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Brownins A and B: Novel Rearranged Limonoids from *Harrisonia brownii*

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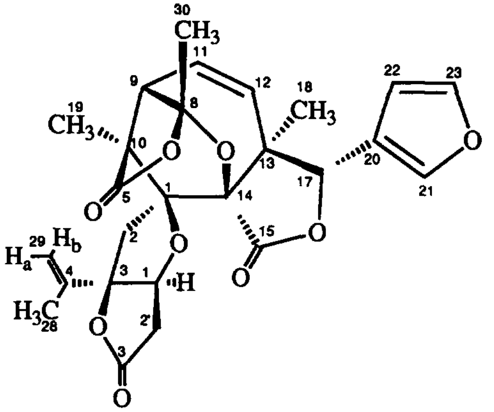
Abstract: Two highly rearranged limonoids of a novel skeletal type, brownins A (**1**) and B (**2**), have been isolated from the bark of *Harrisonia brownii* (Simaroubaceae) Their structures were determined by spectral data and X-ray diffraction analysis

Harrisonia brownii Juss is widely distributed in southeast Asia and its root is used in the treatment of dysentery and cholera.[1,2] No chemical studies have been reported on *H brownii*, but *H abysinica* Oliv. and *H perforata* Merr endemic species widely used in African and southeast Asian tropical medicine, have been reported to contain seven limonoids[3-8] and seven chromans [4,9-13] In this paper, we reported the isolation and structural elucidation of the highly rearranged limonoids of a novel skeletal type, brownin A (**1**) and brwonin B (**2**), from *H brownii* Their structures were elucidated on the basis of spectral data and single crystal X-ray diffraction analysis

RESULTS AND DISCUSSION

Both compounds were obtained from the chloroform extract of the bark and were purified by silica gel chromatography and recrystallization

The first compound, brownin A (**1**), was obtained as colorless prisms, $[\alpha]_D$ -75 0° (pyridine) The molecular formula, $C_{26}H_{26}O_9$ was determined by high-resolution mass measurement (HRMS) of its molecular ion at *m/z* 482 1556 (Δ -1 3 mmu of calcd) The IR spectrum of **1** indicated bands at 1785 (br) (lactone) and 1650 cm^{-1} (olefine) and the UV absorptions at 250 and 255 nm were typical of a βsubstituted furan ring The ^{1}H NMR spectrum (Table 1) showed resonances for four tertiary methyl groups (δ 0 97, 1 23, 1 65 and 1 86), a disubstituted double bond (δ 5 50 and 5 68) and a β-substituted furan (δ 6 56, 7 72 and 7 78) The molecular formula required 14 degrees of unsaturation DEPT spectrum confirmed the presence of three lactone carbons and eight sp^2 carbons (two singlets, five doublets and one triplet) It is thus evident that **1** possesses a heptacyclic skeleton containing a vinylmethylene and a furan ring The ^{1}H and ^{13}C NMR chemical shift assignments and cross-correlation observed in the 2D NMR experiments (HMQC and HMBC) are summarized in Table 1



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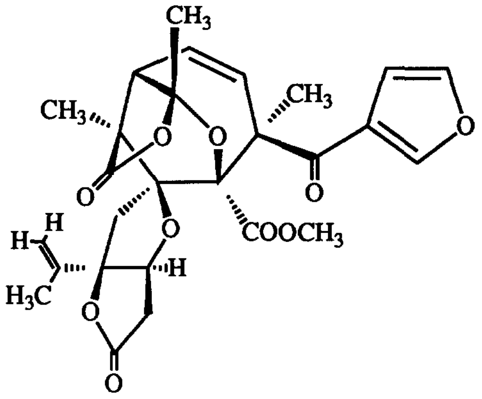

