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Homoisoflavonoids and Related Compounds. II.[1] Isolation and Absolute Configurations of 3,4-Dihydroxylated Homoisoflavans and Brazilins from *Caesalpinia sappan* L.

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Three new phenolic compounds, 3′-*O*-methylsappanol, 3′-*O*-methylepisappanol and 3′-*O*-methylbrazilin, were isolated from Sappan Lignum, the dried heartwood of *Caesalpinia sappan*. The absolute configurations of sappanol, episappanol, 3′-deoxysappanol, 3′-*O*-methylsappanol, 3′-*O*-methylepisappanol, brazilin and 3′-*O*-methylbrazilin were determined by means of Horeau's partial resolution method and chemical correlations.

Sappanol, episappanol, 3′-deoxysappanol, 3′-*O*-methylsappanol and 3′-*O*-methylepisappanol form a novel class of homoisoflavonoids.

Keywords——*Caesalpinia sappan*; heartwood; Sappan Lignum; Leguminosae; homoisoflavonoid; 3,4-dihydroxylated homoisoflavan; brazilin; 3′-*O*-methylbrazilin; absolute configuration

A number of homoisoflavonoids[2,3] have been isolated from several genera in Liliaceae[3,4] and Caesalpinioideae (Legumiosae).[1,5,6] The structural features of homoisoflavonoids can generally be classified into three types, that is, eucomin type (3-benzylidenechroman-4-one), dihydroeucomin type (3-benzylchroman-4-one) and eucomol type (3-hydroxy-3-benzylchroman-4-one). Geometrical (*Z*-isomer) and position ($\Delta^{2(3)}$) isomers[4c] of eucomin-type compounds have also been isolated as natural products. Some unusual compounds were also isolated together with homoisoflavanones. One such group is scillascillin type compounds, which have a characteristic 3-spirocyclobutene ring system. Another example is comosin,[4a] whose 9-carbon is not benzylic, and which is a 3-acetyloxymethyl-3-phenylchroman-4-one. All these compounds have a carbonyl function at the C-4 position. Other novel compounds, reported by Camarda *et al.*,[4b] have a homoisoflavan structure, in which the 4-carbon is a methylene instead of the carbonyl in homoisoflavanones.

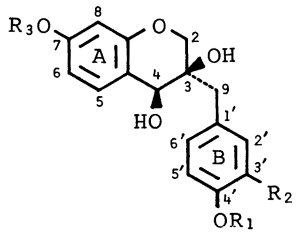
In the course of our studies on homoisoflavonoids and related compounds, we have been investigating the phenolic components of Sappan Lignum, the dried heartwood of *Caesalpinia sappan* L. (Leguminosae), and reported the isolation and structural determination of various homoisoflavonoidal components.[1,5] Among these compounds, newly named sappanol (**1**) and episappanol (**2**) form a novel class of homoisoflavonoids, which have a 3,4-dihydroxyhomoisoflavan structure. In the previous paper,[1] we reported the isolation and structural assignment of three homoisoflavonoids from the same source. One of these compounds, newly named 3′-deoxysappanol (**3**), is the third example of 3,4-dihydroxyhomoisoflavans.

Chemical constituents of Sappan Lignum have also been studied by two other groups. Nagai *et al.* reported the isolation of sappanchalcone[7] and dibenzoxocins[8] (protosappanins A, B and C) in a study on the compounds having a sleeping-time-prolonging effect in mice. Nohara and his co-workers isolated some phenolic components from this source in the course of their systematic screening for antihypercholesteremic activity,[5,9] and reported that

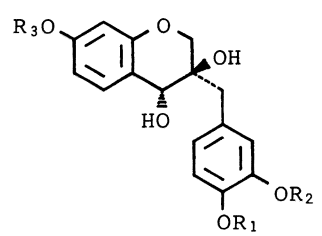
benzofran compounds were responsible for the effect.[9a] They determined the relative configuration of caesalpin J by means of X-ray crystallographic study.[9b]

Thus, Sappan Lignum contains various structural types of phenolic components, that is dibenzoxocins, homoisoflavonoids, brazilin and so on. Brazilin (**14**),[10] a well known main component of this plant, is interesting from the viewpoint of its characteristic skeleton and pharmacological activities.[11,12] Sappan Lignum has been used as an emmenagogue, hemostatic and antiinflammatory agent, and as a medical treatment for contusion and thrombosis prescribed in traditional oriental medicine.[11,13] An extract of Sappan Lignum has a suppressing effect on the central nervous system and has antimicrobial activities against *Staphylococcus*, *Diplococcus*, *Corynebacterium*, *Shigella baydii*, *etc.*[13]

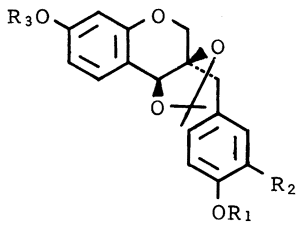
We are interested in structural and biogenetic aspects of these compounds, as well as in the structure–activity relationships. In the present paper we deal with the isolation from Sappan Lignum and the absolute stereochemistries of three new components, 3′-*O*-methylsappanol (**4**), 3′-*O*-methylepisappanol (**5**) and 3′-*O*-methylbrazilin (**15**), and the



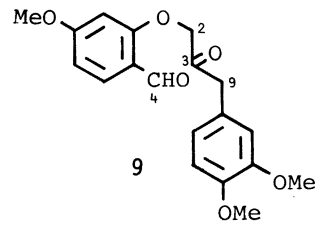
1: $R_1=R_3=H$, $R_2=OH$
3: $R_1=R_2=R_3=H$
4: $R_1=R_3=H$, $R_2=OMe$
6: $R_1=R_3=Me$, $R_2=OMe$
8: $R_1=R_3=Me$, $R_2=H$
26: $R_1=R_3=CH_2Ph$, $R_2=OMe$
28: $R_1=Me$, $R_2=OCH_2Ph$, $R_3=CH_2Ph$
38: $R_1=R_3=CH_2Ph$, $R_2=OCH_2Ph$



2: $R_1=R_2=R_3=H$
5: $R_1=R_3=H$, $R_2=Me$
7: $R_1=R_2=R_3=Me$
27: $R_1=R_3=CH_2Ph$, $R_2=Me$
29: $R_1=Me$, $R_2=R_3=CH_2Ph$
39: $R_1=R_2=R_3=CH_2Ph$



10: $R_1=R_3=H$, $R_2=OH$
11: $R_1=R_3=H$, $R_2=OMe$
12: $R_1=R_2=R_3=H$
13: $R_1=R_3=Me$, $R_2=OMe$
30: $R_1=R_3=CH_2Ph$, $R_2=OMe$
31: $R_1=Me$, $R_2=OCH_2Ph$, $R_3=CH_2Ph$
32: $R_1=Me$, $R_2=OH$, $R_3=H$



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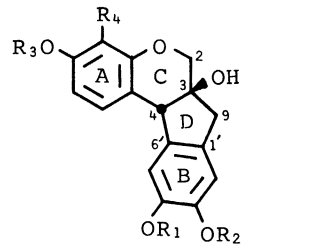

14: $R_1=R_2=R_3=R_4=H$
15: $R_1=R_3=R_4=H$, $R_2=Me$
16: $R_1=R_2=R_3=Me$, $R_4=H$
33: $R_1=Me$, $R_2=R_3=R_4=H$
40: $R_1=R_2=R_3=H$, $R_4=OH$

Chart 1

elucidation of the absolute stereochemistries of sappanol (**1**), episappanol (**2**), 3′-deoxysappanol (**3**) and brazilin (**14**).

Absolute Stereochemistries of Sappanol (1), Episappanol (2) and 3′-Deoxysappanol (3)

Sappanol (**1**) and episappanol (**2**) were obtained as a mixture of epimers at the C-4 position.[5] The ratio of **1** and **2** in the mixture varied between 2 : 1 and 3 : 2 as estimated from the proton nuclear magnetic resonance (^{1}H-NMR) spectra. Compounds **1** and **2** were not chromatographically separable from each other, but could be separated as their methyl derivatives (**6** and **7**), obtained by methylation with dimethyl sulfate. Compounds **6** and **7** were isolated from each other by silica gel column chromatography (hexane–acetone (4 : 1)). Compound **6** has a specific rotation of $+27.6°$ ($c=3.91$, $CHCl_3$), and **7** has $-13.6°$ ($c=1.43$, $CHCl_3$). The ^{1}H-NMR data for **6** and **7** are summarized in Table I. Since **6** and **7** gave a trimethyl ether of brazilin (**16**) upon acid catalyzed ring closure, the difference in the structure of these compounds is the stereochemistry at the C-4 position. On treatment with dry acetone in the presence of an acid catalyst, **6** afforded **13** within 10 h, but about 50% of **7** remained unchanged even after 24 h. In addition, **6** was readily oxidized to form **9** within 20 min upon HIO_4 treatment in methanol, while **7** was not (a half of the starting material remained even after 2 h). These facts suggested that the relative stereochemistries at C-3 and C-4 of **6** and **7** are *cis* and *trans*, respectively.

