[Chem. Pharm. Bull. 34(5)1987—1993(1986)]

Components of *Broussonetia papyrifera* (L.) VENT. III.[1,2] Structures of Two New Isoprenylated Flavonols, Broussoflavonols C and D

TOSHIO FUKAI, JUNKO IKUTA (née MATSUMOTO), and TARO NOMURA*

Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi-shi, Chiba 274, Japan

(Received October 3, 1985)

Two new isoprenylated flavonols, named broussoflavonols C (**1**) and D (**2**), were isolated from the extract of the root bark of *Broussonetia papyrifera* (L.) VENT. (Japanese name "Kazinoki," Moraceae). The structures of broussoflavonols C and D were shown to be **1** and **2**, respectively, on the basis of spectral evidence. The compounds (**1** and **2**) are the first reported examples of trialkenyl flavonols.

Keywords——*Broussonetia papyrifera*; Moraceae; flavonol; isoprenylated flavonol; broussoflavonol C; broussoflavonol D

Previously we reported the structure determination of two isoprenylated chalcones, two isoprenylated flavonols,[3] and two isoprenylated flavans[4] obtained from the cortex of *Broussonetia papyrifera* (L.) VENT. (Japanese name "Kazinoki," Moraceae). In the course of extended studies of the components of the plant, broussoflavonols C (**1**) and D (**2**) were isolated from the root bark. In this paper we report the structure determination of these compounds.

The dried root bark was extracted successively with *n*-hexane and benzene. The *n*-hexane extract was dissolved in methanol. The methanol extract was fractionated sequentially by column chromatography and preparative thin-layer chromatography (preparative TLC) on silica gel to give broussoflavonol D (**2**). The benzene extract was fractionated sequentially by column chromatography and preparative TLC to give broussoflavonols C (**1**) and D (**2**). These compounds (**1** and **2**) were unstable even in crystalline form.[5]

Broussoflavonol C (**1**) was obtained as pale yellow prisms, mp 173—176 °C, $M^+ = 506.2302$, $C_{30}H_{34}O_7$, exhibiting a positive ferric chloride test, magnesium–hydrochloric acid test, sodium molybdate test,[6] and zirconium oxychloride–citric acid test,[7] but a negative Gibbs test. The infrared (IR) spectrum of **1** showed the presence of hydroxyl groups [3430 (br) and 3300 (sh) cm^{-1}], aromatic rings [1595 and 1550 cm^{-1}], and a conjugated carbonyl group [1660 cm^{-1}]. The ultraviolet (UV) spectrum of **1** showed absorption maxima at 212, 261, 294 (sh), 306, and 352 nm. The UV spectrum showed a bathochromic shift in the presence of aluminum chloride as follows: 212, 269, 310, 333 (infl.), and 405 nm. Hypsochromic shifts were observed on adding hydrochloric acid to the aluminum chloride solution as follows: 269, 314, 354, and 394 nm. In the light of the results of the color reaction tests and the UV spectra, **1** was considered to be a flavonol having an *ortho*-dihydroxy moiety and a hydroxyl group at the C-5 position.[8] Treatment of **1** with dimethyl sulfate and potassium carbonate in acetone gave a tetramethyl ether (**1a**) which exhibited a positive ferric chloride test. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** indicated the presence of two 3,3-dimethylallyl groups [δ 1.45, 1.53, 1.77, and 1.83 (each 3H, br s), 3.23 and 3.26 (each 1H, dd,

$J=7$ and 14 Hz), 3.43 (2H, d, $J=7$ Hz), 4.95 (1H, br t, $J=7$ Hz), and 5.20 (1H, br t, $J=7$ Hz)] and a 1,1-dimethylallyl group [δ 1.49 and 1.53 (each 3H, s), 5.36 (1H, br d, $J=11$ Hz), 5.44 (1H, br d, $J=18$ Hz), and 6.41 (1H, dd, $J=11$ and 18 Hz)]. The spectrum also indicated the presence of two aromatic protons and a hydrogen-bonded hydroxyl group as follows: δ 6.30 (1H, s) and 6.81 (1H, s); 12.12 (1H, s), while the characteristic signal of the 3-position of the flavone skeleton was not observed.[8] The mass spectrum (MS) of **1** showed significant fragments at *m/z* 450 ($M^+ - C_4H_8$), 286 (**3**, $C_{18}H_{22}O_3$), 284 ($C_{18}H_{20}O_3$), and 221 (**4**, $C_{12}H_{13}O_4$, base peak). These results supported the presence of both an isoprenyl group and two hydroxyl groups in the A-ring, and both two isoprenyl groups and two hydroxyl groups in the B-ring.[9]

