Helisterculins A and B, Two New (7.5′,8.2′)-Neolignans, and Helisorin, the First (6.4′,7.5′,8.2′)-Neolignan, from the Indonesian Medicinal Plant *Helicteres isora*

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During a chemical study of Indonesian medicinal plants, we examined the constituents of fruits of *Helicteres isora* L. (Sterculiaceae), one of the famous Jamu medicines. From a water extract of the fruits, we isolated three new neolignans, helisterculins A (**1**) and B (**2**) and helisorin (**3**), and elucidated their structures by spectral analyses. Helisterculins A (**1**) and B (**2**) are (7.5′,8.2′)-neolignans with a bicyclo[2.2.2]octene C-framework, while helisorin (**3**) is a (6.4′,7.5′,8.2′)-neolignan with a very rare 4,4a,9,9a-tetrahydro-3,9-methano-3*H*-fluorene C-framework. The natural product with the latter C-framework has no literature precedent. The neolignans **1**–**3** showed weak inhibitory activity against reverse transcriptase from avian myeloblastosis virus.

1. Introduction. – *Helicteres isora* L. is a large arborescent shrub of the family Sterculiaceae, which grows in central and western India, southeast Asia, and the southern part of China. It is one of the best known articles of the Hindu Materia Medica and used as one of Jamu medicines [1]. Timbers of this plant are used as anthelmintic, colic, and aphtha, while fruits are used as colic, anticonvulsant, and abdominalgia [2]. In addition, *Hattori* and co-workers reported an inhibitory activity of a water extract of fruits of *H. isola* against reverse transcriptase from avian myeloblastosis virus (AMV-RT) [3] and anti-human immunodeficiency virus-type-1 (anti-HIV-1) activity [4]. Thus we examined constituents of the water extract of fruits of *H. isora* and isolated three new neolignans[1]), named helisterculins A (**1**) and B (**2**) and helisorin (**3**). This paper reports their structural elucidation by spectroscopic means.

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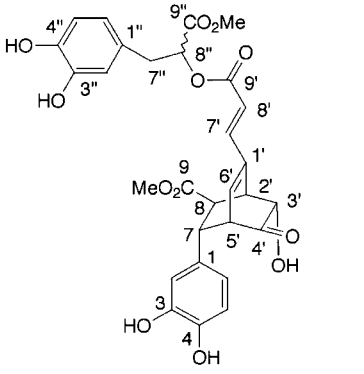
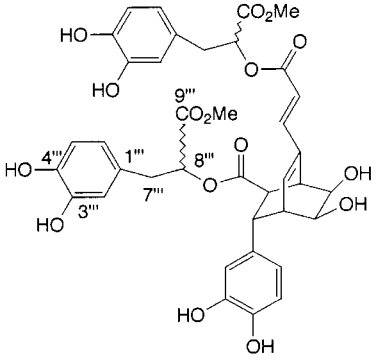
[1]) There are two definitions for 'lignan' and 'neolignan'. Based on structure, 'lignan' is defined as the compound composed of two C_6C_3 fragments linked $\beta-\beta'$ (8–8′) [5] and 'neolignan' as the compound linked otherwise than $\beta-\beta'$ [6]. Based on biosynthetic origin, on the other hand, 'neolignan' is defined as the product of oxidative coupling of allyl- or propenylphenols and 'lignan' as the coupling product of cinnamyl alcohols, *etc.* [7]. In this paper, the former definition is used because of its clarity.

2. Structures of New Neolignans. – *Isolation.* Powdered dried fruits of *H. isora* were extracted with hot water, and the extract was separated into AcOEt-soluble, BuOH-soluble, and H_2O-soluble fractions. Among them, the last one showed an inhibitory activity against AMV-RT, and thus this was subjected to further separation. A preliminary examination suggested that the H_2O-soluble fraction was a complex mixture of very polar acid salts. The fraction was thus treated with an ion-exchange resin *Amberlite IR-120B* (H^+ form) [8], and then a H_2O eluate from the resin was divided into MeOH-soluble and MeOH-insoluble portions. Among these two portions, the former showed stronger AMV-RT inhibition and thus was subjected to further separation by a combination of *Diaion HP-20P*, *Sephadex LH-20*, and *MCI*-gel *CHP-20P* CC, prep. HPLC, and prep. TLC procedures to give helisterculins A (**1**) and B (**2**) and helisorin (**3**), along with two flavonoid glucuronides, 3′,5,7,8-tetrahydroxy-4′-methoxyflavone 8-*O*-*β*-D-glucopyranosiduronic acid methyl ester (**4**) and 4′,5,7,8-tetrahydroxyflavone 8-*O*-*β*-D-glucopyranosiduronic acid methyl ester (**5**). These flavonoid glucuronides[2]) were new compounds, although their corresponding acids had been reported previously as constituents of leaves of *Melva sylvestris* L. (Malvaceae) [9].



1 arbitrary numbering [1])



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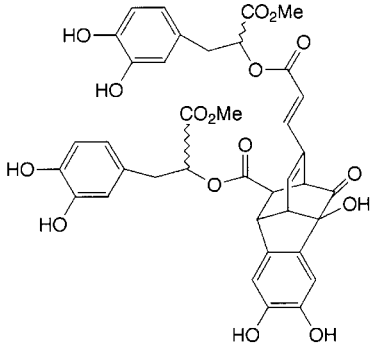

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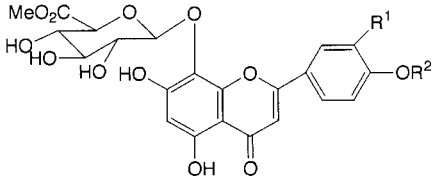

4 R^1 = OH, R^2 = Me

5 $R^1 = R^2$ = H

[2]) The methyl ester might be produced from the corresponding acid during the isolation.