The absolute configurations at C-4 position of **6** and **7** were determined by Horeau's partial resolution method.[14] Compound **6** was treated with two equivalents of racemic 2-phenylbutanoic anhydride in pyridine, and the residual acid was levorotatory. The absolute stereochemistry at C-4 of **6** is thus (*S*) according to Horeau's rule.[14] Compound **6** is therefore (3*R*,4*S*)-3,4-dihydroxy-3-(3,4-dimethoxybenzyl)-7-methoxychroman. Consequently, sappanol (**1**) is (3*R*,4*S*)-3-(3,4-dihydroxybenzyl)-3,4,7-trihydroxychroman, as shown in Chart 1.

Similarly, **7** was treated with racemic 2-phenylbutanoic anhydride and the obtained acid was dextrorotatory. Therefore, the absolute stereochemistry at the C-4 position of **7** is (*R*), and **7** is (3*R*,4*R*)-3,4-dihydroxy-3-(3,4-dimethoxybenzyl)-7-methoxychroman. Accordingly, episappanol (**2**) is (3*R*,4*R*)-3-(3,4-dihydroxybenzyl)-3,4,7-trihydroxychroman (Chart 1).

^{1}H-NMR data for sappanol (**1**) and episappanol (**2**), summarized in Table I, were obtained with synthetic (±)-**1** and (±)-**2** as described later.

3′-Deoxysappanol (**3**) was obtained as a single epimer,[1] and afforded the dimethyl derivative (**8**) upon treatment with dimethyl sulfate. The circular dichroism (CD) curve of **8** showed a negative Cotton effect (Fig. 1). A negative Cotton effect was observed for **6**, while a positive Cotton effect was found for **7** (Fig. 1). Consequently the stereochemical correspondence of **6** and **8** was confirmed. Accordingly, 3′-deoxysappanol (**3**) is (3*R*,4*S*)-3-(4-hydroxybenzyl)-3,4,7-trihydroxychroman, as shown in Chart 1.

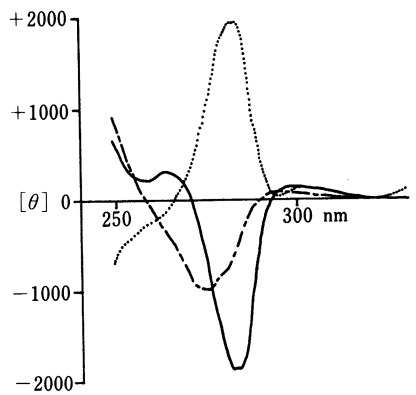

Fig. 1. CD Curves of Compounds **6** (——), **7** (------) and **8** (— · —)

TABLE I. ^{1}H-NMR Data for Compounds **1**, **2**, **4**, **5**, **6** and **7**

	1 (CD_3OD)[a]	**2** (CD_3OD)[a]	**4** (CD_3OD)[a]	**5** (CD_3OD)[a]	**6** ($CDCl_3$)	**7** ($CDCl_3$)
H-2	3.67 dd J=10.6, 1.2[b]	3.74 dd J=11.2, 1.6	3.66 dd J=10.5, 1.3	3.75 dd J=11.2, 1.6	[c]	[c]
	3.88 d J=10.6	4.01 d J=11.2	3.89 d J=10.5	4.02 d J=11.2	[c]	4.07 d J=11.5
H-4	4.21 br s	4.19 br s	4.19 br s	4.18 br s	4.26 br s	4.30 br s
H-5	7.09 d J=8.2	7.07 d J=8.2	7.10 d J=8.2	7.08 d J=8.2	7.20 d J=8.6	7.18 d J=8.6
H-6	6.41 dd J=8.2, 2.3	6.37 dd J=8.2, 2.4	6.42 dd J=8.2, 2.2	6.37 dd J=8.2, 2.4	6.53 dd J=8.6, 2.4	6.50 dd J=8.6, 2.4
H-8	6.26 d J=2.3	6.23 d J=2.4	6.27 d J=2.2	6.24 d J=2.4	6.40 d J=2.4	6.39 d J=2.4
H-9	2.57 d J=14.0 [d]	2.67 d J=14.4	2.65 d J=14.0 [d]	2.72 d J=14.0	2.76 s	2.72 d J=14.0
	2.63 d J=14.0	2.84 d J=14.4	2.69 d J=14.0	2.91 d J=14.0		3.00 d J=14.0
H-2′	6.69 d J=2.0	6.81 d J=1.9	6.77 d J=2.0	6.94 d J=1.8	6.62—6.78	6.76—7.00
H-5′	6.66 d J=8.0	6.70 d J=8.0	6.69 d J=8.0	6.73 d J=8.0	3H m	3H m
H-6′	6.50 dd J=8.0, 2.0	6.65 dd J=8.0, 1.9	6.60 dd J=8.0, 2.0	6.76 dd J=8.0, 1.8		
			3.81 s (3′-OMe)	3.85 s (3′-OMe)	3.76, 3.83, 3.83 3s (OMe)	3.74, 3.84, 3.86 3s (OMe)

a) Measured at 400 MHz. *b*) Coupling constants (J) are given in Hz. *c*) Overlapped with methyl signals. *d*) These signals were observed as a singlet at 100 MHz.

TABLE II. ^{1}H-NMR Data for Compounds **10**, **11**, **12**, **13** and **32**

	10 (Acetone-d_6)[a]	**11** (Acetone-d_6)[a]	**32** (Acetone-d_6)	**12** (Acetone-d_6)	**13** ($CDCl_3$)
H-2	3.61 d J=10.5[c]	3.63 d J=10.5	3.59 d J=10.5	3.59 d J=10.5	[b]
	3.78 dd J=10.5, 1.3	3.79 dd J=10.5, 1.3	3.79 dd J=10.5, 1.3	3.78 dd J=10.5, 1.3	
H-4	4.61 br s	4.62 br s	4.62 br s	4.62 br s	4.58 br s
H-5	7.12 d J=8.5	7.16 d J=8.5	7.16 d J=8.5	7.15 d J=8.5	7.22 d J=8.5
H-6	6.46 dd J=8.5, 2.2	6.52 dd J=8.5, 2.2	6.52 dd J=8.5, 2.2	6.51 dd J=8.5, 2.2	6.58 dd J=8.5, 2.2
H-8	6.33 d J=2.2	6.39 d J=2.2	6.41 d J=2.2	6.39 d J=2.2	6.48 d J=2.2
H-9	2.70 d J=14.5 [d]	2.78 d J=14.4 [d]	2.76 s	2.79 s	2.77 d J=14.5
	2.76 d J=14.5	2.85 d J=14.4			2.95 d J=14.5
H-2′	6.66 d J=2.0	6.81 d J=2.0	6.77 d J=2.0	7.04 d J=8.8	
H-3′	—	—	—	6.75 d J=8.8	— 6.68—6.86
H-5′	6.67 d J=8.0	6.75 d J=8.0	6.80 d J=8.2	6.75 d J=8.8	3H m
H-6′	6.48 dd J=8.0, 2.0	6.63 dd J=8.0, 2.0	6.61 dd J=8.2, 2.0	7.04 d J=8.8	
		3.80 s (3′-OMe)	3.78 s (4′-OMe)		3.76, 3.84 3s (OMe)
$C(CH_3)_2$	1.24, 1.35	1.25, 1.33	1.29, 1.33	1.26, 1.33	1.26, 1.41

a) Measured at 400 MHz. *b*) Overlapped with methyl signals. *c*) Coupling constants (J) are given in Hz. *d*) These signals were observed as a singlet at 100 MHz.

Absolute Stereochemistry of Brazilin (14)

The relative stereochemistry at C-3 and C-4 of brazilin (**14**) has been established by synthetic, spectral and chemical evidence; the compound has a *cis* C/D ring junction.[10a,15] Compounds **1** and **2** were readily cyclized to form brazilin (**14**) on treatment with a catalytic amount of acid. Compounds **6** and **7** were also transformed by similar acid treatment into the trimethyl derivative of brazilin (**16**) which was identical with the compound obtained from brazilin (**14**) upon methylation with dimethyl sulfate. Consequently, the absolute stereochemistry at the C-3 and C-4 positions of brazilin (**14**) must be (3*S*,4*R*), *i.e.*, brazilin is (6a*S*,11b*R*)-3,6a,9,10-tetrahydroxy-6a,11b-dihydro-7*H*-indeno[2,1-*c*]chromene, as shown in Chart 1.

