COMPONENTS OF BROUSSONETIA PAPYRIFERA (L.) VENT. 2.[1] STRUCTURES OF TWO NEW ISOPRENYLATED FLAVANS, KAZINOLS A AND B

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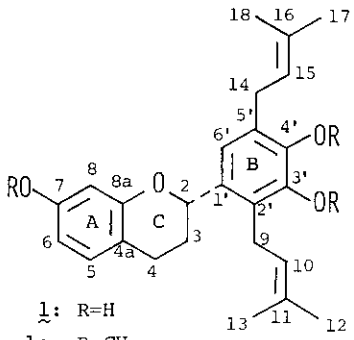
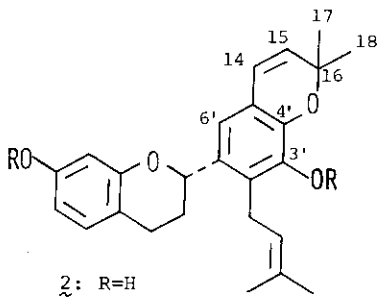
Abstract ——— Two new isoprenylated flavans, named kazinols A (1) and B (2), were isolated from the benzene extract of the cortex of Broussonetia papyrifera (L.) Vent. (Japanese name "Kazinoki", Moraceae). The structures of kazinols A and B were shown to be 1 and 2, respectively, on the basis of spectral evidence.

Previously we reported the structure determinations of two isoprenylated chalcones and two isoprenylated flavonols obtained from the cortex of Broussonetia papyrifera (L.) Vent. (Japanese name "Kazinoki", Moraceae).[2] In the course of extended studies of the benzene extract of the cortex, kazinols A (1) and B (2) were isolated. In this paper, the structure determinations of the compounds are described. The benzene extract of the cortex was dissolved in methanol.[2] The methanol extract was fractionated sequentially by column chromatography and preparative thin-layer chromatography on silica gel to give kazinols A (1) and B (2).

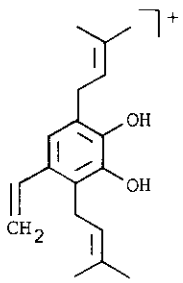
Kazinol A (1) was obtained as an oily substance, M^+=394.2138, $C_{25}H_{30}O_4$, exhibiting positive ferric chloride test and sodium molybdate test,[3] but negative Gibbs test.[4] Treatment of 1 with dimethyl sulfate and potassium carbonate in acetone gave a trimethyl ether (1a) as an oily substance. The compound (1a) gave the ms which showed the molecular ion peak at m/z 436 and exhibited a negative ferric chloride test. The ir spectrum of 1 indicated the presence of hydroxyl group [3595, 3540, 3400 (br) cm^{-1}] and aromatic ring [1620, 1595 cm^{-1}]. The uv spectrum showed absorption maxima at 226 (infl., log ε=4.66), 276 (infl. 3.77), 284 (3.83), and 289 nm (infl. 3.75), which indicated the presence of an unconjugated aromatic system.[5,6] The ^1H nmr spectrum of 1 showed the presence of two γ,γ-dimethylallyl groups as follows: δ 1.71, 1.75 (each 3H, s), 1.76 (6H, s), 3.34 (2H, d, J=7), 3.42 (2H, d, J=6.5),



1: R=H
1a: R=CH_3



2: R=H
2a: R=CH_3
2b: R=$COCH_3$



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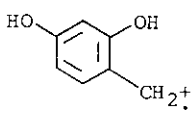

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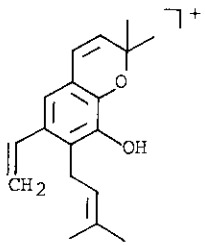

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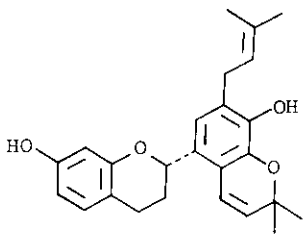

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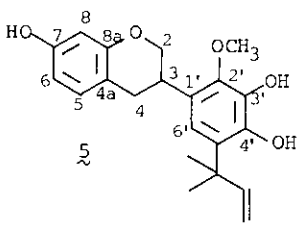

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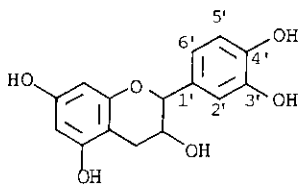