Helisterculin A (**1**). The molecular formula of the yellowish amorphous solid **1** was determined as $C_{29}H_{28}O_{12}$ by high-resolution FAB-MS. The UV and IR spectra showed absorptions of phenyl (276 nm; 1628, 1523 cm^{-1}) and OH groups (3413 cm^{-1}) and of an ester carbonyl group (1724 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table 1*) revealed signals due to two sets of 1,3,4-trisubstituted benzene rings, two (a *trans*-disubstituted and a trisubstituted) olefins, four methine, a methylene, and two MeO groups, and an alcohol and four phenol OH protons. Moreover, its ^{13}C-NMR spectrum (*Table 2*) indicated the presence of a ketone and three ester carbonyl groups. Analyses of these signals by the COSY and HETCOR experiments led to the partial structures depicted in *Fig. 1*.

These partial structures were connected, based on the long-range correlations observed in the HMBC spectrum (*Table 2*), and established the gross structure of helisterculin A, except for the configuration, corresponding to **1**[2]).

Table 1. *^{1}H-NMR Spectral Data for Neolignans* **1**–**3** *in (D_6)Acetone.* Chemical shifts δ in ppm rel. to $SiMe_4$; coupling constants J in Hz. Arbitrary numbering[1]).

	1	**2**	**3**
H–C(2)	6.81 (*d*, *J* = 2)	6.82 (*d*, *J* = 2)	6.740 (*s*)
H–C(5)	6.71 (*d*, *J* = 8)	6.79 (*d*, *J* = 8)	6.78 (*s*)
H–C(6)	6.66 (*dd*, *J* = 8, 2)	6.57 (*dd*, *J* = 8, 2)	
H–C(7)	3.47 (*dd*, *J* = 7.5, 2.5)	*ca.* 2.95[a])	3.54 (*d*[b]), *J* = 5)
H–C(8)	3.56 (*dd*, *J* = 7.5, 2)	*ca.* 2.97[a])	3.02 (*dd*, *J* = 3.5, 1)
MeO–C(9)	3.59 (*s*)		
H–C(2′)	3.82 (*dt*, *J* = 3, 2)	3.62 (br *s*)	3.97 (*dd*, *J* = 3.5, 1.5)
H–C(3′)	3.78 (br *d*, *J* = 3)	4.20 (*dd*, *J* = 7.5, 3)	
H–C(4′)		4.00 (br *d*, *J* = 7.5)	
H–C(5′)	3.18 (*dd*, *J* = 6.5, 2.5)	*ca.* 2.96[a])	3.62 (*dd*, *J* = 6.5, 5)
H–C(6′)	6.83 (*dd*, *J* = 6.5, 2)	*ca.* 6.78[a])	6.70 (*dd*, *J* = 6.5, 2)
H–C(7′)	7.34 (*d*, *J* = 15.5)	7.34 (*d*, *J* = 15.5)	7.24 (*d*, *J* = 15.5)
H–C(8′)	6.12 (*d*, *J* = 15.5)	6.05 (*d*, *J* = 15.5)	6.03 (*d*, *J* = 15.5)
H–C(2″)	6.78 (*d*, *J* = 2)	6.79 (*d*, *J* = 2)	6.727 (*d*, *J* = 2)
H–C(5″)	6.74 (*d*, *J* = 8)	6.73 (*d*, *J* = 8)	6.68 (*d*, *J* = 8)
H–C(6″)	6.61 (*dd*, *J* = 8, 2)	6.60 (*dd*, *J* = 8, 2)	6.55 (*dd*, *J* = 8, 2)
2 H–C(7″)	2.99 (*dd*, *J* = 14.5, 8.5)	2.99 (*dd*, *J* = 14.5, 8.5)	2.98 (*dd*, *J* = 14, 7.5)
	3.08 (*dd*, *J* = 14.5, 4.5)	3.05 (*dd*, *J* = 14.5, 5)	3.04 (*dd*, *J* = 14, 5)
H–C(8″)	5.18 (*dd*, *J* = 8.5, 4.5)	5.16 (*dd*, *J* = 8.5, 5)	5.15 (*dd*, *J* = 7.5, 5)
MeO–C(9″)	3.69 (*s*)	3.65 (*s*)	3.66 (*s*)
H–C(2‴)		6.70 (*d*, *J* = 2)	6.735 (*d*, *J* = 2)
H–C(5‴)		6.69 (*d*, *J* = 8)	6.730 (*d*, *J* = 8)
H–C(6‴)		6.43 (*dd*, *J* = 8, 2)	6.56 (*dd*, *J* = 8, 2)
2 H–C(7‴)		2.84 (*dd*, *J* = 14, 8.5)	2.82 (*dd*, *J* = 14, 9.5)
		2.94 (*dd*, *J* = 14, 4.5)	2.98 (*dd*, *J* = 14, 4)
H–C(8‴)		5.00 (*dd*, *J* = 8.5, 4.5)	4.96 (*dd*, *J* = 9.5, 4)
MeO–C(9‴)		3.56 (*s*)	3.58 (*s*)
OH–Alkyl	5.29 (OH–C(3′))	4.0–4.3 (br., 2 H)	5.07 (br. *s*, OH–C(4′))
OH–Aryl	7.75 (4 H)	7.8–8.1 (br., 6 H)	7.61 (2 H), 7.68 (1 H)
			7.81 (1 H), 7.88 (2 H)

[a]) Chemical shifts were obtained from the COSY experiment. [b]) Slightly broadened by a small, non-resolved coupling with H–C(8).