Isolation, Synthesis and Absolute Stereochemistries of 3′-*O*-Methylsappanol (4) and 3′-*O*-Methylepisappanol (5)

3′-*O*-Methylsappanol (**4**) and 3′-*O*-methylepisappanol (**5**) were obtained as a mixture of two epimers at the C-4 position as in the case of sappanol (**1**) and episappanol (**2**). The ^{1}H-NMR spectrum of the mixture of **4** and **5** resembled that of the mixture of **1** and **2**, except for two three-proton singlets at δ 3.81 and 3.85, due to the *O*-methyl groups at C-3′ of **4** and **5**, respectively. The ratio of **4** and **5** in the mixture was about 2:1. When the separation was performed with the intact compounds, a very small amount of the mixture of **4** and **5** was obtained. Satisfactory amounts were obtained in the form of the isopropylidene derivative (**11**) separated after treatment of the methanolic extract with dry acetone in the presence of an acid catalyst. In a similar way, compound **10**, the isopropylidene derivative of sappanol (**1**) was obtained in greatly improved yield compared with the case of the separation with intact compounds. Compound **12** was also isolated as the isopropylidene derivative of 3′-deoxysappanol (**3**). As the mixture of 3′-*O*-methylsappanol (**4**) and 3′-*O*-methylepisappanol (**5**) was not separable and the yields were very low, the structural elucidation was accomplished by utilizing the isopropylidene derivative (**11**).

The ^{1}H-NMR spectrum of **11** was similar to that of **10**, except for the phenolic methoxy signal (3H, s) at δ 3.80 (summarized in Table II). Compound **11** gave a dimethyl derivative (**13**) on methylation with dimethyl sulfate, and its spectral properties coincided with those of **13** derived from **10**. Therefore, **11** was deduced to be a monomethyl ether of **10**. The fragment ion peaks derived from the A-ring of **10** and **11** were the same (*m/z* 163 and 164), but a fragment ion peak derived from the B-ring of **11** (at *m/z* 137) appeared at a position 14 mass units higher than the corresponding peak of **10** (at *m/z* 123). These facts suggested that the *O*-

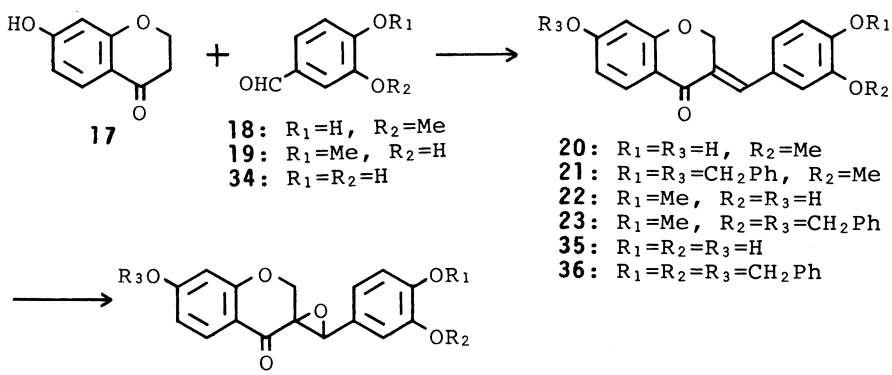

Chart 2

methyl group is attached to either C-3′ or C-4′ in the B-ring of **11**.

A nuclear Overhauser effect (NOE) enhancement was observed for a proton signal at δ 6.81 (H-2′) upon irradiation of the methoxy proton signal at δ 3.80 in the ^{1}H-NMR spectrum of **11**. Therefore, the *O*-methyl group could be attached to the C-3′ position. The structure of **11** was further confirmed by the synthesis of (±)-**11** and (±)-**32**, which has an *O*-methyl group at the C-4′ position.

Vanillin (**18**) reacted with 7-hydroxychroman-4-one[16] (**17**) in dry ethanol saturated with dry HCl gas,[17a] to afford a benzylidenechromanone (**20**). It was transformed into its benzyl ether (**21**) with benzyl chloride in K_2CO_3/DMF. Compound **21**, on epoxidation with alkaline hydrogen peroxide in acetone–methanol,[17a,18] gave (±)-**24**, which was reduced to a mixture of (±)-**26** and (±)-**27** (about 9:1 mixture) with $LiALH_4$ in tetrahydrofuran (THF).[19] The mixture of (±)-**26** and (±)-**27** was treated with dry acetone in the presence of Amberlyst 15 without separation, and yielded the isopropylidene derivative (±)-**30** and a small amount of unchanged (±)-**27**. Compound (±)-**30**, on hydrogenation with palladium-on-charcoal (Pd–C) catalyst in acetone–methanol, afforded (±)-**11**. The physicochemical properties of (±)-**11** coincided with those of **11** isolated from the acetone-treated methanolic extract. Compound (±)-**32** was synthesized from isovanillin (**19**) and **17** in a manner similar to that used for the synthesis of (±)-**11**. Compound (±)-**32** was not distinguishable chromatographically from **11**, but the spectral data of (±)-**32** showed the distinct differences from those of **11**. The ^{1}H-NMR data for (±)-**32** are summarized in Table II together with those for **11**.

On the basis of these results, the position of the *O*-methyl group in **11**, and also in **4** and **5** was determined to be C-3′ of the B-ring.

Compound **13** derived from **11** showed the same levorotatory character as **13** derived from **10**. Consequently, the absolute stereochemistries at C-3 and C-4 of **11** are (3*R*,4*S*), as in **10**. 3′-*O*-Methylsappanol (**4**) and 3′-*O*-methylepisappanol (**5**) are therefore (3*R*,4*S*)-3-(4-hydroxy-3-methoxybenzyl)-3,4,7-trihydroxychroman and (3*R*,4*R*)-3-(4-hydroxy-3-methoxybenzyl)-3,4,7-trihydroxychroman, respectively (Chart 1).

Isolation, Synthesis and Absolute Stereochemistry of 3′-*O*-Methylbrazilin (15)

3′-*O*-Methylbrazilin (**15**) was obtained by repeated column chromatography (silica gel and LH-20) and preparative thin layer chromatography (prep. TLC) from the fractions remaining after separation of the previously reported compounds.[1,5] 3′-*O*-Methylbrazilin (**15**) has the molecular formula $C_{17}H_{16}O_5$ (high-resolution mass spectrum). The ^{1}H-NMR spectrum of 3′-*O*-methylbrazilin (**15**) was similar to that of brazilin (**14**) (summarized in Table III), but it showed the signals due to H-2′ and H-5′ together at δ 6.79, and also a phenolic methoxy signal (3H, s) at δ 3.79. The presence of one *O*-methyl group is consistent with the observation of a 14 mass units increase of the M^+ ion in the mass spectrum of 3′-*O*-methylbrazilin (**15**) compared with brazilin (**14**). The lower field shift (0.14 ppm) of the proton signal due to H-5′ in the ^{1}H-NMR spectrum of **15** suggested that a methoxy group is attached to C-3′ of the B-ring. The structure of **15** was determined on the basis of the following chemical correlations.

3′-*O*-Methylbrazilin (**15**) was readily synthesized from 3′-*O*-methylsappanol (**4**) and 3′-*O*-methylepisappanol (**5**), and also from **11** upon heating in methanol in the presence of an acid. Compound (±)-**33** (4′-*O*-methylbrazilin) was also synthesized from (±)-**32** under similar condition for the comparison of the spectral data. The ^{1}H-NMR spectrum of (±)-**33** was different from that of 3′-*O*-methylbrazilin (**15**) (data are summarized in Table III). The location of the methoxy group was thus determined to be at C-3′ of the B-ring.