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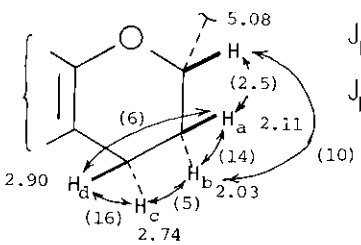
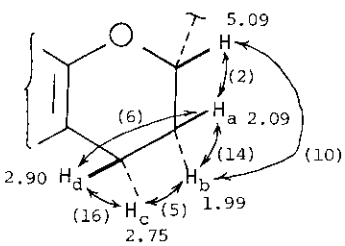
Fig. 1



$J_{H_aH_c}$=2 Hz
$J_{H_bH_d}$=12 Hz

1



$J_{H_aH_c}$=2 Hz
$J_{H_bH_d}$=12 Hz

2

Fig. 2 ( ): J in Hz

5.17 (1H, br t, J=6.5), 5.32 (1H, br t, J=7), and also showed signals of ABC type aromatic protons and a singlet proton as follows: δ 6.36 (1H, d, J=2.5), 6.37 (1H, dd, J=2.5 and 8), 6.93 (1H, d, J=8); δ 6.81 (1H, s). These results suggest that kazinol A (1) possesses a pentasubstituted benzene ring and a 1,2,4-trisubstituted benzene ring. The remaining five aliphatic proton signals were analysed with the aid of of sequential decoupling experiment and the deduced partial structure is shown in Fig. 2 along with the chemical shift values and the coupling constants of the protons of the moiety. The ms of 1 showed the peaks at m/z 272 (3) and 123 (4, base peak).[5,7] From the above spectral data and the result of sodium molybdate test, it is suggested that kazinol A is a flavan derivative having two γ,γ-dimethylallyl groups and an ortho-dihydroxyl moiety both on the B ring, and a hydroxyl group on the A ring.

In order to corroborate the structure of 1, the ^{13}C nmr spectrum of 1 was measured, and the carbon atoms were assigned by the off-resonance decoupling technique as well as by comparison of the ^{13}C nmr spectrum of 1 with those of model compounds[8] (5 and 6) (Table 1). In the ^{13}C nmr spectrum of 1, the chemical shift values of the carbon atoms of the A and C rings were similar to those of the relevant carbon atoms of 5 except the chemical shift values of the carbon atoms at the C-2 and C-3 positions which were affected by substituent effects. The chemical shift values of the oxygenated carbon atoms of the B ring of 1 were similar to those of the carbon atoms at the C-3' and C-4' positions of 6[8] and other 3',4'-dioxygenated flavonoids[9] (Table 1). The substitution pattern in the B ring was further supported by the following results. Dahmi et al. reported that the signal of the diortho-substituted methoxyl carbon nucleus appears at δ ~60 ppm, while that of the mono-ortho-substituted methoxyl nucleus at δ ~55 ppm.[10] In the case of 1a the signals of the methoxyl carbons appeared at δ 55.0, 59.9, and 60.1 ppm suggesting that two of the methoxyl groups are diortho-substituted methoxyl groups. On the basis of the CD spectrum, 1 was shown to have the (S)-configuration at C-2 position.[11] From these results, kazinol A is represented by the formula (1).

Kazinol B (2) was obtained as an oily substance, M^+=392.1977, $C_{25}H_{28}O_4$, $[\alpha]_D^{24}$ -20°, exhibiting positive ferric chloride test and Gibbs test. The ir spectrum of 2 indicated the presence of hydroxyl group [3615, 3560, 3340 (br) cm^{-1}] and aromatic ring [1625, 1590 cm^{-1}]. Treatment of 2 with dimethyl sulfate and potassium carbonate in acetone gave a dimethyl ether (2a) as colorless prisms, mp 89-92 °C. The compound (2a) gave the ms spectrum which showed the molecular ion peak at m/z

Table 1 ^{13}C nmr spectra of 1, 1a, 2, 2a, 5 and 6 (ppm)