The ^{13}C-nuclear magnetic resonance (^{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.[2-4,10,11] In the ^{13}C-NMR spectrum of **1**, the chemical shift value of the carbonyl carbon atom (δ 174.9) was similar to those of relevant carbon atoms of flavonols,[10] and the chemical shift values of the oxygenated carbon atoms in the B-ring were similar to those of the corresponding carbon atoms of 3′,4′-dioxygenated flavonoids[3,4,10,11] and 3″,4″-dioxygenated-1,3-diphenylpropane derivatives[2] [*e.g.* broussoflavonol B (**5**, 6,8-bis-γ,γ-dimethylallyl-3-*O*-methylquercetin)[3] and kazinol F (**6**, 5″,6″-bis- γ,γ-dimethylallyl-2′,4′,3″,4″-tetrahydroxy-1,3-diphenylpropane),[2] Table I]. Compound **1** seems to be flavonol having hydroxyl groups at C-3′ and -4′ on the basis of the ^{13}C-NMR spectrum of **1**, the result of the sodium molybdate test, and the biogenetic analogy to the flavonoids and 1,3-diphenylpropane derivatives obtained from the plants of *Broussonetia* species.[2-4] The substitution patterns of the A- and B-rings were supported by the detailed nuclear Overhauser effect (NOE) measurements[12] (Fig. 2). When the hydrogen-bonded hydroxyl group (δ 12.12) was irradiated, NOE was observed at the C-6 proton (δ 6.30). When the C-12′-CH_3 protons (δ 1.49) were irradiated, NOE was observed at the C-15′ (δ 6.41), C-16′ (δ 5.44), and C-5′ (δ 6.81) protons. When the C-12′- and C-11-CH_3 protons (δ 1.53) were irradiated, NOE was observed at the C-15′ (δ 6.41), C-16′ (δ 5.44), C-5′, and C-10 (δ 4.95) protons. Dhami and Stothers reported that the signal of the di-*ortho*-substituted methoxyl carbon nucleus appears at δ *ca.* 60 ppm, while that of the mono-*ortho*-substituted methoxyl carbon nucleus appears at δ *ca.* 55 ppm.[13] In the case of **1a**, the signals of the methoxyl carbon atoms appeared at δ 55.7 ($OCH_3 \times 2$) and 60.6 ($OCH_3 \times 2$) ppm. This result suggests that two of the methoxyl groups are di-*ortho*-substituted and one of them is at the C-3 position.[10,14] All these results indicated that broussoflavonol C is represented by formula **1**. This structure is consistent with the observation that, in the ^{1}H-NMR spectrum, the olefinic proton signals of the 1,1-dimethylallyl groups of **1a** appear at a higher applied magnetic field than the corresponding proton signals of **1** (Δ 0.30—0.72 ppm). This is because the B-ring of **1a** is twisted out of conjugation with the pyrone ring system by the bulky groups at the C-2′ and -6′ positions.[15]

Broussoflavonol D (**2**) was obtaind as pale yellow prisms, mp 102—110 °C, $M^+ =$ 504.2127, $C_{30}H_{32}O_7$, exhibiting a positive ferric chloride test, magnesium–hydrochloric acid test, and zirconium oxychloride test,[7] but a negative sodium molybdate test[6] and Gibbs test. The IR spectrum of **2** showed the presence of hydroxyl groups [3300 (br) cm^{-1}], aromatic rings [1590 and 1555 cm^{-1}], and a conjugated carbonyl group [1660 cm^{-1}]. The UV spectrum of **2** showed absorption maxima at 206, 222 (sh), 263, 283 (infl.), 304 (sh), and 350 nm. The spectrum showed a bathochromic shift in the presence of aluminum chloride: 206, 224 (sh), 269, 310 (sh), and 405 nm. Treatment of **2** with dimethyl sulfate and potassium carbonate in acetone gave a trimethyl ether (**2a**) which exhibited a positive ferric chloride test. Acetylation of **2** with acetic anhydride in pyridine gave a triacetate (**2b**) which exhibited a positive ferric chloride test. The ^{1}H-NMR spectrum of **2** indicated the presence of a 3,3-dimethylallyl group