Table 2. *^{13}C-NMR Spectral Data for Neolignans* **1**–**3** *in (D_6)Acetone.* δ in ppm relative to $SiMe_4$. Arbitrary numbering[1]).

	1		**2**		**3**	
	δ	HMBC[a])	δ	HMBC[a])	δ	HMBC[a])
C(1)	134.1	H–C(5), H–C(7), H–C(8), H–C(5′)	133.4	H–C(8), H–C(5′)	137.4	H–C(2), H–C(5), H–C(7), H–C(8)
C(2)	116.7	H–C(6), H–C(7)	116.2		111.3	
C(3)	146.1[b])	H–C(5)	146.3	H–C(5)	147.2	H–C(2), H–C(5)
C(4)	145.3	H–C(2), H–C(6)	145.0	H–C(2), H–C(6)	145.8	H–C(2), H–C(5)
C(5)	116.3		116.6		111.0	
C(6)	121.1	H–C(2), H–C(7)	120.1		134.6	H–C(2), H–C(5), H–C(7), H–C(5′)
C(7)	49.6	H–C(2), H–C(2′), H–C(6), H–C(8)	44.9	H–C(2), H–C(6)	43.7	H–C(2), H–C(5′)
C(8)	43.5	H–C(3′), H–C(7)	44.8		52.8	H–C(7), H–C(5′)
C(9)	175.1	H–C(7), H–C(8), MeO–C(9)	174.0	H–C(7), H–C(8), H–C(8‴)	170.5	H–C(7), H–C(8), H–C(8‴)
*Me*O–C(9)	52.8					
C(1′)	141.5	H–C(8), H–C(2′), H–C(3′), H–C(5′), H–C(7′), H–C(8′)	139.0	H–C(8), H–C(5′), H–C(7′), H–C(8′)	137.9	H–C(8), H–C(2′), H–C(7′), H–C(8′)
C(2′)	44.2	H–C(8), H–C(6′), H–C(7′)	42.5	H–C(6′), H–C(7′)	52.8	H–C(7), H–C(8), H–C(6′)
C(3′)	70.2	H–C(8), H–C(2′), H–C(5′)	70.4		204.6	H–C(8), H–C(2′), H–C(5′)
C(4′)	207.3	H–C(7), H–C(2′), H–C(5′), H–C(6′)	66.2		82.0	H–C(5), H–C(7), H–C(5′)
C(5′)	57.5	H–C(7), H–C(6′)	47.2		57.4	H–C(7), H–C(8), H–C(6′)
C(6′)	138.1	H–C(7), H–C(2′), H–C(5′), H–C(7′)	142.1	H–C(7′)	140.6	H–C(2′), H–C(5′), H–C(7′)
C(7′)	143.5	H–C(6′), H–C(8′)	146.1		142.8	H–C(2′)
C(8′)	118.4	H–C(7′)	116.9		118.2	H–C(7′)
C(9′)	166.9	H–C(7′), H–C(8′), H–C(8″)	167.5	H–C(7′), H–C(8′), H–C(8″)	166.7	H–C(7′), H–C(8′), H–C(8″)
C(1″)	129.0	H–C(5″), H–C(7″), H–C(8″)	128.9	H–C(5″), H–C(8″)	128.6	H–C(5″), H–C(7″), H–C(8″)
C(2″)	117.7	H–C(6″), 2H–C(7″)	117.5	2H–C(7″)	117.3	H–C(6″), H–C(7″)
C(3″)	146.2[b])	H–C(5″)	146.2	H–C(5″)	145.8	H–C(2″), H–C(5″)
C(4″)	145.3	H–C(2″), H–C(6″)	145.3	H–C(2″), H–C(6″)	145.0	H–C(2″), H–C(5″), H–C(6″)
C(5″)	116.4		116.4		116.2	
C(6″)	121.9	H–C(2″), 2H–C(7″)	121.9	2H–C(7″)	121.8	H–C(2″), 2H–C(7″)
C(7″)	37.9	H–C(2″), H–C(6″), H–C(8″)	37.9	H–C(2″), H–C(6″)	37.7	H–C(2″), H–C(6″), H–C(8″)
C(8″)	74.5	2H–C(7″)	74.4		74.3	2H–C(7″)
C(9″)	171.0	2H–C(7″), H–C(8″), MeO–C(9″)	171.2	2H–C(7″), H–C(8″), MeO–C(9″)	170.8	2H–C(7″), H–C(8″), MeO–C(9″)
*Me*O–C(9″)	52.8		52.8		52.7	
C(1‴)			128.7	H–C(5‴), H–C(8‴)	128.6	H–C(5‴), 2H–C(7‴), H–C(8‴)
C(2‴)			117.5	2H–C(7‴)	117.3	H–C(6‴), 2H–C(7‴)
C(3‴)			146.0	H–C(5‴)	145.9	H–C(2‴), H–C(5‴)
C(4‴)			145.2	H–C(2‴), H–C(6‴)	145.0	H–C(2‴), H–C(5‴), H–C(6‴)
C(5‴)			116.5		116.3	
C(6‴)			122.0	2H–C(7‴)	121.8	H–C(2‴), H–C(5‴), 2H–C(7‴)
C(7‴)			37.7	H–C(2‴), H–C(6‴)	37.6	H–C(2‴), H–C(6‴), H–C(8‴)
C(8‴)			74.7		75.0	2H–C(7‴)
C(9‴)			170.8	2H–C(7‴), H–C(8‴), MeO–C(9‴)	170.2	2H–C(7‴), H–C(8‴), MeO–C(9‴)
*Me*O–C(9‴)			52.6		52.7	

[a]) Long-range correlated protons in the HMBC spectra. [b]) Assignments interchangeable.