	1	1a	2	2a	5[8]	6[8]
C-2	75.6	74.5	74.8	74.8	69.6	81.2
C-3	25.0	24.8	24.8	25.2	32.5	66.6
C-4	29.4	29.5	29.9	29.9	30.1	28.1
C-4a	113.5	113.4	113.9	114.0	114.1	99.4
C-5	129.5	129.3	130.0	130.0	130.1	156.3*
C-6	107.6	106.9	108.1	107.4	108.1	95.5
C-7	154.3	158.4	155.1	159.1	154.9	156.6*
C-8	103.2	101.3	103.7	101.7	103.0	94.3
C-8a	155.5	155.6	156.1	156.4	154.5	155.5
C-1'	130.7	134.8	131.6	132.0	122.0	130.8
C-2'	124.6	130.7	125.8	131.2	145.8	114.7
C-3'	141.7	150.4	142.2	146.0	137.1	145.0
C-4'	141.3	150.1	138.6	145.1	142.2	145.0
C-5'	123.0	130.5	119.0	120.8	132.4	115.4
C-6'	118.5	123.2	122.3	122.4	116.3	118.8
C-9	25.1	25.1	25.3	25.4		
C-10	122.0	122.5	122.9	123.5		
C-11	132.3	131.5	132.3	133.3		
C-12	25.6	25.6	25.7	25.7		
C-13	17.8	17.9	17.9	18.0		
C-14	29.3	28.6	115.1	119.2		
C-15	121.5	121.9	130.1	130.1		
C-16	133.6	133.3	77.4	77.5		
C-17	25.5	25.5	28.0	28.0		
C-18	17.8	17.7	28.2	28.3		
OCH_3		55.0 59.9 60.1		55.3 60.6	60.4	

solvent: $CDCl_3$ *: Assignments may be reversed.

Table 2 Acetylation shifts of aromatic protons of 2

	C-6'-H	C-5-H	C-6-H	C-8-H
2	6.75	6.49	6.39	6.39
2a	7.07	7.12	6.64	6.65
Δ	-0.32	-0.18	-0.25	-0.26

measured in $CDCl_3$ (ppm)

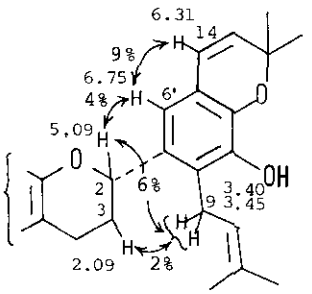


Fig. 3 The NOE experiments of 2
%: An increase in the signal area.
(measured in $CDCl_3$)

420, and exhibited negative ferric chloride test. Acetylation of 2 with acetic anhydride in pyridine gave a diacetate (2b) as colorless needles, mp 140-143 °C, the ms spectrum of which showed the molecular ion peak at m/z 476. The uv spectrum of 2 showed the absorption maxima at 233 (log ε =4.49), 268 (infl. 3.86), 276 (4.00), 285 (4.00), and 312 nm (sh 3.18), which was similar to that of 1. The ms spectrum of 2 showed the peaks at m/z 392 (M^+), 377 (M^+-CH_3), 336 ($M^+-C_4H_8$), 321 (336-CH_3), 270 (7),[5] 255 (270-CH_3), 199 (255-C_4H_8). From these results, it is suggested that kazinol B is a flavan derivative having a hydroxyl group in the A ring, and a hydroxyl group as well as a 2,2-dimethylpyran ring system in the B ring. The ^1H nmr spectrum of 2 indicated the presence of a 3,3-dimethylallyl group and a 2,2-dimethylpyran ring system as follows: δ 1.68, 1.73 (each 3H, s), 3.40 (1H, dd, J=7.5 and 15), 3.45 (1H, dd, J=7 and 15), 5.15 (1H, dd, J=7 and 7.5); δ 1.44, 1.47 (each 3H, s), 5.57 (1H, d, J=10), 6.31 (1H, d, J=10), and also indicated signals of four aromatic protons as follows: δ 6.39 (1H, d, J=2.5), 6.39 (1H, dd, J=2.5 and 9), 6.94 (1H, d, J=9); δ 6.75 (1H, s). The remaining five aliphatic proton signals were assigned by decoupling experiment as described in Fig. 2. In the ^{13}C nmr spectrum of 2, the chemical shift values of the carbon atoms of the A and C rings were similar to those of the relevant carbon atoms of 1, while the chemical shift values of the oxygenated carbon atoms of the B ring were similar to those of the relevant carbon atoms of the 3',4'-dioxygenated flavonoids.[8,9] In the ^{13}C nmr spectrum of 2a, the signals of the methoxyl carbons appear at δ 55.3 and 60.6 ppm, suggesting that one of the methoxyl groups is di*ortho*-substituted.

The CD spectrum of 2 supports the (*S*)-configuration at the C-2 position.[11] From above results, two possible structures (2 and 2') were proposed.