3′-*O*-Methylbrazilin (**15**) showed a specific rotation of +113.2° (methanol), and the compound derived from **11** showed the same dextrorotatory character. Moreover, **15**, on methylation with dimethyl sulfate, afforded **16**, whose optical rotatory character was the same

TABLE III. ^{1}H-NMR Data for Compounds **14**, **15**, **16** and **33**

	14 (Acetone-d_6)	**15** (Acetone-d_6)	**16** ($CDCl_3$)	**33** (Acetone-d_6)
H-2	3.72 d J=11.5[a]	3.74 d J=11.5	[b]	3.77 d J=11.5
	3.96 dd J=11.5, 2.0	3.97 dd J=11.5, 2.0	3.98 dd J=12.0, 2.0	4.01 d J=11.5
H-4	4.01 d J=2.0	4.02 br s	4.04 br s	4.06 br s
H-5	7.21 d J=8.6	7.23 d J=8.6	7.24 d J=8.8	7.30 d J=8.6
H-6	6.50 dd J=8.6, 2.4	6.51 dd J=8.6, 2.4	6.58 dd J=8.8, 2.5	6.53 dd J=8.6, 2.4
H-8	6.32 d J=2.4	6.31 d J=2.4	6.41 d J=2.5	6.35 d J=2.4
H-9	2.80 d J=16.0	2.88 d J=16.0	2.81 d J=16.0	2.88 d J=16.0
	3.06 d J=16.0	3.10 d J=16.0	3.20 d J=16.0	3.10 d J=16.0
H-2′	6.79 s	6.79 s	6.76 s	6.90 s
H-5′	6.65 s	6.79 s	6.69 s	6.69 s
		3.79 s (3′-OMe)	3.72 s 3.79 2s (OMe)	3.79 s (4′-OMe)

a) Coupling constants (J) are given in Hz. *b*) Overlapped with methyl signals.

as that of **16** derived from brazilin (**14**). Therefore, the absolute stereochemistries at C-3 and C-4 of 3′-*O*-methylbrazilin (**15**) are (3*S*,4*R*), *i.e.*, **15** is (6a*S*,11b*R*)-9-methoxy-3,6a,10-trihydroxy-6a,11b-dihydro-7*H*-indeno[2,1-*c*]chromene, as shown in Chart 1.

The stereochemical correspondence of brazilin (**14**) and haematoxylin[10b] (**40**), a constituent of *Haematoxylon campechianum*, was established in an optical rotatory dispersion (ORD) experiment carried out on their peracetates.[10a] The great similarity of the ORD curves of the two acetates revealed the stereochemistry at C-3 and C-4 of haematoxylin (**40**) to be (3*S*,4*R*), as shown in Chart 1 ((6a*S*,11b*R*)-3,4,6a,9,10-pentahydroxy-6a,11b-dihydro-7*H*-indeno[2,1-*c*]chromene). Studies on the absolute stereochemistries of the other components obtained from Sappan Lignum are in progress.

In the preceding paper,[1] we reported the isolation of three flavonoidal components, quercetin, rhamnetin and ombuin, from this source. The contents of these flavonoids are significantly less than those of homoisoflavonoidal components. Therefore, methylation at the C-2′ position of a chalcone to form a 2′-methoxychalcone is preferred to isomerization of the chalcone into a flavonoidal skeleton in the heartwood of this plant. 2′-Methoxychalcone is thought to be important in the biogenetic transformation into the homoisoflavonoidal skeleton, based on an isotope labeling study reported by Dewick.[20] He also proposed the biogenetic route to brazilin (**14**) from 2′-methoxychalcone via homoisoflavonoids;[20] this was supported recently by the isolation of homoisoflavanones and 3,4-dihydroxyhomoisoflavans.[5] Among these biogenetic transformations, the hydrogenation of a carbonyl group at the C-4 position to a hydroxyl group, that is, the formation of sappanol (**1**) and/or episappanol (**2**), will be a key step for brazilin biosynthesis.[5,20] Hydrogenation at the C-4 carbonyl group has never been recognized in homoisoflavonoids isolated from other sources. Sappanol (**1**) and episappanol (**2**) are readily transformed into brazilin (**14**) in the presence of a catalytic amount of acids or bases, and upon heating even under neutral conditions.[5] 3′-*O*-Methylsappanol (**4**) and 3′-*O*-methylepisappanol (**5**) afforded 3′-*O*-methylbrazilin (**15**) under similar conditions. However 3′-deoxysappanol (**3**) was not transformed into a brazilin-type compound by similar treatments. This can be attributed to the lack of an oxygen function at the C-3′ position, and 3′-deoxysappanol (**3**) should not biogenetically transform into a brazilin-type compound on the basis of this result.[3,20] When a methanol solution of 3′-deoxysappanol (**3**) was refluxed in the presence of an acid catalyst, two methylated compounds were formed, which were found to be 3′-deoxy-4-*O*-

methylsappanol and its C-4 epimer.[21] A similar phenomenon was observed with sappanol (**1**) and episappanol (**2**), which both gave a mixture of two epimers at the C-4 position of 4-*O*-methylated sappanol together with brazilin (**14**) upon heating in methanol.[5] 4-*O*-Methylsappanol and 4-*O*-methylepisappanol were also obtained from the methanolic extract of Sappan Lignum (compounds **5** and **6** in ref. 5), and were proposed to be activated precursors for brazilin biosynthesis, like sappanol (**1**) and episappanol (**2**).[3,20,22] Studies on the details of biosynthesis of brazilin and related compounds originating from homoisoflavonoids have been started in our laboratory.

From this series of studies, it has been shown that there are three substitution patterns of oxygen functions on aromatic rings in homoisoflavonoidal components of this plant material. One group consists of 3′,4′,7-trihydroxy compounds,[5] and the second consists of 4′,7-dihydroxy compounds,[1] which could be synthesized biogenetically from the corresponding methylated chalcones.[1,5] The third group consists of 4′,7-dihydroxy-3′-methoxy compounds as reported in this paper. The isolation of the biosynthetic precursors of 3′-*O*-methylsappanol (**4**) and 3′-*O*-methylepisappanol (**5**) is in progress.

The spectral data for sappanol (**1**), episappanol (**2**), 3′-*O*-methylsappanol (**4**) and 3′-*O*-methylepisappanol (**5**) were obtained by utilizing the synthetic compounds. The epimers **1** and **2**, and **4** and **5** could not be separated from each other, as already mentioned. The methyl ethers and the benzyl ethers of these compounds were separable. The mixtures of benzyl ethers (±)-**26** and (±)-**27**, and (±)-**38** and (±)-**39** were each subjected to column chromatography on silica gel with a hexane–acetone mixture (4 : 1). The hydrogenation of (±)-**26**, (±)-**27**, (±)-**37** and (±)-**38** in acetone–methanol in the presence of Pd–C catalyst afforded the free hydroxyl compounds (±)-**4** and (±)-**5**, and (±)-**1** and (±)-**2**, respectively. (±)-Sappanol (**1**) and (±)-3′-*O*-methylsappanol (**4**) gave a trimethyl ether (±)-**6** upon methylation with dimethyl sulfate, and on similar methylation, (±)-episappanol (**2**) and (±)-3′-*O*-methylepisappanol (**5**) yielded (±)-**7**. The synthesis of other components of Sappan Lignum will be reported elsewhere.

Experimental

All melting points were taken on a Yanagimoto micro melting point determination apparatus and are not corrected. Specific rotations were measured with a JASCO DIP-181 digital polarimeter. Circular dichroism (CD) spectra were observed on a JASCO J-20A automatic recording spectropolarimeter, equipped with a JASCO J-DPY data processor. ^{1}H-NMR spectra were recorded at 100 MHz on a Varian XL-100 instrument and at 400 MHz on a JEOL JNM-GX 400 spectrometer. Chemical shifts are reported on the δ scale (ppm) relative to tetramethylsilane (TMS) as an internal standard and coupling constants (J) are given in Hz. Abbreviations used are as follows: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet, br = broad. Electron impact mass spectra (EI-MS) were measured with a JEOL JMS-D 300 at 70 eV. Ultraviolet (UV) spectra were obtained with a Shimadzu UV-240 spectrometer. TLC was performed on precoated Silica gel 60 F_{254} plates (0.25 mm thickness, Merck), and the spots were visualized by UV irradiation (254 nm) and by spraying 10% H_2SO_4 followed by heating.

Extraction and Isolation——Sappan Lignum, the dried heartwood of *Caesalpinia sappan* L. (500 g), purchased in Tokyo, was extracted 3 times with methanol at room temperature for 3 d. The methanolic extract (48.9 g) was repeatedly subjected to column chromatography and prep. TLC as described in the preceding papers.[1,5] Compounds **4** and **5**, and **15** were obtained by further separation from the fractions remaining after isolation of the previously reported compounds,[1,5] giving a mixture of **4** and **5** (1.2 mg), $[\alpha]_D^{25}$ +2.6° (c = 0.39, MeOH), in a ratio of about 2 : 1 estimated from the ^{1}H-NMR spectrum, and 5.6 mg of **15**.