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A convenient starting point for the spectral analysis was the methine proton (H-17, δ 5 64) This showed long-range correlation (^{1}H-^{1}H COSY) with H-21 (δ 7 78) and H-22 (δ 6 56) Long-range ^{1}H-^{13}C correlation (HMBC) was observed between H-17 and the quaternary carbon (C-13, δ 55.84) and the lactone carbonyl carbon (C-15, δ 166 56) The ring juncture methyl signal at H-18 (δ 0.97) showed ^3J cross-peaks with the two oxygenated carbons at C-14 (δ 84 94) and C-17 (δ 77 76) and the olefinic carbon at C-12 (δ 133 33) The singlet methyl signal (H-30, δ 1 65) showed a ^2J cross-peak with the ketalic carbon at C-8 (δ 110 22) On the other hand, the ring juncture methyl signal at H-19 (δ 1 23) showed ^3J cross-peaks with the γ-lactone carbonyl carbon at C-5 (δ 173 20) and the spyro carbon bearing an oxygen atom at C-1 (δ 86 07) The bridgehead methine proton at H-1' showed ^2J and ^3J cross-peaks with C-1 as well as with the γ-lactone carbonyl carbon at C-3' (δ 173 54) and the quaternary oxygenated carbon at C-3 (δ 96 77), indicating that the γ-lactone carbonyl was adjacent to the carbon at C-2' (δ 36 01) and that there was ether linkage between C-1 and C-1' The methyl protons at H-28 (δ 1 86) of the isopropenyl group showed a ^3J cross-peak at C-3, demonstrating that the isopropenyl group and C-3 were connected by the bond between the carbons at δ 96 77 and 140 70 (C-4) One of the nonequivalent methylene protons at H-2β (δ 2 49) showed long-range correlations with C-1 and the quaternary carbon at

C-14 (δ 84 94) On the other hand, long-range correlations of the other nonequivalent methylene proton at H-2α (δ 2.94) were observed with C-1 and C-3 These observations indicated that the spyro carbon at the C-1 of furanofuranone moiety connected with C-10 and C-14 Finally, the remaining ether linkage must be from C-8 to C-14 These data clearly demonstrated that the novel limonoid brownin A was compound **1** The relative configuration of **1** was determined by NOE studies (Figure 1) In order to confirm unambiguously the structure and to establish the relative stereochemisty of all nine chiral C atoms, a single crystal X-ray diffraction analysis on **1** was performed. A computer-generated perspective drawing of the final X-ray model of brownin A (**1**) is given in Figure 2 or its antipode

Table 1 ^{13}C and ^{1}H Chemical Shift Assignments and Long-range Correlation Responses of Brownin A (**1**) in Dimethylsulfoxide-d_6 at 350K

Assignment	δ_C	DEPT	δ_H	mult	(*J*, Hz)	Long-range connectivity in HMBC
1	86 07	C				H-2α,β, H-19, H-1'
2	41 32	CH_2	2 96 α	d	(15 4)	H-1'
			2 49 β	d	(15 4)	
3	96 77	C				H-2β, H-28, H-1', H-2'β
4	140 70	C				H-2α, H-28, H-1'
5	173 20	C				H-19
8	110 22	C				H-9, H-11, H-30
9	50 09	CH	3 02	d	(9 2)	H-19, H-30
10	58 45	C				H-2β, H-9, H-19
11	120 81	CH	5 68	dd	(10 6, 9 2)	H-9, H-12
12	133 33	CH	5 50	d	(10 6)	H-11, H-18
13	55 84	C				H-9, H-11, H-17
14	84 94	C				H-2β, H-18
15	166 56	C				H-17
17	77 76	CH	5 64	s		H-18, H-21, H-22
18	21 59	CH_3	0 97	s		H-12, H-17
19	16 94	CH_3	1 23	s		H-9
20	118 57	C				H-17, H-21, H-22, H-23
21	141 57	CH	7 78	qui	(0 7)	H-17, H-22, H-23
22	109 54	CH	6 56	dd	(1 8, 0 7)	H-17, H-21, H-23
23	144 03	CH	7 72	t	(1 8)	H-21
28	19 15	CH_3	1 86	s		H-29
29	114 24	CH_2	5 14 a	brd	(1 8)	
			5 23 b	s		
30	21 35	CH_3	1 65	s		
1'	78 07	CH	4 76	d	(5 9)	H-2'
2'	36 01	CH_2	2 93 α	dd	(18 7, 5 9)	H-1'
			2 58 β	d	(18 7)	
3'	173 54	C				H-1', H-2'