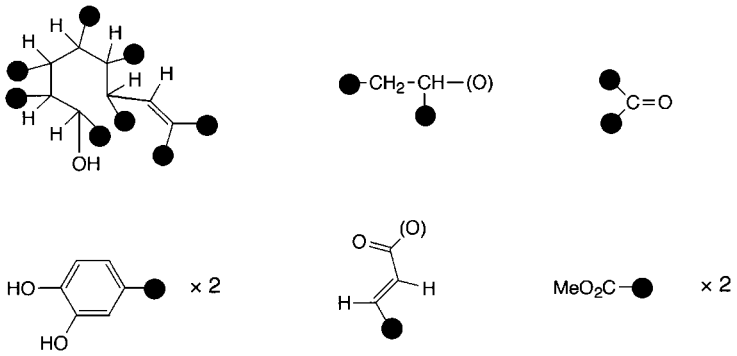

Fig. 1. *Partial structures for helisterculin A* (**1**)

Two 3,4-dihydroxyphenyl moieties (C(1) to C(6), C(1″) to C(6″)) were each connected to a benzylic C-atom (C(7) and C(7″), resp.), as shown by the long-range correlations between the benzylic proton(s) or C-atom and the corresponding C_2 or H_2, respectively (H–C(7)/C(2), C(6); 2 H–C(7″)/C(2″), C(6″); C(7)/H–C(2), H–C(6); C(7″)/H–C(2″), H–C(6″)) (arbitrary numbering, see *Formula* **1**). Likewise, the structures of the ester moieties were deduced from the three-bond correlations of the ester-carbonyl C-atoms (C(9)/H–C(7), MeO–C(9); C(9′)/H–C(7′), H–C(8″); C(9″)/H–C(7″), MeO–C(9″)). Furthermore, the ketone-carbonyl C-atom and the sp^2 quaternary C-atom were assigned as C(5′) and C(1′), respectively, based on the long-range correlations with H–C(2′), H–C(5′), H–C(6′), and H–C(7), and with H–C(8), H–C(2′), H–C(3′), H–C(5′), H–C(7′), and H–C(8′).

The relative configuration of **1** was determined by a series of NOE experiments (at 20°: H–C(2)/OH–C(3′) (1%), H–C(5′) (2%), H–C(7) (6%), and H–C(8) (9%); at 0°: H–C(3′)/H–C(8′) (2%), H–C(6′)/H–C(7) (2%) and *vice versa* (1%), H–C(7′)/H–C(3′) (7%); see *Table 3*). The absolute configuration of **1** could not be determined

Table 3. *NOE Results of Neolignans* **1**–**3**. Arbitrary numbering[1]).

^{1}H Irradiated	^{1}H Enhanced (%)		
	1 (in (D_6)acetone, 0°)	**2** (in CD_3OD, 25°)	**3** (in (D_6)acetone, 25°)
H–C(2)	H–C(7) (4), H–C(8) (6), H–C(5′) (1)	H–C(4′) (1), H–C(7) and H–C(8) (total 5)[a])	H–C(8″) (1), H–C(7) (3), H–C(8) (1)
H–C(2)[b])	H–C(7) (6), H–C(8) (9), H–C(5′) (2), OH–C(3′) (1)		
H–C(6)	H–C(7) (4), H–C(8) (5)	H–C(4′) (2), H–C(7) and H–C(8) (total 9)[a])	H–C(5) (16), H–C(7′) (9), H–C(6″) (10)
H–C(7)	H–C(2) (5), H–C(6) (5), H–C(5′) (6), H–C(6′) (1)		H–C(2) (3), H–C(8) (3)
H–C(8)	H–C(2) (7), H–C(6) (6), H–C(2′) (4)		H–C(2) (2), H–C(7) (3), H–C(2′) (6), H–C(6‴) (3)
H–C(3′)	H–C(8′) (2)	H–C(8) (4)	
H–C(4′)		H–C(2) (2), H–C(6) (1), H–C(3′) (2)	
H–C(6′)	H–C(7) (2), H–C(5′) (6), H–C(7′) (8)		
H–C(7′)	H–C(3′) (7)		

[a]) The individual enhancement values (%) could not be obtained due to the partial overlapping. [b]) Data at 20°.

due to the small quantity of compound available; however, the configuration at C(8″) was supposed to be (*R*) based on the isolation of methyl (*R*)-3-(3,4-dihydroxyphenyl)lactate[3]) from the same extract in a preliminary examination.

Helisterculin B (**2**). The yellowish amorphous solid **2** had the molecular formula $C_{38}H_{38}O_{16}$. The UV and IR spectra were similar to those of **1**, suggesting the presence of phenyl, OH, and ester groups. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Tables 1* and *2*) also resembled those of **1**, but analyses of the COSY and HETCOR spectra revealed the presence of an additional OH group and an additional 3-(3,4-dihydroxyphenyl)lactic acid moiety, and the absence of the ketone group in **2**. In addition, they suggested the presence of the partial structure C(1′)=CH(6′)−CH(5′)−CH(7)−CH(8)−CH(2′)−CH(3′)(OH)−CH(4′)(OH)−CH(5′), instead of C(1′)=CH(6′)−CH(5′)−CH(7)−CH(8)−CH(2′)−CH(3′)−OH in **1**. The HMBC spectrum of **2** showed long-range correlations similar to those of **1** (*Table 2*) and indicated that two 3-(3,4-dihydroxyphenyl)lactic-acid moieties should be located at C(9) and C(9′) (C(9)/H−C(7), H−C(8‴); C(9′)/H−C(7′), H−C(8‴)) and two methyl esters at C(9″) and C(9‴) (C(9″)/2 H−C(7″), MeO−C(9″); C(9‴)/2 H−C(7‴), MeO−C(9‴)). These and other long-range correlations from *Table 2* led to the gross structure of helisterculin B (**2**)[2]).