Well-dried methanolic extract (52.6 g) was dissolved in dry acetone (300 ml), and 2 ml of Amberlyst 15 was added. The mixture was stirred at room temperature for 24 h, then the catalyst was filtered off, and the filtrate was evaporated to dryness. Compounds **10**, **11** and **12** were detectable on TLC ($CHCl_3$–MeOH (9 : 1), benzene–acetone (4 : 1), hexane–acetone (3 : 2)) of the crude reaction mixture. Repeated column chromatography (silica gel and LH-20) and prep. TLC of the crude product gave **10** (782 mg), **11** (26 mg) and **12** (254 mg).

Compound 10——$[\alpha]_D^{25}$ −24.7° (c = 1.50, MeOH). UV λ_{max}^{MeOH} nm (log ε): 283 (3.79), 278 (3.76). EI-MS m/z: 344.1257 (M^+, Calcd for $C_{19}H_{20}O_6$: 344.1257), 269, 220, 164, 163, 123, 107. ^{1}H-NMR data are summarized in Table II.

From the Mixture of Sappanol (**1**) and Episappanol (**2**): The mixture of **1** and **2** (38 mg) in dry acetone (20 ml) was stirred at room temperature for 24 h in the presence of Amberlyst 15 (0.2 ml). The mixture was filtered, and the filtrate was evaporated to dryness. The residue was purified by prep. TLC (benzene–acetone (7 : 3)) to afford **10** (36 mg). The physical and spectral properties coincided with those of an authentic sample of **10**.

Compound 11——$[\alpha]_D^{25}$ −24.0° (c = 1.04, MeOH). UV λ_{max}^{MeOH} nm (log ε): 283 (3.69), 278 (3.71), 225 (4.10). EI-MS m/z: 358.1420 (M^+, Calcd for $C_{20}H_{22}O_6$: 358.1415), 283, 220, 164, 163, 137, 122, 107. ^{1}H-NMR data are summarized in Table II. A difference NOE spectrum was measured on a 400 MHz ^{1}H-NMR spectrometer. Irradiating at δ 3.80 (3′-OMe) [one of geminal methylene signals due to H-2 (δ 3.79) overlapped] caused NOE enhancements at δ 6.81 (H-2′) and at δ 3.63 (the other geminal methylene signal due to H-2).

Compound 12——$[\alpha]_D^{25}$ −21.1° (c = 3.56, MeOH). UV λ_{max}^{MeOH} nm (log ε): 283 (3.58), 278 (3.63), 224 (4.20). EI-MS m/z: 328.1311 (M^+, Calcd for $C_{19}H_{20}O_5$: 328.1311), 164, 163, 107, 77. ^{1}H-NMR data are summarized in Table II.

Methylation of the Mixture of Sappanol (1) and Episappanol (2) (Compounds 6 and 7)——Dimethyl sulfate (180 mg) and anhydrous K_2CO_3 (3 g) were added to a solution of the mixture of **1** and **2** (138 mg) in dry acetone (50 ml). The mixture was refluxed for 3 h, then filtered, and the filtrate was evaporated to dryness. The residue was applied to a silica gel column and eluted with hexane–acetone (9 : 1) to afford **6** (83 mg) and **7** (45 mg).

Compound **6** was obtained as colorless needles. mp 108—109 °C. $[\alpha]_D^{25}$ +27.6° (c = 3.90, $CHCl_3$). EI-MS m/z: 346 (M^+), 328, 262, 261, 194, 193, 153, 152, 151, 121, 86, 84. CD (c = 0.00026 mol/l, $CHCl_3$) $[\theta]^{25}$ (nm): +583 (250), +202 (257), +291 (264), −1830 (282), −1830 (284), +132 (300). ^{1}H-NMR data are summarized in Table I.

Compound **7** was obtained as colorless prisms. mp 95—96 °C. $[\alpha]_D^{25}$ −13.7° (c = 1.41, $CHCl_3$). EI-MS m/z: 346 (M^+), 344, 272, 271, 257, 194, 193, 152, 151. CD (c = 0.00025 mol/l, $CHCl_3$) $[\theta]^{25}$ (nm): −629 (250), +1920 (281), +1930 (283), +117 (302). ^{1}H-NMR data are summarized in Table I.

Compound 13——From Compound **10**: Compound **10** (186 mg) was methylated as described above. The crude product was purified on a silica gel column (benzene–acetone (98 : 2)) to yield **13** (203 mg). $[\alpha]_D^{25}$ −29.6° (c = 4.36, $CHCl_3$). EI-MS m/z: 386 (M^+), 311, 234, 177, 151, 121. ^{1}H-NMR data are summarized in Table II.

From Compound **11**: Compound **11** (6 mg), on methylation in a similar manner, gave **13** (5 mg). The physicochemical properties of this compound coincided with those of **13** derived from **10**.

From Compound **6**: A solution of **6** (12 mg) in dry acetone (10 ml) was stirred at room temperature for 10 h in the presence of Amberlyst 15 (0.2 ml). The reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was purified by prep. TLC (benzene–acetone (30 : 1)) to yield pure **13** (13 mg). The physical and spectral properties coincided with those of **13** derived from **10**.

From Compound **7**: Compound **7** (16 mg) was treated as described above. After 24 h, about 50% of unchanged **7** was detected on TLC. The product was separated to afford **13** (8 mg) and **7** (7 mg). The physicochemical properties of **13** coincided with those of **13** derived from **10**, **11** and **6**.

Compound **13** gave a mixture of **6**, **7** and **16** (small amount) upon heating at 60 °C for 5 h in 80% AcOH.

The HIO_4 Oxidation of Compound 6——A solution of HIO_4 in H_2O (10 mg, 1 ml) was added to a solution of **6** (11 mg) in methanol (9 ml) with stirring. After 20 min, H_2O (50 ml) was added, and the mixture was extracted with EtOAc (50 ml × 3). The EtOAc extract was washed twice with H_2O (60 ml) and dried over Na_2SO_4. After evaporation of the solvents, the residue was purified by prep. TLC (hexane–acetone (1 : 1)) to afford **9** (9 mg), EI-MS m/z: 344 (M^+), 326, 193, 178, 175, 165, 164, 162, 152, 151, 107. ^{1}H-NMR ($CDCl_3$) δ: 3.79 (2H, s, H-9), 3.83 and 3.84 (3H and 6H, each s, OMe × 3), 4.68 (2H, s, H-2), 6.14 (1H, d, J = 2.2 Hz, H-8), 6.58 (1H, dd, J = 8.8, 2.2 Hz, H-6), 6.68—6.88 (3H, m, H-2′, 5′ and 6′), 7.82 (1H, d, J = 8.8 Hz, H-5), 10.38 (1H, s, H-4).

The HIO_4 Oxidation of Compound 7——Compound **7** (10 mg) was treated with HIO_4 (10 mg) as described above. About 50% of starting material remained even after stirring for 2 h. The residue, obtained after EtOAc extraction, was purified by prep. TLC (hexane–acetone (1 : 1)) to give **9** (4 mg), whose physicochemical properties coincided with those of **9** derived from **6**, and recovered **7** (3 mg).

Compound 8——Compound **3** (10 mg) was methylated as described for **6** and **7**. The product was purified by prep. TLC (hexane–acetone (3 : 2)) to afford **8** (9 mg) as colorless needles, mp 154—156 °C. $[\alpha]_D^{25}$ +31.0° (c = 0.29, $CHCl_3$). EI-MS m/z: 316 (M^+), 177, 164, 153, 122, 121. CD (c = 0.00027 mol/l, $CHCl_3$) $[\theta]^{25}$ (nm): +677 (250), −1000 (275), −818 (280), +104 (295). ^{1}H-NMR ($CDCl_3$) δ: 2.78 (2H, s, H-9), 3.74 (1H, dd, J = 10.5, 1.3 Hz, H-2), 3.77 (3H, s, OMe), 3.79 (3H, s, OMe), 3.93 (1H, d, J = 10.5 Hz, H-2), 4.35 (1H, br s, H-4), 6.41 (1H, d, J = 2.2 Hz, H-8), 6.56 (1H, dd, J = 8.2, 2.2 Hz, H-6), 6.84 (2H, d, J = 8.8 Hz, H-3′ and 5′), 7.15 (2H, d, J = 8.8 Hz, H-2′ and 6′). 7.24 (1H, d, J = 8.2 Hz, H-5).

Brazilin (14)——From the Mixture of Sappanol (**1**) and Episappanol (**2**): A solution of the mixture of **1** and **2** in methanol (26 mg, 10 ml) and conc. HCl (0.1 ml) was refluxed for 1 h. The mixture was evaporated to dryness and the residue was purified by prep. TLC ($CHCl_3$–MeOH (9 : 1)) to afford **14** (18 mg), $[\alpha]_D^{25}$ +125.6° (c = 0.61, MeOH).

From Compound **10**: Compound **10** (42 mg) was treated in a similar manner to yield dextrorotatory brazilin (**14**) (21 mg).