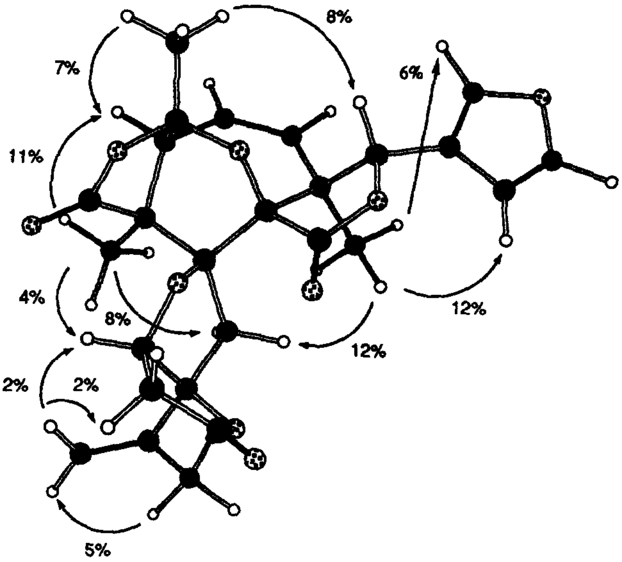

Figure 1 The conformation of brownin A (1) was calculated by molecular mechanics (MM2)[14] and the arrows illustrate the most relevant experimental NOE effects. Enhancements (%) are obtained for those nuclei at the arrow heads on irradiating those at the arrow tails

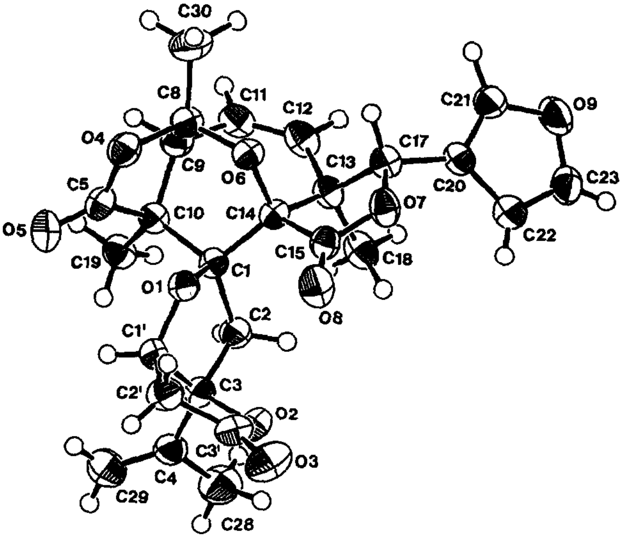

Figure 2 The ORTEP drawing of the molecular structure of brownin A (1)

The second compound, brownin B (**2**), was obtained as colorless prisms, $[\alpha]_D$ -145 2° (pyridine) Its molecular formula $C_{27}H_{28}O_{10}$ was determined by positive FAB mass spectrum (*m/z* 551 $[M+K]^+$ and *m/z* 513 $[M+H]^+$) and HRMS of its pseudo molecular ion at *m/z* 513 1768 ($[M+H]^+$, Δ +1.6 mmu of calcd) The IR spectrum showed the presence of ketone, lactone and olefinic functions. The ^1H and ^{13}C NMR spectra of **2** were similar to those of brownin A (**1**), except that the singlet signal at δ 5.64 due to the methine proton at H-17 was absent The presence of a new methoxycarbonyl group (δ_H 3 58 (3H, s), δ_C 51 45 and 168 48) and ketone (δ_C 194.85) due to cleavage of γ-lactone with loss of H-17 was observed The remaining NMR assignments for **2**, shown in Table 2, were based on ^1H-^1H COSY, HMQC, HMBC and phase-sensitive NOESY spectra The stereochemistry of **2** was deduced from NOESY results to be the same that of brownin A (**1**) Thus, the structure of brownin B is **2** or its antipode