The relative configuration of **2**, except for that at the (3,4-dihydroxyphenyl)lactic acid moieties, was determined by a difference NOE experiment in CD_3OD (*Table 3*) which showed better separation of several signals than in the solvent (D_6)acetone.

Helisorin (**3**). The molecular formula of **3** was determined as $C_{38}H_{34}O_{16}$ by high-resolution FAB-MS. The UV spectrum of **3** displayed a phenyl (279 nm) absorption, and the IR spectrum OH (3390 cm^{-1}), C=O (1720 cm^{-1}), and phenyl (1620, 1520, 1445 cm^{-1}) absorptions. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **1** and **2** (*Tables 1* and *2*) and showed signals due to a *trans*-disubstituted and a trisubstituted olefin, to four methine, a ketone, and two ester-carbonyl groups, and to two methyl 3-(3,4-dihydroxyphenyl)lactate moieties. However, the signals due to a 3,4-dihydroxyphenyl group and one of two hydroxymethine moieties, observed in the spectra of **2** were absent, and instead of them, signals due to a 1,2,4,5-tetrasubstituted phenyl group and a tertiary-alcohol C-atom were present. These data and a comparison of their compositions indicated that **3** has one more ring (C−C bond) than **2**. To ascertain the location of the new C−C bond and to determine the structure of **3**, COSY, HETCOR, and HMBC experiments were performed; the long-range correlations C(4′)/H−C(5) and C(6)/H−C(5′) were consistent with a new C(4′)−C(6) bond in **3**. The former two experiments indicated presence of the partial structures depicted in *Fig. 2*.

The HMBC spectrum confirmed the presence of two (3,4-dihydroxyphenyl)lactic-acid moieties (2 H−C(7″)/C(2″), C(6″); 2 H−C(7‴)/C(2‴), C(6‴); C(7″)/H−C(2″), H−C(6″); C(7‴)/H−C(2‴), H−C(6‴)) and the location of each ester group (C(9)/H−C(7), H−C(8‴); C(9′)/H−C(7′), H−C(8″); C(9″)/2 H−C(7″), MeO−C(9″); C(9‴)/2 H−C(7‴), MeO−C(9‴)). Similarly, the ketone-carbonyl atom C(3′) and the tertiary-alcohol atom C(4′) were attributed by long-range correlations with H−C(2′), H−C(5′), and H−C(8), and with H−C(5), H−C(7), and H−C(5′), respectively.

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[3]) This compound led to a trimethylated derivative which was identified by comparison of its data with published ones [8].

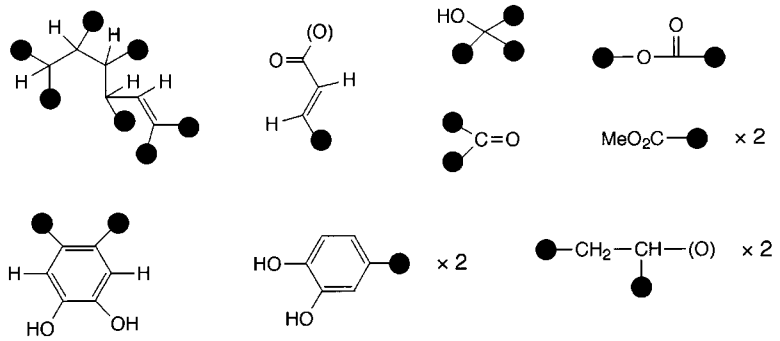


Fig. 2. *Partial structures for helisorin* (**3**)

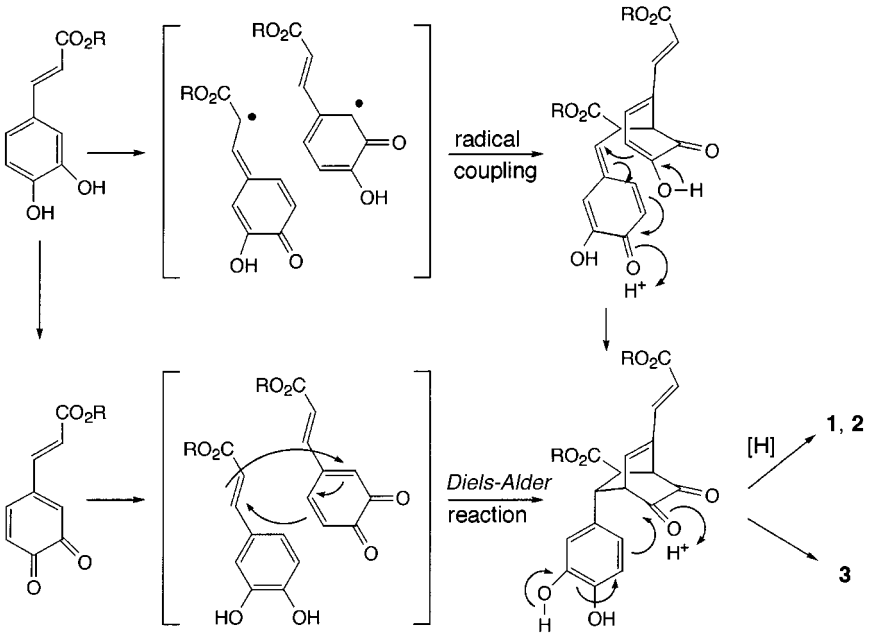
The configuration at C(8) of **3** was deduced from the coupling constant and the results of difference NOE experiments. The coupling constant for H–C(7) and H–C(8) ($J = 1$ Hz) indicated a dihedral angle of 65–110° [10], *i.e.*, *trans* configuration. This was confirmed by NOE experiments (H–C(2)/H–C(7) (3%) and H–C(8) (1%); H–C(8)/H–C(2) (2%) and H–C(7) (3%); see *Table 3*).