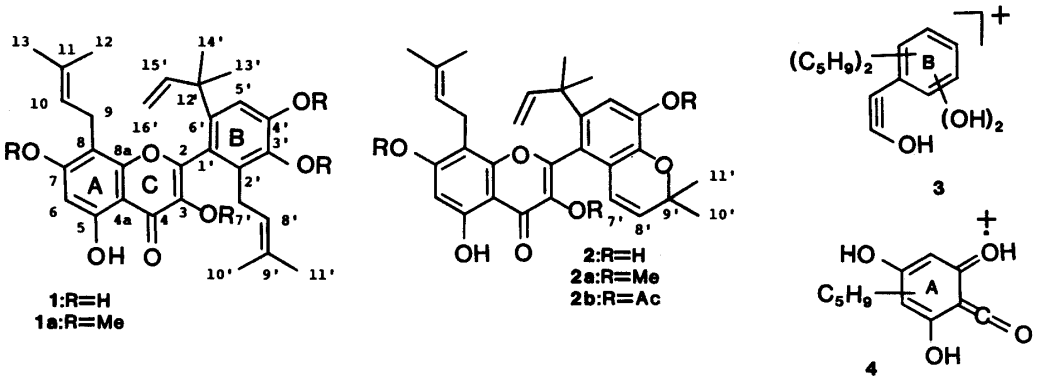

Fig. 1

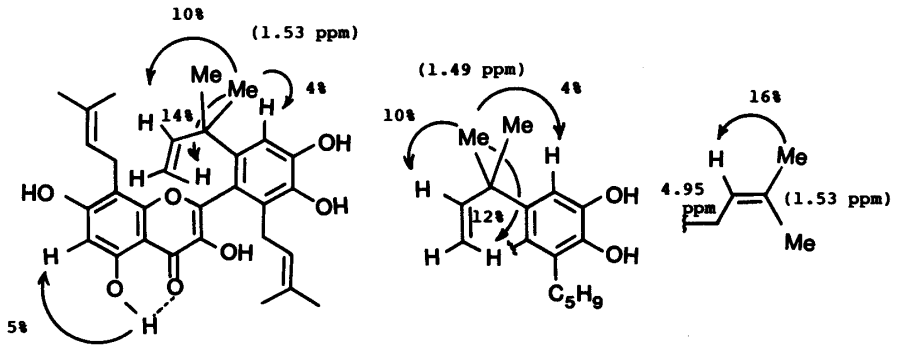

Fig. 2. NOE Values for Broussoflavonol C (1)

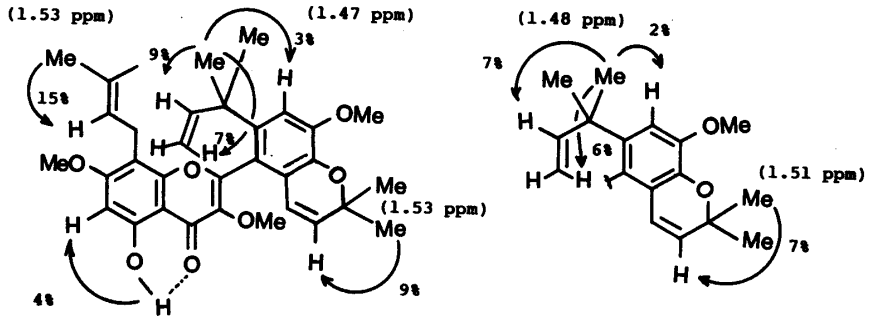

Fig. 3. NOE Values for Broussoflavonol D Trimethyl Ether (2a)