To our knowledge this is the first report on the isolation of a new class of highly rearranged limonoids

Table 2 ^{13}C and ^1H Chemical Shift Assignments and ^1H-NOEs of Brownin B (**2**) in Dimethylsulfoxide-d_6 at 350 K

Assignment	δ_C	δ_H	mult	(*J*, Hz)	NOEs in NOESY
1	88 13				
2	39 82	3 34 α	d	(15 0)	
		2 67 β	d	(15 0)	H-18
3	96 61				
4	140 49				
5	173 68				
8	108 37				
9	48 97	2 95	d	(9 0)	H-19, H-30
10	56 85				
11	121 50	5 90	dd	(11 4, 9 0)	
12	136 49	6 15	d	(11 4)	H-18, H-21
13	63 80				
14	87 94				
15	168 48				
17	194 85				
18	26 00	1 66	s		H-2β, H-12, H-19
19	18 30	1 19	s		H-2α, H-9, H-18, H-29, H-1'
20	124 82				
21	147 57	8.46	brs		H-12
22	110 59	6 75	d	(1 4)	
23	143 53	7 72	t	(1 4)	
28	19 32	1 87	s		H-29a
29	114 73	5 13 a	brd	(1 1)	H-28
		5 18 b	s		H-19
30	22 08	1 27	s		H-9
1'	78 77	4 76	d	(5 3)	H-19, H-29b
2'	36 09	2 97 α	dd	(18 7, 5 3)	H-19
		2 32 β	d	(18 7)	
3'	173 11				
OMe	51 45	3 58	s		

EXPERIMENTAL SECTION

General procedures Melting points were determined on a Yanagimoto micromelting point hot-stage type apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a JASCO 7300 FTIR spectrophotometer UV spectra were recorded on a Hitachi 340 spectrometer in MeOH Low-resolution EIMS were measured on a JEOL D-300 mass spectrometer. HRMS and FABMS were measured on a JEOL D-JEOL DX-303 mass spectrometer Optical rotations were determined on a JASCO DIP-4 digital polarimeter ^{1}H, ^{13}C and two dimensional NMR spectra were recorded on a JEOL EX-400 spectrometer, using tetramethylsilane as an internal standard.

Isolation of Brownins A (1) and B (2) Dried aerial bark (3 0 kg) of *Harrisonia brownii* collected in Indonesia, May, 1990, was extracted with $CHCl_3$ (45 L) and MeOH (45 L) The $CHCl_3$ extract was concentrated under reduced pressure to give a residue (165 g) which was chromatographed on silica gel (2 kg) with $CHCl_3$ as eluent containing increasing amounts of MeOH (1, 5, 10, 20, 50 and 100%) Fractions (1-107) (500 ml each) were collected and the chromatographic separation was monitored by TLC (Merck silica gel 60 F_{256}) The fractions (28-54) obtained by elution with $CHCl_3$ MeOH (19 1 and 9 1) afforded a crude extract containing a mixture of limonoids (58 g), which was repeatedly chromatographed on silica gel (BW-820 MH, Fuji Davison) and then recrystallized from MeOH to gave brownin A (**1**, 260 mg) and brownin B (**2**, 76 mg)

Brownin A (1) Colorless prisms (MeOH), mp >300°, $[\alpha]^{26}_{D}$ -75 0° (*c*, 1 0, pyridine) UV λmax nm (log ε) 242 (sh, 2.84), 250 (2 96), 255 (2 99) 260 (sh, 2 81) IR νmax cm^{-1} 1785 (br), 1650, 1505, 1390, 1200, 1150, 1060, 1020, 900 EIMS *m/z* (relative intensity) 482 (M^{+}, 1), 386 (9), 358 (27), 330 (49), 316 (12), 287 (85), 265 (35), 247 (15), 221 (31), 201 (26), 43 (100) HRMS, *m/z* 482 1556 [M]$^{+}$ ($C_{26}H_{26}O_9$, Δ -1 3 mmu of calcd)