The result of Gibbs test being taken into consideration, the structure (2) seems to be more favorable than the structure (2'). This result was further supported by observation of the acetylation shift of the aromatic proton signals of 2b.

The NOE measurement of 2 was carried out as follows: when the aromatic proton signal at δ 6.75 (C-6'-H) was irradiated, the NOE was observed in the proton at δ 6.31 (C-14-H, 9%) and the proton at δ 5.09 (C-2-H, 4%). The NOE between the methylene protons at δ 3.40 and 3.45 (C-9-H), and the protons at δ 5.09 (C-2-H, 6%) and δ 2.09 (C-3-H, 2%) was observed by the irradiation of the methylene proton signals at C-9 position. These results are shown in Fig. 3. Comparison of the ^1H nmr spectra of 2 and 2b indicates that the acetylation of the phenolic hydroxyl group on the B ring of 2 caused a remarkable downfield shift (-0.32 ppm) of the

proton at C-6' position. On the other hand, acetylation of the hydroxyl group at C-7 position caused smaller downfield shift (-0.18 ppm) of the proton at C-5 position (Table 2). These results suggest that the hydroxyl group in the B ring is not located at the C-4' position but at the C-3' position. From these results, we propose the formula (2) for the structure of kazinol B.

EXPERIMENTAL

All melting points were uncorrected. Abbreviations: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad, sh=shoulder, infl.=inflection. The general experimental procedures used are described in the previous paper.[2] The following instruments were used: ^{1}H nmr spectra; JEOL GX-400 and Varian XL-200 FT NMR spectrometers: ^{13}C nmr spectra; JEOL GX-270 and GX-400 FT NMR spectrometers: optical rotation; JASCO DIP-4; CD spectra; JASCO J-20 ORD spectrometer.

Isolation of Kazinols A (1) and B (2)

The benzene extract, described in the previous paper,[2] of the cortex (3.50 Kg) of Broussonetia papyrifera (L.) Vent., was dissolved in methanol, and the methanol extract (28.0 g) was chromatographed on silica gel (340 g) using benzene-acetone as an eluent, each fraction being monitored by tlc. One of the fractions eluted with benzene was evaporated to give the residue (979 mg), which was fractionated by preparative tlc (chloroform : ether=4 : 1, acetone : hexane=1 : 3, benzene : ethyl acetate=3 : 1) to give kazinol A (1, 334 mg) and kazinol B (2, 7 mg).

Kazinol A (1)

The compound (1) was obtained as an oily substance, $[\alpha]_D^{17}$ -11° (c=0.13 in chloroform), $FeCl_3$ test: brown, sodium molybdate test: orange, Gibbs test: brown (negative). High-resolution ms: Calcd. for $C_{25}H_{30}O_4$ (M^+, m/z): 394.2143. Found: 394.2138. uv λ_{max}^{EtOH} nm (log ε): 226 (infl. 4.66), 276 (infl. 3.77), 284 (3.83), 289 (infl. 3.75). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3595, 3540, 3400 (br), 1620, 1595. ms m/z: 394 (M^+), 338 ($M^+-C_4H_8$), 272 (3), 271, 216 ($272-C_4H_8$), 215 ($271-C_4H_8$), 123 (4, base peak). ^{1}H nmr (400 MHz, $CDCl_3$): δ 1.71, 1.75 (each 3H, s, C-11 or C-16-CH_3), 1.76 (6H, s, C-11 and C-16-CH_3), 2.03 (1H, m, C-3-H), 2.11 (1H, m, C-3-H), 2.74 (1H, m, C-4-H), 2.90 (1H, m, C-4-H), 3.34 (2H, d, J=7, C-14-H x2), 3.42 (2H, d, J=6.5, C-9-H x2), 5.08 (1H, dd, J=2.5 and 10, C-2-H), 5.17 (1H, br t, J=6.5, C-10-H), 5.28 (1H, br s, OH), 5.32 (1H, br t, J=7, C-15-H), 5.55 (1H, br s, OH), 5.56 (1H, br s, OH), 6.36 (1H, d, J=2.5, C-8-H), 6.37 (1H, dd, J=2.5 and 8, C-6-H), 6.81 (1H, s, C-6'-H), 6.93 (1H, d, J=8, C-5-H). CD spectrum : $[\theta]_{292}$ -50, $[\theta]_{287}$ +50 (c=3.8 x 10^{-4}, MeOH). ^{13}C nmr spectrum is

described in Table 1.