The physicochemical properties of **14** coincided with those of the natural product. ^{1}H-NMR data are summarized in Table III.

3′-*O*-Methylbrazilin (15)——$[\alpha]_D^{25}$ +113.2° (c = 0.21, MeOH). UV λ_{max}^{MeOH} nm (log ε): 288 (3.82), 282 (sh). EI-MS

m/z: 300.0994 (M^+, Calcd for $C_{17}H_{16}O_5$: 300.0995), 283, 282, 281, 267, 243. ^{1}H-NMR data are summarized in Table III.

From Compound **11**: Compound **11** (12 mg) was treated as described for **14** to yield **15** (7 mg). The physical and spectral properties coincided with those of **15**.

Compound 16——From Brazilin (**14**): Brazilin (**14**) (270 mg) was methylated as described for **6** and **7**, to afford **16** (246 mg) as colorless needles from benzene, mp 136—137 °C. $[\alpha]_D^{25}$ +127.4° (c=0.51, $CHCl_3$). EI-MS *m/z*: 328 (M^+), 310, 309, 297, 279, 271, 204, 155, 151, 121. ^{1}H-NMR data are summarized in Table III.

From 3′-*O*-Methylbrazilin (**15**): Compound **15** (5 mg) on similar methylation afforded **16** (4 mg). The physical and spectral properties coincided with those of **16** derived from **14**.

From Compound **6**: A mixture of **6** (18 mg), methanol (9 ml) and conc. HCl (1 ml) was refluxed for 2 h. The solution was evaporated to dryness and the residue was purified by prep. TLC to yield **16** (12 mg).

From Compound **7**: Compound **7** (22 mg) was treated in a similar manner to give **16** (15 mg).

From Compound **13**: Similar treatment of **13** (10 mg) afforded **16** (6 mg).

Determination of the Absolute Configurations by Horeau's Partial Resolution Method[14]——Compound **6**: Racemic 2-phenylbutanoic anhydride (22.6 mg) was added to a solution of **6** (12.6 mg) in pyridine (1 ml). The mixture was allowed to stand at room temperature for 20 h, then 0.5 ml of water was added and the mixture was stirred for 1 h. After addition of further water (3 ml), the solution was extracted with benzene (3 ml × 3), and the benzene extract was extracted with 5% Na_2CO_3 aq. (3 ml × 3). The free acid (10.4 mg) was obtained after acidification of the alkaline extract and extraction with benzene (4 ml × 3). The residual acid had a specific rotation of −12.0° (c=0.42, benzene).

Compound **7**: Compound **7** (14.7 mg) was similarly reacted with racemic 2-phenylbutanoic anhydride (26.3 mg) in pyridine (1 ml), and worked up in a similar manner to afford the free acid (16.1 mg), which had a specific rotation of +6.2° (c=0.64, benzene).

Compounds 20, 22 and 35——A mixture of 7-hydroxychroman-4-one[16] (**17**) (10 mmol) and the aldehyde (11 mmol) in dry EtOH (25 ml) was saturated with dry HCl gas under cooling and then stirred at room temperature. After 20 h, the mixture was poured into water (100 ml), and 1 N NaOH aq. (20 ml) was added. The mixture was stirred at room temperature for 5 h, and the precipitate was filtered off, washed sufficiently with H_2O and dried over P_2O_5.

Compound **20** (2.86 g) was obtained by the reaction of **17** (1.64 g) and vanillin (**18**) (1.67 g). EI-MS *m/z*: 298 (M^+), 297, 162, 147, 137, 119, 108, 102, 91, 89, 80, 77, 69, 65, 63. ^{1}H-NMR (CD_3OD) δ: 3.89 (3H, s, 3′-OMe), 5.36 (2H, d, J=1.8 Hz, H-2), 6.29 (1H, d, J=2.2 Hz, H-8), 6.51 (1H, dd, J=8.5, 2.2 Hz, H-6), 6.80—7.00 (3H, m, H-2′, 5′ and 6′), 7.70 (1H, t, J=1.8 Hz, H-9), 7.80 (1H, d, J=8.5 Hz, H-5).

Compound **22** (2.88 g) was obtained by the reaction of **17** (1.64 g) and isovanillin (**19**) (1.67 g). EI-MS *m/z*: 298 (M^+), 297, 273, 181, 167, 161, 151, 147, 137, 136, 119, 108, 91. ^{1}H-NMR (CD_3OD) δ: 3.92 (3H, s, 4′-OMe), 5.36 (2H, d, J=1.8 Hz, H-2), 6.32 (1H, d, J=2.2 Hz, H-8), 6.53 (1H, dd, J=8.5, 2.2 Hz, H-6), 6.85 (1H, d, J=2.0 Hz, H-2′), 6.87 (1H, dd, J=8.8, 2.0 Hz, H-6′), 7.02 (1H, d, J=8.8 Hz, H-5′), 7.68 (1H, t, J=1.8 Hz, H-9), 7.81 (1H, d, J=8.5 Hz, H-5).

Compound **35** (2.70 g) was obtained by the reaction of **17** (1.64 g) and 3,4-dihydroxybenzaldehyde (**34**) (1.52 g). EI-MS *m/z*: 284 (M^+), 272, 268, 255, 237, 175. ^{1}H-NMR (CD_3OD) δ: 5.39 (2H, d, J=1.8 Hz, H-2), 6.34 (1H, d, J=2.2 Hz, H-8), 6.55 (1H, dd, J=8.9, 2.2 Hz, H-6), 6.60—7.00 (3H, m, H-2′, 5′ and 6′), 7.68 (1H, br s, H-9), 7.83 (1H, d, J=8.9 Hz, H-5). These data are identical with those of the natural product.[5]

These products each showed a single spot on TLC ($CHCl_3$–MeOH (9 : 1), benzene–acetone (3 : 2), hexane–acetone (1 : 1)), and the following benzylation was performed without separation.

Compounds 21, 23 and 36——A mixture of compound **20** (602 mg), benzyl chloride (280 mg) and anhydrous K_2CO_3 (3 g) in dry dimethylformamide (DMF) (30 ml) was heated at 100 °C for 2 h with stirring. The mixture was cooled and poured into H_2O, and the precipitate was filtered off, washed with H_2O and digested with methanol to afford **21** (867 mg). EI-MS *m/z*: 478 (M^+), 388, 387, 92, 91, 65. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (3H, s, 3′-OMe), 5.09 and 5.20 (each 2H, s, OCH_2Ph × 2), 5.34 (2H, d, J=1.8 Hz, H-2), 6.47 (1H, d, J=2.2 Hz, H-8), 6.69 (1H, dd, J=8.5, 2.2 Hz, H-6), 6.78—7.00 (3H, m, H-2′, 5′ and 6′), 7.28—7.50 (10H, m, OCH_2Ph × 2), 7.77 (1H, t, J=1.8 Hz, H-9), 7.96 (1H, d, J=8.5 Hz, H-5).

Compound **23** (613 mg) was obtained from **22** (422 mg) by similar treatment. EI-MS *m/z*: 478 (M^+), 388, 387, 92, 91, 65. ^{1}H-NMR ($CDCl_3$) δ: 3.94 (3H, s, 4′-OMe), 5.10 (2H, s, OCH_2Ph), 5.17 (2H, d, J=1.8 Hz, H-2), 5.18 (2H, s, OCH_2Ph), 6.46 (1H, d, J=2.2 Hz, H-8), 6.69 (1H, dd, J=8.5, 2.2 Hz, H-6), 6.76—7.02 (3H, m, H-2′, 5′ and 6′), 7.28—7.74 (10H, m, OCH_2Ph × 2), 7.71 (1H, t, J=1.8 Hz, H-9), 7.95 (1H, d, J=8.5 Hz, H-5).

Compound **36** (1.62 g) was obtained from **35** (850 mg) by similar benzylation. EI-MS *m/z*: 554 (M^+), 464, 463, 91. ^{1}H-NMR ($CDCl_3$) δ: 5.10 and 5.20 (2H and 4H, each s, OCH_2Ph × 3), 5.22 (2h, s, H-2), 6.47 (1H, d, J=2.2 Hz, H-8), 6.70 (1H, dd, J=8.8, 2.2 Hz, H-6), 6.78—6.92 (2H, m, H-2′ and 6′), 6.99 (1H, d, J=9.0 Hz, H-5′), 7.26—7.58 (15H, m, OCH_2Ph × 3), 7.72 (1H, br s, H-9), 7.97 (1H, d, J=8.8 Hz, H-5).

These products were used for the following epoxidation without purification.