Brownin B (2) Colorless prisms (MeOH), mp 287-288°, $[\alpha]^{26}_{D}$ -145 2° (*c*, 1 0, pyridine) UV λmax nm (log ε) 256 (3 69) IR νmax cm^{-1} 1790 (br), 1760, 1730, 1660, 1560, 1510, 1270, 1215, 1180, 1160, 1060, 1040, 920 Positive FABMS *m/z* 551 [M+K]$^{+}$, 513 [M+H]$^{+}$ EIMS *m/z* (relative intensity) 513 ([M+1]$^{+}$, 2), 481 (8), 453 (15), 358 (17), 326 (89), 315 (51),283 (44), 253 (10), 221 (25), 95 (100), 43 (75) HRMS, m/z 513 1768 [M+H]$^{+}$ ($C_{27}H_{29}O_{10}$, Δ +1 6 mmu of calcd)

Crystal data for 1. (Crystallized from methanol/water) $C_{26}H_{26}O_9$, M=482 49, colorless prisms, orthorhombic (no 19), a=10 404(2) Å, b=24 480(1) Å, c=8 8470(9) Å, space group $P2_1P2_1P2_1$, Z=4, D_c=1 422 g/cm^3, Cu radiation, λ= 1 54178 Å, μ(Cu-Kα)=8 60 cm^{-1}, F(000)=1016 00 Data were measured on a Rigaku AFC7R diffractometer with Cu-Kα radiation (graphite monochrometer) using ω-2θ scans A total of 1962 independent reflections (2θ<120 1°) were measured, of which 1810 had (I>3 00σ(I)), and were considered to be observed All calculations were performed using the TEXSAN system [15] The structure was solved by direct method with SHELXS-86[16] and refined by using full-matrix least-squeares technique to R=0 027 (Rw=0 035) The maximum and minimum residual electron densities in peaks on the final difference Fourier map corresponded to 0 16 and -0 15 $e^-/Å^3$, respectively Atomic coordinates, anisotropic and isotropic thermal parameters, bond angles, bond lengths and torsion angles have been deposited at the Cambridge Crystallographic Data Centre [17]

Final Positional Parameters ($\times 10^4$) and Equivalent Isotropic Thermal Parameters with Estimated Standard Deviations in Parentheses for Brownin A (**1**)