3. Phytochemistry. – The three new compounds helisterculins A (**1**) and B (**2**) and helisorin (**3**) belong to the class of the neolignans[1]). Previously, β-sitosterol, diosgenin, α- and β-amyrin, lupeol, friedelin, epifriedelinol, taraxerone, lupeol acetate, bauerenol acetate, and cytotoxic cucurbitacin B and isocucurbitacin B were reported as constituents of *H. isola* [11], but no lignans or neolignans had yet been isolated from *H. isola*.

Helisterculins (**1** and **2**) are (7.5′,8.2′)-neolignans with a bicyclo[2.2.2]octene C-framework, while helisorin (**3**) is a (2.8′,4.6′,5.7′)-neolignan with a very rare 3,9-methano-3*H*-fluorene C-framework. Although many neolignans have been reported [12], only six belong to the (7.5′,8.2′)-neolignan: silydianin, a flavonolignan from *Sylybium marianum* (Compositae) [13] and yunnaneic acids A–D and F from *Salvia yunnanensis* (Labiatae) [14]. On the other hand, the (2.8′,4.6′,5.7′)-neolignan has no literature precedent; the 1,2,3,9a-tetrachloro-4,4a,9,9a-tetrahydro-3,9-methano-3*H*-fluorene, a *Diels-Alder* reaction product of tetrachlorothiophene dioxide with 1,2-divinylbenzene [15], is the only previously reported compound having the same parent structure as **3**.

4. Biogenetic Consideration. – The structures of **1**–**3** suggest two probable biogenetic pathways (*Scheme*), *i.e.*, a radical-coupling pathway and a *Diels-Alder* reaction pathway. The former was recognized as a biosynthetic route for lignans and neolignans [12], and enzymes catalyzing the reaction were also reported [16]. On the other hand, the latter pathway was suggested for neolignans containing the bicyclo[2.2.2]octene structure [14][17]. Most *Diels-Alder* reactions involve an electron-rich diene carrying electron-donating substituents (*e.g.*, alkyl, alkoxy) and an electron-deficient dienophile bearing electron-attracting substituents (*e.g.*, CO, CN) [18]. In the case of **1**–**3**, the plausible intermediates are a diene carrying three electron-attracting groups and a dienophile bearing both an electron-attracting ester group and

Scheme



Two Possible Biogenetic Pathways for the New Neolignans **1**–**3**

an electron-donating 3,4-dihydroxyphenyl group. Thus the reaction might be difficult without any forcing condition(s) such as the presence of an enzyme, *i.e.*, a *Diels-Alder*ase. Although two enzymes were reported as being *Diels-Alder*ases [19], they catalyzed oxidation and enol formation, thus leading to an acceleration of the *Diels-Alder* reaction, but they did not catalyze the *Diels-Alder* reaction itself. Thus, it seems more reasonable to suggest that **1**–**3** are biosynthesized by the radical-coupling pathway.

5. Reverse Transcriptase Inhibitory Activity. – The inhibitory activity of **1**–**3** against AMV-RT was examined with [*methyl*-^{3}H]thymidine 5′-triphosphate (dTTP) or [8-^{3}H]deoxyguanosine 5′-triphosphate (dGTP) as a template primer [3]. The inhibitory activities of **1**–**3** were weak (IC_{50}: **1**, 1.6 mM; **2**, 1.0 mM; **3**, 0.46 mM; adriamycin as positive control, 60 μM), but it should be noted here that (2.8′,4.6′,5.7′)-neolignan **3** showed stronger activity than (7.5′,8.2′)-neolignans **1** and **2**.

This work was supported in part by a *Grant-in-Aid for Scientific Research* from the Ministry of Education, Science, Sports, and Culture of Japan (No. 06780464).

Experimental Part

General. Prep. TLC: *Merck* precoated silica gel *60F$_{254}$* plate (0.5-mm thick); 20% MeOH/$CHCl_3$. Prep. HPLC: *Japan-Analytical-Industry-LC-908* system; column: *ODS-80TM*™, 215 × 300 mm (*Toso*); mobile phase: 25% MeCN/1M AcOH. Optical rotations ($[\alpha]_D^{26}$): *Jasco-DIP-140* digital polarimeter; *c* in g sample/100 ml solvent. UV Spectra: *Shimadzu-UV-160A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco-IRA-2* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR Spectra: *Jeol-JNM-GX400* (400 (^{1}H) and 100 MHz (^{13}C)) instrument

using CD_3OD, (D_6)acetone, or (D_6)DMSO solns. with $SiMe_4$ as an internal standard; δ in ppm, J in Hz. FAB-MS and HR-FAB-MS: *Jeol-JMS-SX102* mass spectrometer with glycerol 3-nitrobenzyl alcohol as matrix.