[δ 1.48 (3H, br s), 1.55 (3H, br s), *ca.* 3.3 (2H, br signal), and 4.97 (1H, br t, J=7 Hz)], a 1,1-dimethylallyl group [δ 1.46 and 1.48 (each 3H, s), 5.36 (1H, br d, J=11 Hz), 5.44 (1H, br d, J=18 Hz), and 6.42 (1H, dd, J=11 and 18 Hz)], and 2,2-dimethylpyran ring system [δ 1.529 and 1.532 (each 3H, s), 5.71 (1H, d, J=10 Hz), and 6.54 (1H, d, J=10 Hz)]. The spectrum also indicated the presence of two aromatic protons [δ 6.31 (1H, s) and 6.87 (1H, s)] and a hydrogen-bonded hydroxyl group (δ 12.11), while no proton signal from the 3-position of the flavone skeleton was observed.[8] The MS of **2** showed significant fragments at m/z 448 ($M^+ - C_4H_8$), 221 (**4**, $C_{12}H_{13}O_4$), and 165 ($C_8H_5O_4$). In the ^{13}C-NMR spectrum of **2**, the chemical shift value of the carbonyl carbon atom (δ 175.0) was similar to those of the relevant carbon atoms of flavonols.[10] These results suggested that broussoflavonol D is a flavonol having both an isoprenyl group and two hydroxyl groups in the A-ring, and an isoprenyl group, a hydroxyl group, and a 2,2-dimethylpyran ring system in the B-ring. The substitution patterns of the A- and B-rings were supported by the following spectral data. Detailed NOE

TABLE I. ^{13}C-NMR Data for Brossoflavonols C (**1**) and D (**2**), and Related Compounds (**1a**, **2a**, **5**, and **6**)

Carbon No.	1	1a	2	2a	5[a]	6[b]	
2	147.8	154.8	147.5	154.1	153.1		
3	135.6	138.9	135.7	138.4	139.0		
4	174.9	179.4	175.0	178.5	179.8		
4a	104.7	106.6	104.7	106.1	106.0		
5	158.9	161.0	158.8	160.2			
6	100.5	96.3	100.4	95.9			
7	161.1	164.3	161.1	163.5			
8	109.6	113.6	109.7	113.0			
8a	155.1	159.1	155.1	157.9	157.7		
9	29.2	29.1	28.0	28.3			
10	122.9[c]	123.2[c]	122.9	122.8			
11	132.1[d]	131.7[d]	130.1	130.9			
12	17.7[e]	17.8[e]	17.7	17.8			
13	25.4[f]	25.5[f]	25.4	25.3			
1′	121.4	122.0	121.5	120.1	123.1	(1′′)[g]	132.7
2′	126.4	125.9	119.4	121.4	116.5	(2′′)	114.6
3′	141.5	149.0	140.9	143.6	146.2	(3′′)	143.0
4′	144.0	150.5	142.3	145.8	149.4	(4′′)	142.2
5′	113.8	111.6	115.3	112.9	116.5	(5′′)	130.4
6′	130.8[d]	131.4[d]	128.7	129.3	122.0	(6′′)	127.7
7′	26.2	25.8	118.9	119.0			
8′	121.1[c]	123.5[c]	130.7	130.4			
9′	134.2[d]	133.1[d]	76.6	75.6			
10′	17.9[e]	18.0[e]	28.0	29.35			
11′	25.6[f]	25.8[f]	28.0	29.37			
12′	40.5	41.0	40.5	40.9			
13′	27.4	29.4	27.6	27.7			
14′	28.0	29.4	27.8	27.8			
15′	148.5	150.1	148.5	149.4			
16′	113.1	107.4	112.7	107.0			
3-OMe		60.6		60.3	59.6		
7-OMe		55.7		55.4[c]			
3′-OMe		60.6					
4′-OMe		55.7		56.2[c]			
Solvent	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$(CD_3)_2CO$	$(CD_3)_2CO$	

a) Data from reference 3. *b*) Data from reference 2. *c*—*f*) Assignments may be interchanged in each column. *g*) The numbers are those of the corresponding carbons of **6**.

