pasakbumin-B 2. *mp* > 300°C; $[\alpha]_D$ + 30.0°; IR (KBr) 3440, 3395, 1737, 1666, 1630 cm^{-1}; SI-MS m/z 425 (M^+ + H); ^{1}H-NMR δ 6.16 (br-s, H-3), 5.83 (s, H-15), 5.13 (t-like, H-7), 4.88, 4.07 (ABq, H-20), 4.55 (s, H-1), 4.04 (s, H-12), 3.82 (s, H-9), 3.80, 3.04 (ABq, H-18), 3.26 (br-d, H-5), 2.34 (br-d, H-6α), 2.03 (br-t, H-6β), 1.80 (s, Me-17), 1.63 (s, Me-19); ^{13}C-NMR δ 197.4 (C-2), 173.8 (C-16), 162.5 (C-4), 126.1 (C-3), 109.6 (C-11), 84.4 (C-1), 81.7 (C-12), 75.6 (C-7), 75.4 (C-14), 71.4 (C-15), 66.9 (C-20), 59.2 (C-13), 53.5 (C-8), 48.4 (C-9), 46.5 (C-18), 45.8 (C-10), 42.2 (C-5), 25.5 (C-6), 22.4 (C-17), 10.4 (C-19).

The mother liquor of pasakbumin-A **1** was rechromatographed on ODS column to separate **1** [Develosil ODS, MeOH-H_2O (2/8)] and the remaining fractions were further chromatographed on silica gel [Lobar B, AcOEt-MeOH-H_2O (50/5/1)] to yield pasakbumin-C **4**, which upon crystallisation from MeOH gave pure **4**. In the same manner, the mother liquor of pasakbumin-B **2** was rechromatographed on ODS column [Develosil ODS, MeOH-H_2O (2/8)] and silica gel column [Lobar B, AcOEt-MeOH-H_2O (30/5/1)] successively to give pasakbumin-D **7**, which on recrystallisation from MeOH gave pure **7**.

Pasakbumin-C 4. $C_{20}H_{26}O_9 \cdot 2H_2O \cdot$MeOH (after X-ray analysis) *mp* = 256–257°C; $[\alpha]_D$ – 12.6°; IR (KBr) 3520, 3420, 1723, 1665, 1618 cm^{-1}; SI-MS m/z 411 (M^+ + H); ^{1}H-NMR δ 6.12 (br-s, H-3), 5.62 (s, H-15), 5.21 (t-like, H-7), 4.65, 4.05 (ABq, H-20), 4.40 (s, H-1), 4.15 (d, H-12), 3.52 (s, H-9), 3.19 (br-d, H-5), 2.86 (d-q, H-13), 2.29 (br-d, H-6α), 2.03 (br-t, H-6β), 1.86 (d, Me-18), 1.77 (s, Me-17), 1.63 (s, Me-19); ^{13}C-NMR δ 197.5 (C-2), 174.8 (C-16), 162.7 (C-4), 126.1 (C-3), 110.3 (C-11), 84.6 (C-1), 79.7 (C-12), 76.6 (C-14), 75.1 (C-7), 71.9 (C-15), 67.3 (C-20), 52.8 (C-8), 47.0 (C-9), 45.7 (C-10), 42.2 (C-13 and C-5), 25.8 (C-6), 22.4 (C-17), 14.0 (C-18), 10.7 (C-19).

Pasakbumin-D 7. *mp* = 280–282°C (dec); $[\alpha]_D$ + 12.9°; IR (KBr) 3480, 3400, 1725, 1672, 1625 cm^{-1}; SI-MS m/z 443 (M^+ + H); ^{1}H-NMR δ 6.13 (br-s, H-3), 5.58 (s, H-15), 5.22, 3.97 (ABq, H-20), 5.12 (t-like, H-7), 5.03, 4.64 (ABq, H-18), 4.60 (s, H-12), 4.46 (s, H-1), 3.60 (s, H-9), 3.20 (br-d, H-5), 2.28 (br-d, H-6α), 2.07 (br-t, H-6β), 1.77 (s, Me-17), 1.65 (s, Me-19); ^{13}C-NMR δ 197.6 (C-2), 173.5 (C-16), 162.5 (C-4), 126.1 (C-3), 110.0 (C-11), 84.7 (C-1), 79.6 (C-12), 78.3 (C-13), 77.9 (C-14), 74.9 (C-7), 70.6 (C-15), 67.6 (C-20), 66.8 (C-18), 53.5 (C-8), 47.4 (C-9), 45.5 (C-10), 42.3 (C-5), 25.7 (C-6), 22.4 (C-17), 11.0 (C-19).