Kazinol A Trimethyl Ether (1a)

A mixture of kazinol A (1, 60 mg), dimethyl sulfate (0.7 ml) and potassium carbonate (5 g) in acetone (30 ml) was refluxed for 2 h and filtered. The filtrate was evaporated. Water was added to the residue, which was then extracted with ether. The ether layer was treated as usual and finally evaporated. The residue was purified by prepararive tlc (benzene) to give trimethyl ether (1a, 37 mg) which showed only one spot on tlc. $FeCl_3$ test: negative. ms m/z: 436 (M^+). ^{13}C nmr spectrum is described in Table 1.

Kazinol B (2)

The compound (2) was obtained as an oily substance, $[\alpha]_D^{24}$ -20° (c=0.38 in chloroform), $FeCl_3$ test: negative, Gibbs test: blue (positive). High-resolution ms: Calcd. for $C_{25}H_{28}O_4$ (M^+, m/z): 392.1985. Found: 392.1977. uv λ_{max}^{EtOH} nm (log ε): 233 (4.48), 268 (infl. 3.86), 276 (4.00), 285 (4.00), 312 (sh 3.18). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3615, 3560, 3340 (br), 1650 (sh), 1635 (sh), 1630 (sh), 1625, 1590. ms m/z: 392 (M^+), 377 (M^+-CH_3), 336 ($M^+-C_4H_8$), 321 (336-CH_3), 270 (7), 255 (270-CH_3), 199 (255-C_4H_8), 123. ^1H nmr (400 MHz, $CDCl_3$): δ 1.44, 1.47 (each 3H, s, C-16-CH_3), 1.68, 1.73 (each 3H, s, C-11-CH_3), 1.99 (1H, m, C-3-H), 2.09 (1H, m, C-3-H), 2.75 (1H, m, C-4-H), 2.90 (1H, m, C-4-H), 3.40 (1H, dd, J=7.5 and 15, C-9-H), 3.45 (1H, dd, J=7 and 15, C-9-H), 5.09 (1H, dd, J=2 and 10, C-2-H), 5.15 (1H, dd, J=7 and 7.5, C-10-H), 5.25 (1H, br s, OH), 5.57 (1H, d, J=10, C-15-H), 5.57 (1H, br s, OH), 6.31 (1H, d, J=10, C-14-H), 6.39 (1H, dd, J=2.5 and 9, C-6-H), 6.39 (1H, d, J=2.5, C-8-H), 6.75 (1H, s, C-6'-H), 6.94 (1H,d, J=9, C-5-H). CD spectrum : $[\theta]_{289}$ -198, $[\theta]_{280}$ +33 (c=6.2 x 10^{-4}, MeOH). ^{13}C nmr spectrum is described in Table 1.

Kazinol B Dimethyl Ether (2a)

A mixture of kazinol B (2, 32 mg), dimethyl sulfate (0.5 ml) and potassium carbonate (5 g) in acetone (30 ml) was refluxed for 2 h and treated as usual. The reaction product was purified by preparative tlc (benzene) to give dimethyl ether (2a, 19 mg). The compound (2a) was obtained as colorless prisms, mp 89-92 °C, $FeCl_3$ test: negative. The ^{13}C nmr spectrum is described in Table 1.

Kazinol B Diacetate (2b)

A mixture of kazinol B (2, 5.5 mg), acetic anhydride (0.9 ml) and pyridine (0.3 ml) was kept at room temperature for 30 min, and treated as usual. The reaction product was purified by preparative tlc (acetone : hexane=1 : 3) to give colorless needles (2b, 6.1 mg). The compound (2b) was recrystallized from chloroform to give

colorless needles, mp 140-143 °C. ^{1}H nmr (200 MHz, $CDCl_3$): δ 1.40 (6H, s, C-16-CH_3 x 2), 1.69, 1.72 (each 3H, s, C-11-CH_3), 2.12 (2H, m, C-3-H x 2), 2.29, 2.32 (each 3H, s, OAc), 2.89 (2H, m, C-4-H x 2), 3.32 (2H, d, J=7.5, C-9-H x 2), 5.06 (1H, br t, J=7.5, C-10-H), 5.12 (1H, m, C-2-H), 5.33 (1H, d, J=10, C-15-H), 6.34 (1H, d, J=10, C-14-H), 6.64 (1H, dd, J=2 and 8, C-6-H), 6.65 (1H, d, J=2, C-8-H), 7.07 (1H, s, C-6'-H), 7.12 (1H, d, J=8, C-5-H).

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