Compounds (±)-24 and (±)-25——Hydrogen peroxide (1 ml) and 2 N NaOH aq. (1.2 ml) were added to a solution of **21** (520 mg) in acetone (40 ml) and methanol (10 ml). The mixture was stirred at room temperature for 24 h, then 25 ml of water was added and the organic solvents were evaporated off. The precipitate was filtered off and washed

with H_2O and methanol successively to yield (±)-**24** (516 mg). EI-MS *m/z*: 494 (M^+), 404, 403, 227, 92, 91. ^{1}H-NMR ($CDCl_3$) δ: 3.88 (3H, s, 3′-OMe), 4.14 (1H, d, J=12.5 Hz, H-2), 4.52 (1H, d, J=12.5 Hz, H-2), 4.52 (1H, s, H-9), 5.09 and 5.16 (each 2H, s, $OCH_2Ph \times 2$), 6.45 (1H, d, J=2.2 Hz, H-8), 6.72 (1H, dd, J=8.8, 2.2 Hz, H-6), 6.80—7.00 (3H, m, H-2′,5′ and 6′), 7.28—7.52 (10H, m, $OCH_2Ph \times 2$), 7.91 (1H, d, J=8.8 Hz, H-5).

Compound (±)-**25** (438 mg) was obtained from **23** (450 mg) on similar epoxidation. EI-MS *m/z*: 494 (M^+), 404, 403, 387, 227 177, 92, 91, 65. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (3H, s, 4′-OMe), 3.94 (1H, d, J=12.5 Hz, H-2), 4.38 (1H, d, J=12.5 Hz, H-2), 4.46 (1H, s, H-9), 5.08 and 5.16 (each 2H, 2s, $OCH_2Ph \times 2$), 6.43 (1H, d, J=2.2 Hz, H-8), 6.70 (1H. dd, J=8.8, 2.2 Hz, H-6), 6.78—6.96 (3H, m, H-2′, 5′ and 6′), 7.26—7.74 (10H, m, $OCH_2Ph \times 2$), 7.90 (1H, d, J= 8.8 Hz, H-5).

These compounds each showed a single spot on TLC (benzene–acetone (50 : 1), $CHCl_3$, hexane–acetone (9 : 1)).

Compound (±)-37——Compound **36** (1.22 g) in dioxane (120 ml) was treated with alkaline hydrogen peroxide as described for (±)-**24** to afford (±)-**37** (1.08 g). EI-MS *m/z*: 570 (M^+), 479, 318, 268, 254, 228, 181, 92, 91. ^{1}H-NMR ($CDCl_3$) δ: 3.95 (1H, d, J=12.5 Hz, H-2), 4.37 (1H, d, J=12.5 Hz, H-2), 4.44 (1H, s, H-9), 5.07 and 5.15 (2H and 4H, each s, $OCH_2Ph \times 3$), 6.42 (1H, d, J=2.2 Hz, H-8), 6.68 (1H, dd, J=8.8, 2.2 Hz, H-6), 6.84—7.00 (3H, m, H-2′, 5′ and 6′), 7.20—7.56 (15H, m, $OCH_2Ph \times 3$), 7.78 (1H, d, J=8.8 Hz, H-5).

The product showed a single spot on TLC, and was used for the following reduction without separation.

Compounds (±)-26 and (±)-27——A solution of (±)-**24** in THF (496 mg, 15 ml) was added dropwise to a solution of $LiAlH_4$ (36 mg) in THF (10 ml) with stirring at 0 °C. The mixture was stirred at room temperature for 2 h, then 100 ml of 1 N HCl aq. was added and the products were extracted with CH_2Cl_2 (100 ml × 3). The organic extract was washed twice with H_2O (each 100 ml), dried over Na_2SO_4 and evaporated to afford a mixture of (±)-**26** and (±)-**27**. The ratio of (±)-**26** and (±)-**27** was about 9 : 1,[19] as estimated from the ^{1}H-NMR spectrum and TLC behavior (benzene–acetone (9 : 1), benzene–EtOAc (7 : 1)).

The mixture of (±)-**26** and (±)-**27** was chromatographed on silica gel with benzene–acetone (9 : 1) to yield the pure compounds (±)-**26**, EI-MS *m/z*: 498 (M^+), 480, 270, 254, 253, 229, 228, 227, 137, 92, 91, ^{1}H-NMR ($CDCl_3$) δ: 2.75 (2H, s, H-9), 3.74 (1H, br d, J=10.5 Hz, H-2), 3.84 (3H, s, 3′-OMe), 3.92 (1H, d, J=10.5 Hz, H-2), 4.33 (1H, br s, H-4), 5.02 and 5.11 (each 2H, s, $OCH_2Ph \times 2$), 6.48 (1H, d, J=2.2 Hz, H-8), 6.61 (1H, dd, J=8.5, 2.2 Hz, H-6), 6.65 (1H, dd, J=8.0, 2.0 Hz, H-6′), 6.78 (1H, d, J=2.0 Hz, H-2′), 6.82 (1H, d, J=8.0 Hz, H-5′), 7.21 (1H, d, J=8.5 Hz, H-5), 7.20—7.54 (10H, m, $OCH_2Ph \times 2$), and (±)-**27**, EI-MS *m/z*: 498 (M^+), 271, 270, 254, 253, 227, 180, 179, 137, 92, 91, ^{1}H-NMR ($CDCl_3$) δ: 2.73 (1H, d, J=14.0 Hz, H-9), 2.99 (1H, d, J=14.0 Hz, H-9), 3.82 (1H, br d, J=11.5 Hz, H-2), 3.88 (3H, s, 3′-OMe), 4.09 (1H, d, J=11.5 Hz, H-2), 4.27 (1H, br s, H-4), 5.00 and 5.12 (each 2H, s, $OCH_2Ph \times 2$), 6.48 (1H, d, J=2.4 Hz, H-8), 6.58 (1H, dd, J=8.5, 2.4 Hz, H-6), 6.76—7.00 (3H, m, H-2′, 5′ and 6′), 7.14 (1H, d, J= 8.5 Hz, H-5), 7.20—7.54 (10H, m, $OCH_2Ph \times 2$).

Compound (±)-30——The mixture of (±)-**26** and (±)-**27** (168 mg) in dry acetone (30 ml) was stirred for 24 h in the presence of Amberlyst 15 (0.5 ml). The reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (eluted with benzene–EtOAc (99 : 1)) to afford (±)-**30** (146 mg). EI-MS *m/z*: 538 (M^+), 301, 300, 254, 253, 228, 227, 137, 92, 91. ^{1}H-NMR ($CDCl_3$) δ: 1.22 and 1.39 (each 3H, 2s, $C(CH_3)_2$), 2.75 (1H, d, J=14.5 Hz, H-9), 2.92 (1H, d, J=14.5 Hz, H-9), 3.69 (1H, d, J=10.5 Hz, H-2), 3.82 (3H, s, 3′-OMe), 3.85 (1H, br d, J=10.5 Hz, H-2), 4.54 (1H, br s, H-4), 5.04 and 5.11 (each 2H, s, $OCH_2Ph \times 2$), 6.53 (1H, d, J= 2.2 Hz, H-8), 6.62 (1H, dd, J=8.0, 2.0 Hz, H-6′), 6.64 (1H, dd, J=8.2, 2.2 Hz, H-6), 6.76 (1H, d, J=2.0 Hz, H-2′), 6.79 (1H, d, J=8.0 Hz, H-5′), 7.21 (1H, d, J=8.2 Hz, H-5), 7.20—7.54 (10H, m, $OCH_2Ph \times 2$). A small amount of unchanged (±)-**27** was also obtained after the elution of (±)-**30** (eluted with a 9 : 1 mixture of the same solvents).

Compound (±)-11——Compound (±)-**30** (62 mg) was hydrogenated in acetone–methanol (4 : 1, 24 ml) under a hydrogen atmosphere in the presence of 5% Pd–C catalyst (50 mg). After 2 h, the mixture was filtered, and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (eluted with benzene–acetone (9 : 1)) to give (±)-**11** (32 mg). The spectral properties of (±)-**11** coincided with those of (−)-**11**.

Compound (±)-32——Compound (±)-**25** (402 mg) was treated with $LiAlH_4$ (32 mg) in THF as described for (±)-**26** and (±)-**27** to give a mixture of (±)-**28** and (±)-**29** (294 mg), which was transformed into its isopropylidene derivative (±)-**31** as described for (±)-**30** without separation. Compound (±)-**31** (85 mg) was hydrogenated as described for (±)-**11**, yielding (±)-**32** (48 mg). EI-MS *m/z*: 358 (M^+), 283, 220, 181, 176, 164, 163, 147, 138, 137, 135, 122, 107, 94. ^{1}H-NMR data are summarized in Table II.