Epoxidation of pasakbumin-A 1

To a solution of **1** (101 mg) in acetonitrile (16 ml), *m*-chloroperbenzoic acid (81 mg, 1.5 eq) was added and the mixture was stirred for 70 h at room temperature. Evaporation of the solvent followed by trituration of the residue with ether afforded a solid which was chromatographed on ODS column [Develosil ODS, MeOH-H_2O (3/7)], giving the starting material **1** (24 mg, 23%), β-epoxide (= pasakbumin-B **2**, 18 mg, 17%), and α-epoxide **3** (58 mg, 55%).

α-Epoxide 3. *mp* = 260–261°C; $[\alpha]_D$ + 18.2°; IR (KBr) 3400, 1735, 1670, 1623 cm^{-1}; SI-MS m/z 425 (M^+ + H); ^{1}H-

NMR δ 6.15 (br-s, H-3), 6.06 (s, H-15), 5.28 (t-like, H-7), 4.58, 4.09 (ABq, H-20), 4.52 (s, H-1), 4.08 (s, H-12), 3.80 (s, H-9), 3.68, 2.88 (ABq, H-18), 3.27 (br-d, H-5), 2.32 (br-d, H-6α), 2.03 (br-t, H-6β), 1.79 (s, Me-17), 1.63 (s, Me-19); ^{13}C-NMR δ 197.4 (C-2), 173.1 (C-16), 162.6 (C-4), 126.1 (C-3), 109.8 (C-11), 84.5 (C-1), 81.3 (C-12), 75.1 (C-7), 74.1 (C-14), 72.4 (C-15), 67.1 (C-20), 62.7 (C-13), 52.0 (C-8), 50.6 (C-18), 47.4 (C-9), 45.8 (C-10), 42.1 (C-5), 25.6 (C-6), 22.4 (C-17), 10.5 (C-19).

Hydrogenation of pasakbumin-A ***1***

The solution of **1** (48 mg) in MeOH (8 ml) was hydrogenated in the presence of $(Ph_3P)_3RhCl$ (43 mg) for 24 h. The reaction mixture was separated by RP-HPLC (Develosil ODS, MeOH-H_2O (3/7)], giving 13α-methyl epimer (= pasakbumin-C 4, 7 mg, 16%), 13β-methyl epimer **5** (6 mg, 13%), 3,4ξ-dihydroeurycomanone **6** (1 mg, 2%) and the starting material **1** (31 mg, 65%).

13β-Methyl isomer ***5***. Amorphous; ^{1}H-NMR δ 6.13 (br-s, H-3), 5.45 (s, H-15), 5.21 (t-like, H-7), 4.76, 4.02 (ABq, H-20), 4.43 (s, H-1), 4.29 (d, H-12), 3.64 (s, H-9), 3.55 (dq, H-13), 3.18 (br-d, H-5), 2.28 (br-d, H-6α), 2.00 (br-t, H-6β), 1.78 (s, Me-17), 1.68 (d, Me-18), 1.63 (s, Me-19).

3,4ξ-Dihydroeurycomanone ***6***. Amorphous; ^{1}H-NMR δ 6.09, 5.62 (each d, =CH2), 5.64 (s, H-15), 5.23 (t-like, H-7), 4.78 (s, H-12), 4.61 (s, H-1), 4.50, 3.99 (ABq, H-20), 3.69 (s, H-9), 1.84 (br-d, H-6α), 1.67 (s, Me-19), 0.92 (d, Me-17).

Epoxidation of ailanthone ***8***

To a solution of **8** (112 mg) in CH_2Cl_2 (10 ml) was added *m*-chloroperbenzoic acid (97 mg) and the mixture was refluxed for 44 h. Evaporation of the solvent followed by trituration with ether gave a residue, which on RP-HPLC [Develosil, ODS, MeOH-H_2O (3/7)] gave α-epoxide **9** (48 mg, 44%), β-epoxide **10** (35 mg, 32%), and the starting material **8** (11 mg, 10%).