measurement of **2a** was carried out, and the results are shown in Fig. 3.[16] When the hydrogen-bonded hydroxyl group (δ 13.10 ppm) was irradiated, NOE was observed at the C-6 proton (δ 6.41). When the C-12′-CH_3 protons (δ 1.47) were irradiated, NOE was observed at the C-15′ (δ 6.11), C-16′ (δ 4.72), and C-5′ (δ 6.76) protons. When the C-12′-CH_3 protons (δ 1.48) were irradiated, NOE was observed at the C-15′ (δ 6.11), C-16′ (δ 4.72), and C-5′ (δ 6.76) protons. NOE was observed between the C-9′-CH_3 protons (δ 1.51 ppm) and the C-8′ proton (δ 5.71) on irradiation of the signal at δ 1.51 ppm. NOE was also observed between the C-9′-CH_3 protons (δ 1.53) and C-8′-H (δ 5.71), and between the C-11-CH_3 protons (δ 1.53) and C-10-H (δ 4.94) on irradiation of the C-11-CH_3 and C-9′-CH_3 protons (δ 1.53). In the ^{13}C-NMR spectrum of **2a**, the signals of the methoxyl carbon atoms appeared at δ 55.4, 56.2, and 60.3 ppm. This result suggests that one of the methoxyl groups is a di-*ortho*-substituted methoxyl group (C-3-OCH_3).[10,12,13] In the ^{13}C-NMR spectrum of **2**, the chemical shift values of the carbon atoms of the flavonol skeleton as well as those of the carbon atoms of a 3,3-

dimethylallyl group and a 1,1-dimethylallyl group are in good agreement with those of the relevant carbon atoms of **1** except those of the carbon atoms at C-2′ and -4′ positions. All these results indicate that broussoflavonol D can be represente by formula **2**.

To the authors' knowledge, broussoflavonols C (**1**) and D (**2**) are the first reported examples of trialkenyl flavonols. They have unique substitution patterns in the B-rings.

Experimental

Abbreviations: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, sh = shoulder, and infl. = inflection. The general experimental procedures used are described in the previous papers.[2-4] The following instruments were used: melting points, Yazawa micromelting point apparatus (hot-stage type); ^{1}H-NMR spectra, JEOL GX-400 FT NMR spectrometer; ^{13}C-NMR spectra, JEOL GX-400 and Hitachi R-900 FT NMR spectrometers. For TLC, Wakogel B5-FM and B5-F were used.

Isolation of Broussoflavonols C (1) and D (2)——The dried root bark (4.5 kg) of *Broussonetia papyrifera* (L.) VENT., collected in the Botanical Gardens, Faculty of Science, University of Tokyo, Japan, in June, 1984, was finely cut and extracted with *n*-hexane and then with benzene. Evaporation of the *n*-hexane and the benzene extracts to dryness yielded 65 and 50 g of residue, respectively. The residue of the hexane extract (65 g) was extracted with MeOH, and the MeOH extract (30 g) was chromatographed on silica gel (350 g) using benzene as an eluent, each fraction (500 ml) being monitored by TLC. The fractions (frs. 10 and 11) eluted with benzene were evaporated to give a residue (0.75 g), which was fractionated by preparative TLC (solvent systems, $(CH_3)_2CO$: *n*-hexane = 1 : 1; $CHCl_3$: $(CH_3)_2CO$ = 50 : 1; benzene: $(CH_3)_2CO$: *n*-hexane = 20 : 3 : 4) to give broussoflavonol D (61 mg). The benzene extract (17 g) was chromatographed on deactivated silica gel[17] (300 g) with a benzene (saturated with water)–$(CH_3)_2CO$ system (frs. 1—20; benzene, frs. 21—35; benzene: $(CH_3)_2CO$ = 99 : 1), each fraction (500 ml) being monitored by TLC. The fractions (frs. 24—31) eluted with benzene (saturated with water) containing 1% $(CH_3)_2CO$ were evaporated to give a residue (620 mg), which was fractionated by preparative TLC ($CHCl_3$: $(CH_3)_2CO$ = 12 : 1; *n*-hexane : Et_2O = 3 : 2) to give broussoflavonol C (**1**, 45 mg). The fraction (fr. 3) eluted with benzene (saturated with water) was evaporated to give a residue (1.9 g), which was fractionated by preparative TLC (Et_2O : *n*-hexane = 1 : 1; $CHCl_3$: $(CH_3)_2CO$ = 50 : 1; benzene : $(CH_3)_2CO$: *n*-hexane = 20 : 3 : 4) to give broussoflavonol D (**2**, 18 mg).