Reverse Transcriptase Inhibitory Activity. Inhibitory activities of extracts, fractions, and compounds against AMV-RT were examined by the literature method [3], using dTTP or dGTP as a template primer.

Isolation. The powdered dried fruits of *H. isora* (2.5 kg), purchased in 1991 in Java, Indonesia, was extracted twice with hot H_2O (each 9 l, 1 h). The H_2O extract was concentrated *in vacuo* and the residue successively extracted with AcOEt and BuOH (each 6 l × 2) to give an AcOEt-soluble fraction (*A*, 2.4 g; inhibition at 0.1 mg/ml, 1.1 ± 1.3%) and a BuOH-soluble fraction (*B*, 5.8 g; inhibition at 0.1 mg/ml, 6.4 ± 1.6%), while the H_2O layer was lyophilized to give a H_2O-soluble fraction (*C*, 210 g; inhibition at 0.1 mg/ml, 87.9 ± 0.7%). A portion of the fraction *C* (160 g) was dissolved in H_2O and treated with ion-exchange resin *Amberlite IR-120B* (H^+ form, 400 g; *Organo*) for 12 h. The resin was separated by filtration and washed several times with H_2O. The filtrate and washing solns. were combined and evaporated. MeOH was added to the obtained residue, and an insoluble material was separated by filtration to give a MeOH-insoluble fraction (*D*, 40 g; inhibition at 0.1 mg/ml, 97.8 ± 0.4%) and a MeOH-soluble fraction (*E*, 95 g; inhibition at 0.1 mg/ml, 69.5 ± 1.2%). *Fraction D* (80 g) was then chromatographed on a *Diaion-HP-20P* column (8.3 × 22 cm) with H_2O (7 l), MeOH/H_2O (20%, 7 l; 40%, 10 l; 70%, 8 l) gradient mixtures, and MeOH (11 l) to give five fractions: *D.1* (H_2O eluate, 17.6 g; inhibition at 0.1 mg/ml, −3.3 ± 1.4%), *D.2* (20% MeOH/H_2O eluate, 5 g; inhibition at 0.1 mg/ml, 1.0 ± 0.1%), *D.3* (40% MeOH/H_2O eluate, 4.5 g; inhibition at 0.1 mg/ml, 1.5 ± 1.1%), *D.4* (70% MeOH/H_2O eluate, 12.6 g; inhibition at 0.1 mg/ml, 81.9 ± 1.4%), *D.5* (MeOH eluate, 15.8 g; inhibition at 0.1 mg/ml, 98.3 ± 0.3%).

Fraction D.4 (12.6 g) was separated on a *Sephadex-LH-20* column (7.0 × 30 cm) with EtOH to give four fractions (*Fr. 1*, 0.19 g; *Fr. 2*, 4.2 g; *Fr. 3*, 5.0 g; *Fr. 4*, 1.9 g). *Fr. 3* was subjected to *MCI*-gel *CHP-20P* CC (3.2 × 43 cm) with 50% and then 60% MeOH/H_2O to give ten fractions (*Fr. 3.1–3.10*). Among them, *Fr. 3.7* (183 mg) was subjected to prep. HPLC, followed by prep. TLC to give *helisterculin B* (**2**, 29 mg). *Fr. 3.8* (615 mg) was also subjected to prep. HPLC to afford two fractions having t_R 54 and 68 min. The former fraction, after prep. TLC, gave *3′,5,7,8-tetrahydroxy-4′-methoxyflavone 8-O-β-*D*-glucopyranosiduronic acid methyl ester* (**4**; 7 mg) and *4′,5,7,8-tetrahydroxyflavone 8-O-β-*D*-glycopyranosiduronic acid methyl ester* (**5**; 13 mg), while the latter fraction (t_R 68 min), after prep. TLC, afforded *helisterculin A* (**1**, 14 mg) and *helisorin* (**3**, 48 mg).

Helisterculin A (= *3-(3,4-Dihydroxyphenyl)-6-{3-{1-[(3,4-dihydroxyphenyl)methyl]-2-methoxy-2-oxoethoxy}-3-oxoprop-1-enyl}-7-hydroxy-8-oxobicyclo[2.2.2]oct-5-ene-2-carboxylic Acid Methyl Ester;* **1**). Yellowish amorphous solid. $[\alpha]_D^{26} = 239.2$ ($c = 0.75$, MeOH). UV (MeOH): 276 (4.28). IR (KBr): 3413, 1724, 1628, 1523, 1422, 1368, 1286. ^{1}H- and ^{13}C-NMR ((D_6)acetone): *Tables 1* and *2*. FAB-MS: 569 ($[M+H]^+$), 357, 291. HR-FAB-MS: 569.1654 ($C_{29}H_{29}O_{12}^+$; calc. 569.1659).

Helisterculin B (= *3-(3,4-Dihydroxyphenyl)-6-{3-{1-[(3,4-dihydroxyphenyl)methyl]-2-methoxy-2-oxoethoxy}-3-oxoprop-1-enyl}-7,8-dihydroxybicyclo[2.2.2]oct-5-ene-2-carboxylic Acid {1-[(3,4-Dihydroxyphenyl)methyl]-2-methoxy-2-oxoethyl} Ester*; **2**). Yellowish amorphous solid. $[\alpha]_D^{26} = 51.9$ ($c = 1.5$, MeOH). UV (MeOH): 279 (4.36). IR (KBr): 3424, 1736, 1628, 1523, 1443, 1368. ^{1}H- and ^{13}C-NMR ((D_6)acetone): *Tables 1* and *2*. FAB-MS: 751 ($[M+H]^+$), 539, 291. HR-FAB-MS: 751.2244 ($C_{38}H_{39}O_{16}^+$; calc. 751.2238).