α-Epoxide ***9***. *mp* = 298–300°C (dec); $[\alpha]_D$ −52.8°; IR (KBr) 3500, 3445, 1718, 1660, 1621 cm^{-1}; SI-MS m/z 393 (M^+ + H); ^{1}H-NMR δ 6.14 (br-s, H-3), 4.68 (t-like, H-7), 4.51 (s, H-1), 4.26, 3.96 (ABq, H-20), 3.94 (s, H-12), 3.89 (dd, H-15α), 3.64 (s, H-9), 3.15 (br-d, H-5), 3.12 (dd, H-15β), 3.07, 3.01 (ABq, H-18), 2.25 (br-d, H-6α), 2.16 (dd, H-14), 2.06 (br-t, H-6β), 1.77 (s, Me-17), 1.59 (s, Me-19); ^{13}C-NMR d 197.4 (C-2), 169.5 (C-16), 162.1 (C-4), 126.3 (C-3), 110.2 (C-11), 84.3 (C-1), 81.3 (C-12), 78.3 (C-7), 71.6 (C-20), 59.2 (C-13), 58.0 (C-18), 45.4 (C-8, C-9, C-10), 44.6 (C-14), 42.4 (C-5), 31.0 (C-15), 26.1 (C-6), 22.4 (C-17), 10.4 (C-19).

β-Epoxide ***10***. *mp* > 300°C; $[\alpha]_D$ −21.6°; IR (KBr) 3420, 1727, 1673, 1623 cm^{-1}; SI-MS m/z 393 (M^+ + H); ^{1}H-NMR δ 6.12 (br-s, H-3), 4.69 (t-like, H-7), 4.50 (s, H-1), 4.32, 4.23 (ABq, H-20), 3.82 (s, H-12), 3.71 (dd, H-15α), 3.54 (s, H-9), 3.12 (br-d, H-5), 3.01 (dd, H-15β), 2.91, 2.84 (ABq, H-18), 2.23 (br-d, H-6α), 2.06 (dd, H-14), 2.04 (br-t, H-6β), 1.76 (s, Me-17), 1.59 (s, Me-19); ^{13}C-NMR δ 197.5 (C-2), 169.5 (C-16), 162.3 (C-4), 126.2 (C-3), 110.2 (C-11), 84.3 (C-1), 81.9 (C-12), 78.5 (C-7), 71.4 (C-20), 59.8 (C-13), 46.8 (C-18), 46.7 (C-9), 46.3 (C-8), 45.5 (C-10), 44.9 (C-14), 42.5 (C-5), 31.9 (C-15), 25.9 (C-6), 22.4 (C-17), 10.4 (C-19).

X-ray results

Crystal data for **3**: $C_{20}H_{24}O_{10}$, orthorhombic, space group $P2_12_12_1$, a = 12.089(1), b = 19.371(1), c = 7.916(1) Å, Z = 4. **4**: $C_{20}H_{26}O_9 \cdot 2H_2O \cdot CH_3OH$, orthorhombic, space group $P2_12_12_1$, a = 10.739(1), b = 21.809(1), c = 9.403(1) Å, Z = 4. **9**: $C_{20}H_{24}O_8$, orthorhombic, space group $P2_12_12_1$, a = 12.217(1), b = 18.338(1), c = 7.895(1), Å, Z = 4.

The structures were solved by direct methods and refined by a block-diagonal least-squares technique to R = 0.030 (for 1615 reflections), 0.045 (1715) and 0.029 (1542), respectively.

The molecular structures are shown in figures 1, 2 and 3 respectively. Hydrogen atoms of methyl and methylene groups were omitted for clarity, and no absolute configuration is implied.

Pharmacology

Antiulcer activity

Male SD rats (220–260 g) were used. Animals were deprived of food for 24 h but allowed free access to water.

Indomethacin ulcer. Indomethacin (Sigma) suspended in 5% acacia was given subcutaneously (sc) at a dose of 30 mg/kg. Animals were sacrificed 7 h later, and the stomach was removed and inflated with 6 ml of 1% formalin. The stomach was incised along the greater curvature and examined for mucosal lesions. The test compound was given intra-peritoneally 30 min before administration of indomethacin.

Water-immersion stress ulcer. Rats were placed in a restraint cage and immersed vertically to the level of the xiphoid process in a water bath (23°C). Animals were sacrificed 7 h later and the stomachs were examined for mucosal lesions. The test compound was given intraperitoneally (ip) or orally (*po*) 30 min before restraint.

Gastric secretion. Under ether anesthesia, the abdomen was incised and the pylorus was ligated. Four h after the ligation, the animals were sacrificed and the gastric content was collected, then analysed for volume and acidity. Acidity was determined by titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Autoburette, Radiometer). The test compound was given into the duodenum immediately after the pylorus ligation.

Acute toxicity

Five male mice (Slc ddY) were used for each dose. The test compound was administrated intra-peritoneally, and after 72 h the number of deaths were examined. Values of LD_{50} were calculated by the probit method [7].

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