Compounds (±)-38 and (±)-39——Compound (±)-**37** (980 mg) was treated with $LiAlH_4$ (75 mg) as described for (±)-**26** and (±)-**27** to give a mixture of (±)-**38** and (±)-**39** (768 mg), which was chromatographed on silica gel (eluted with benzene–EtOAc (92 : 8)) to afford (±)-**38**, EI-MS *m/z*: 556 ($M^+ - H_2O$), 304, 303, 254, 211, 182, 181, 151, 123, 92, 91 ^{1}H-NMR ($CDCl_3$) δ: 2.68 (2H, s, H-9), 3.62 (1H, br d, J=10.5 Hz, H-2), 3.79 (1H, d, J=10.5 Hz, H-2), 4.24 (1H, br s, H-4), 5.00 and 5.11 (2H and 4H, each s, $OCH_2Ph \times 3$), 6.46 (1H, d, J=2.2 Hz, H-8), 6.59 (1H, dd, J= 8.5, 2.2 Hz, H-6), 6.67 (1H, dd, J=8.0, 2.0 Hz, H-6′), 6.80 (1H, d, J=2.0 Hz, H-2′), 6.85 (1H, d, J=8.0 Hz, H-5′), 7.15 (1H, d, J=8.5 Hz, H-5), 7.20—7.54 (15H, m, $OCH_2Ph \times 3$), and (±)-**39**, EI-MS *m/z*: 574 (M^+), 556, 312, 303, 254, 181, 152, 151, 91, ^{1}H-NMR ($CDCl_3$) δ: 2.64 (1H, d, J=14.0 Hz, H-9), 2.92 (1H, d, J=14.0 Hz, H-9), 3.74 (1H, br d, J=11.5 Hz, H-2), 4.00 (1H, d, J=11.5 Hz, H-2), 4.08 (1H, br s, H-4), 4.98, 5.12 and 5.16 (each 2H, s, $OCH_2Ph \times 3$), 6.46 (1H, d, J=2.4 Hz, H-8), 6.54 (1H, dd, J=8.5, 2.4 Hz, H-6), 6.68—7.00 (3H, m, H-2′, 5′ and 6′), 7.05

(1H, d, J=8.5 Hz, H-5), 7.20—7.54 (15H, m, $OCH_2\underline{Ph} \times 3$).

(±)-Sappanol (1)——Compound (±)-**38** (126 mg) was hydrogenated as described for (±)-**11** to give (±)-**1** (52 mg). UV λ_{max}^{MeOH} nm (log ε): 284 (3.73), 279 (3.73). EI-MS m/z: 286 ($M^+ - H_2O$), 285, 269, 268, 267, 123, 111. ^{1}H-NMR data are summarized in Table I.

(±)-Episappanol (2)——Compound (±)-**39** (86 mg) was hydrogenated as described for (±)-**11** to afford (±)-**2** (32 mg). UV λ_{max}^{MeOH} nm (log ε): 284 (3.83), 280 (3.83). EI-MS m/z: 286 ($M^+ - H_2O$), 269, 268, 267, 229, 164, 163, 123, 111, 91. ^{1}H-NMR data are summarized in Table I.

(±)-3′-*O*-Methylsappanol (4)——Compound (±)-**26** (72 mg) was similarly hydrogenated to yield (±)-**4** (36 mg). UV λ_{max}^{MeOH} nm (log ε): 284 (3.67), 279 (3.89), 224 (sh). EI-MS m/z: 318 (M^+), 300, 180, 164, 163, 138, 137, 122, 107, 91. ^{1}H-NMR data are summarized in Table I.

(±)-3′-*O*-Methylepisappanol (5)——Hydrogenation of (±)-**27** (58 mg) gave (±)-**5** (28 mg). UV λ_{max}^{MeOH} nm (log ε): 285 (3.80), 279 (3.84), 224 (4.23). EI-MS m/z: 318 (M^+), 300, 180, 164, 163, 147, 139, 138, 137, 128, 127, 107. ^{1}H-NMR data are summarized in Table I.

Compound (±)-6——A mixture of (±)-**1** (18 mg), dimethyl sulfate (29 mg) and anhydrous K_2CO_3 (0.4 g) in dry acetone (15 ml) was refluxed for 3 h. The reaction mixture was filtered, and the filtrate was evaporated. The residue was purified by prep. TLC (hexane–acetone (3:2)) to afford (±)-**6** (12 mg).

Compound (±)-**4** (10 mg) gave (±)-**6** (6 mg) on similar methylation. The spectral properties of (±)-**6** coincided with those of (+)-**6**.

Compound (±)-7——Compound (±)-**2** (16 mg) and (±)-**5** (13 mg) were each methylated as described for (±)-**6** to give (±)-**7** (9 mg and 5 mg, respectively). The spectral properties of (±)-**7** coincided with those of (−)-**7**.

Compound (±)-33 (4′-*O*-Methylbrazilin)——A mixture of (±)-**32** (86 mg), conc. HCl (0.2 ml) and methanol (20 ml) was refluxed for 1 h. The solution was evaporated to dryness, and the residue was purified on a silica gel column (eluted with benzene–acetone (7:3)) to give (±)-**33** (48 mg). EI-MS m/z: 300 (M^+), 285, 283, 282, 281, 243. ^{1}H-NMR data are summarized in Table III.

Acid Treatment of (±)-Sappanol (1) and (±)-Episappanol (2) [(±)-Brazilin (14)]——A mixture of (±)-**1** (38 mg), methanol (10 ml) and conc. HCl (0.1 ml) was refluxed for 1 h. The solution was evaporated to dryness, and the residue was purified by prep. TLC ($CHCl_3$–MeOH (9:1)) to afford 23 mg of (±)-brazilin (**14**).

(±)-Episappanol (**2**) (12 mg) was treated in a similar manner to yield (±)-**14** (6 mg). The spectral properties of (±)-**14** coincided with those of (+)-**14**.

Acid Treatment of (±)-3′-*O*-Methylsappanol (4) and (±)-3′-*O*-Methylepisappanol (5) [(±)-3′-*O*-Methylbrazilin (15)]——A solution of (±)-**4** (19 mg) in methanol (10 ml) and conc. HCl (0.1 ml) was refluxed for 1 h, then the solvent was evaporated off. The residue was purified by prep. TLC (benzene–acetone (7:3)) to gave 10 mg of (±)-3′-*O*-methylbrazilin (**15**).

Similarly, (±)-**5** (11 mg) was treated with conc. HCl/methanol to give (±)-**15** (6 mg). The spectral properties of (±)-**15** coincided with those of (+)-**15**.

Acid Treatment of 3′-Deoxysappanol (3) and Compound 12——Compound **12** (36 mg) in 60% AcOH was stirred at 50 °C for 5 h. The solution consisted of an about 4:1 mixture of two compounds (**3** and its epimer at the C-4 position). When the mixture was heated at 100 °C for 3 h, the solution contained an about 1:1 mixture of **3** and its epimer. Similar treatment of **3** resulted in an identical mixture of these compounds.

Compound **3** and its epimer were separable on TLC (benzene–acetone (7:3), hexane–acetone (1:1)). The Rf values of these compounds obtained with the different solvent mixtures were reversed. The compound eluted from the lower spot on prep. TLC (benzene–acetone (7:3)) was identified as **3** and the compound eluted from the upper spot proved to be the C-4 epimer of **3**, having the following physical and chemical properties: $[\alpha]_D^{25}$ −77.5° (c=0.42, MeOH). EI-MS m/z: 288 (M^+), 270, 165, 164, 163, 151, 108, 107, 77. ^{1}H-NMR (CD_3OD) δ: 2.71 (1H, d, J=14.4 Hz, H-9), 2.93 (1H, d, J=14.4 Hz, H-9), 3.74 (1H, dd, J=11.2, 1.6 Hz, H-2), 4.02 (1H, d, J=11.2 Hz, H-2), 4.20 (1H, br s, H-4), 6.24 (1H, d, J=2.5 Hz, H-8), 6.36 (1H, dd, J=8.2, 2.5 Hz, H-6), 6.72 (2H, d, J=8.8 Hz, H-3′ and 5′), 7.08 (1H, d, J=8.2 Hz, H-5), 7.17 (2H, d, J=8.8 Hz, H-2′ and 6′).

When **3** and **12** were each treated under conditions similar to those used for acid treatment of (±)-**1** (reflux in methanol with conc. HCl), the reaction solution contained mainly two components (two spots on TLC (benzene–acetone (4:1))). These compounds were found to be the 4-*O*-methyl derivative of **3** and its C-4 epimer.[21]

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