Helisorin (= *2-{3-{1-[(3,4-Dihydroxyphenyl)methyl]-2-methoxy-2-oxoethoxy}-3-oxoprop-1-enyl}-4,4a,9,9a-tetrahydro-4a,6,7-trihydroxy-4-oxo-3,9-methano-3*H*-fluorene-10-carboxylic Acid {1-[(3,4-Dihydroxyphenyl)-methyl]-2-methoxy-2-oxoethyl} Ester*; **3**). Yellowish amorphous solid. $[\alpha]_D^{26} = 28.4$ ($c = 0.94$, MeOH). UV (MeOH): 279 (4.03), 317 (sh, 3.23). IR (KBr): 3390, 1720, 1620, 1520, 1445. ^{1}H- and ^{13}C-NMR ((D_6)acetone): *Tables 1* and *2*. FAB-MS: 747 ($[M+H]^+$), 718, 399. HR-FAB-MS: 747.1918 ($C_{38}H_{35}O_{16}^+$; calc. 747.1925).

*3′,5,7,8-Tetrahydroxy-4′-methoxyflavone 8-*O*-β-*D*-Glucopyranosiduronic Acid Methyl Ester* (= *5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran-8-yl β-*D*-Glucopyranosiduronic Acid Methyl Ester;* **4**). Yellow amorphous solid. $[\alpha]_D^{26} = 38.4$ ($c = 0.12$, MeOH). UV (MeOH): 213 (4.18), 274.5 (4.16), 343 (3.92). IR (KBr): 3416, 1739, 1658, 1607, 1506. ^{1}H-NMR ((D_6)acetone): 3.65 (t, $J = 9.5$, H−C(3″)); 3.65 (s, MeO−C(6″)); 3.74 (dd, $J = 9.5$, 8.0, H−C(2″)); 3.76 (t, $J = 9.5$, H−C(4″)); 3.97 (s, MeO−C(4′)); 4.06 (d, $J = 9.5$, H−C(5″)); 4.88 (d, $J = 8.0$, H−C(1″)); 6.25 (s, H−C(6)); 6.66 (s, H−C(3)); 7.11 (d, $J = 8.5$, H−C(5′)); 7.57 (d, $J = 2.1$, H−C(2′)); 7.82 (dd, $J = 8.5, 2.1$, H−C(6′)). ^{13}C-NMR ((D_6)DMSO): 181.8 (s, C(4)); 169.2 (s, C(6″)); 163.5 (s, C(2)); 157.3 (s, C(5), C(7)); 151.3 (s, C(4′)); 146.7 (s, C(3′)); 149.5 (s, C(9)); 125.0 (s, C(8)); 123.0 (s, C(1′)); 119.3 (d, C(6′)); 113.3 (d, C(2′)); 111.8 (d, C(5′)); 106.0 (d, C(1″)); 103.4 (d, C(3)); 103.3 (s, C(10)); 103.0 (d, C(6)); 75.7 (d, C(5″)); 75.2 (d, C(3″)); 73.7 (d, C(2″)); 71.4 (d, C(4″)); 55.7 (q, *Me*O−C(4′)); 51.9 (q, *Me*O−C(6″)). FAB-MS: 507 ($[M+H]^+$).

*4′,5,7,8-Tetrahydroxyflavone 8-O-β-*D*-Glucopyranosiduronic Acid Methyl Ester* (= *5,7-Dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-1-benzopyran-8-yl β-*D*-Glucopyranosiduranoic Acid Methyl Ester;* **5**). Yellow

amorphous solid. $[\alpha]_D^{26} = 40.9$ ($c = 0.69$, MeOH). UV (MeOH): 211.0 (3.83), 274.0 (3.78), 346.0 (3.70). IR (KBr): 3423, 1750, 1657, 1608, 1581, 1506. ^{1}H-NMR ((D$_6$)acetone): 3.64 (*s*, MeO–C(6″)); 3.65 (*t*, *J* = 9.5, H–C(3″)); 3.75 (*dd*, *J* = 9.5, 8.0, H–C(2″)); 3.77 (*t*, *J* = 9.5, H–C(4″)); 4.06 (*d*, *J* = 9.5, H–C(5″)); 4.88 (*d*, *J* = 8.0, H–C(1″)); 6.24 (*s*, H–C(6)); 6.67 (*s*, H–C(3)), 7.02 (*d*, *J* = 8.9, H–C(3′), H–C(5′)); 8.10 (*d*, *J* = 8.9, H–C(2′), H–C(6′)). ^{13}C-NMR ((D$_6$)DMSO): 181.8 (*s*, C(4)); 169.4 (*s*, C(6″)); 163.8 (*s*, C(2)); 157.4 (*s*, C(5)); 157.2 (*s*, C(7)); 151.3 (*s*, C(4′)); 149.3 (*s*, C(9)); 128.9 (*d*, C(2′), C(6′)); 125.2 (*s*, C(8)); 121.1 (*s*, C(1′)); 115.9 (*d*, C(3′), C(5′)); 106.4 (*d*, C(1″)); 102.4 (*d*, C(3)); 103.4 (*s*, C(10)); 99.0 (*d*, C(6)); 75.6 (*d*, C(5″)); 75.1 (*d*, C(3″)); 73.8 (*d*, C(2″)); 71.5 (*d*, C(4″)); 51.9 (*q*, *Me*O–C(6″)). FAB-MS: 477 ($[M+H]^+$).

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Received December 27, 1998