Atom	x	y	z	B_{eq}(Å^2)
C(1)	4366 (2)	8743 (9)	1252 (3)	2 51 (5)
C(2)	5840 (2)	8809 (1)	1121 (3)	3 04 (5)
C(3)	6075 (2)	9174 (10)	-253 (3)	2 91 (5)
C(4)	7110 (3)	9597 (1)	-61 (3)	3 43 (6)
C(5)	2566 (2)	9370 (10)	1690 (3)	3 02 (5)
C(8)	1890 (2)	8523 (9)	2395 (3)	3 08 (5)
C(9)	2813 (2)	8735 (10)	3585 (3)	3 00 (5)
C(10)	3671 (2)	9092 (9)	2535 (3)	2 72 (5)
C(11)	3333 (3)	8297 (1)	4611 (3)	3 44 (6)
C(12)	3961 (3)	7862 (1)	4176 (3)	3 50 (6)
C(13)	4454 (3)	7757 (10)	2602 (3)	2 94 (5)
C(14)	3899 (2)	8145 (10)	1365 (3)	2 61 (5)
C(15)	4116 (2)	7792 (1)	-68 (3)	2 94 (6)
C(17)	3932 (3)	7203 (10)	1973 (3)	3 03 (5)
C(18)	5919 (3)	7729 (1)	2729 (3)	3 63 (6)
C(19)	4501 (3)	9502 (10)	3369 (3)	3 35 (6)
C(20)	4624 (2)	6693 (10)	2428 (3)	3 01 (5)
C(21)	4219 (3)	6340 (1)	3470 (3)	3 68 (6)
C(22)	5799 (3)	6476 (1)	1869 (3)	3 77 (6)
C(23)	6016 (3)	6013 (1)	2600 (4)	4 03 (6)
C(28)	8392 (3)	9386 (1)	382 (4)	5 13 (8)
C(29)	6901 (3)	10118 (1)	-301 (5)	5 59 (8)
C(30)	635 (3)	8271 (1)	2903 (4)	4 54 (7)
C(1')	4738 (2)	9348 (10)	-770 (3)	2 90 (5)
C(2')	4765 (3)	9296 (1)	-2469 (3)	3 80 (6)
C(3')	5842 (3)	8906 (1)	-2766 (3)	3 47 (6)
O(1)	3890 (2)	8941 (6)	-177 (2)	2 70 (3)
O(2)	6519 (2)	8815 (7)	-1486 (2)	3 17 (4)
O(3)	6137 (2)	8694 (9)	-3939 (2)	4 81 (5)
O(4)	1582 (2)	9014 (7)	1542 (2)	3 38 (4)
O(5)	2488 (2)	9835 (7)	1258 (2)	3 98 (4)
O(6)	2504 (1)	8137 (6)	1438 (2)	2 83 (3)
O(7)	4050 (2)	7261 (7)	335 (2)	3 52 (4)
O(8)	4280 (2)	7922 (7)	-1350 (2)	4 32 (5)
O(9)	5056 (2)	5917 (7)	3627 (3)	4 58 (5)

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REFERENCES AND NOTES

1. White, T C *Tree Flora of Malaya*; Longman, **1973**, Vol II, pp 349-350

2 Ridley, N H. *The Flora of the Malay Peninsula*, A Sher, Amsterdam, **1976**, Vol. I, pp360-361.

3 Kubo, I , Tanis, S P , Lee, Y -W , Miura, I.; Nakanishi, K *Heterocycles* , **1976**, 5, 485

4 Liu, H -W , Kubo, I , Nakanishi, K *Heterocycles* , **1982**, 17, 67

5 Okone, D A *Phytochemistry* , **1982**, 21, 2424

6 Chapya, A W *Phytochemistry* , **1987**, 26, 573

7 Balde, A M , Vanhaelen, M , Daloze, D *Phytochemistry* , **1988**, 27, 942

8 Byme, L T , Tri, M , Phuong, N M , Sargent, M V , Skelton, B. W , White, A H *Aust J Chem* , **1991**, 44, 165

9 Liu, H -W , Kusumi, T , Nakanishi, K *J C S Chem Comm* , **1981**, 24, 1271

10 Meixin, W , Minsheng, Z , Yuanlong, Z *Yaoxue Xuebao* , **1983**, 18, 113

11 Meixin, W , Minsheng, Z , Wenzi, L , Yuanlong Z *Yaoxue Xuebao* , **1984**, 19, 760

12 Xincheng, W , Zuohua, P , Xiaocheng, G., Meixin, W , Yuanlong, Z *Jiegou Huaxae*, **1985**, 4, 281

13 Balde, A M , Vanhaelen, M , Ottinger, R *Phytochemistry* , **1987**, 26, 2415

14 Allinger, N L *J Am Chem Soc* , **1977**, 99, 8127

15 TEXSAN Crystal structure analysis package, Molecular Structure Corporation **(1985 &1992)**

16 SHELXS86· Sheldrick, G M , *Crystallographic Computing 3* (Eds M Sheldrick, C Kruger and R Goddard) Oxford University Press, **1985**, pp 175-189

17 Archival X-ray crystallographic data have been deposited and can be ordered from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U K (please provide complete reference when ordering)