Broussoflavonol C (1)——Compound **1** was obtained as pale yellow prisms, mp 173—176 °C (from benzene–$(CH_3)_2CO$). $FeCl_3$ test: dark green→dark brown. Mg–HCl test: orange, $ZrOCl_2$–citric acid test: positive. Na_2MoO_4 test: orange. Gibbs test: negative. High-resolution MS: Calcd for $C_{30}H_{34}O_7$ (M^+, *m/z*): 506.2302. Found: 506.2297, Calcd for $C_{26}H_{26}O_7$: 450.1677. Found: 450.1694, Calcd for $C_{18}H_{22}O_3$ (**3**): 286.1567. Found: 286.1545, Calcd for $C_{12}H_{13}O_4$ (**4**, base peak): 221.0812. Found: 221.0811. Electron impact-mass spectra (EI-MS) (75 eV) *m/z* (relative intensity): 507 (M^+ +1, 13%), 506 (M^+, 40), 489 (8), 450 (19), 448 (7), 435 (11), 286 (6, **3**), 284 (10), 228 (10), 221 (32, **4**), 201 (10), 179 (7), 165 (9), 79 (8), 78 (100), 77 (23). UV λ_{max}^{EtOH} nm (log ε): 212 (4.81), 261 (4.44), 294 (sh 3.98), 306 (4.01), 352 (4.03); $\lambda_{max}^{EtOH+AlCl_3}$: 212 (4.82), 269 (4.56), 310 (3.96), 333 (infl. 3.81), 405 (4.08); $\lambda_{max}^{EtOH+AlCl_3+HCl}$: 269 (4.43), 314 (4.08), 354 (sh 3.94), 394 (sh 3.83). IR ν_{max}^{KBr} cm^{-1}: 3430 (br), 3300 (sh), 1660, 1640, 1635 (sh), 1595, 1550. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, br s, C-11-CH_3), 1.49 (3H, s, C-12′-CH_3), 1.53 (6H, br s, C-11-CH_3 and C-12′-CH_3), 1.77, 1.83 (each 3H, br s, C-9′-CH_3), 3.23, 3.26 (each 1H, dd, *J* = 7, 14 Hz, C-9-H), 3.43 (2H, d, *J* = 7 Hz, C-7′-H × 2), 4.95, 5.20 (each 1H, br t, *J* = 7 Hz, C-10-H and C-8′-H), 5.36 (1H, br d, *J* = 11 Hz, C-16′-H, *cis*), 5.44 (1H, br d, *J* = 18 Hz, C-16′-H, *trans*), 5.52, 5.80, 6.05 (each 1H, br, OH), 6.30 (1H, s, C-6-H), 6.41 (1H, dd, *J* = 11, 18 Hz, C-15′-H), 6.81 (1H, s, C-5′-H), 7.31 (1H, s, OH), 12.12 (1H, s, C-5-OH). The ^{13}C-NMR (100.4 MHz) data are listed in Table I.

Broussoflavonol C Tetramethyl Ether (1a)——A mixture of broussoflavonol C (**1**, 5 mg), $(CH_3)_2SO_4$ (0.2 ml) and K_2CO_3 (5 g) in $(CH_3)_2CO$ (30 ml) was refluxed for 40 min and treated as usual. The reaction product was purified by preparative TLC (benzene only) to give the tetramethyl ether (**1a**, 2.3 mg). Compound **1a** was obtained as an oily substance. ^{1}H-NMR spectrum ($CDCl_3$) δ: 1.42 (3H, br s, C-11-CH_3), 1.47 (6H, br s, C-12′-CH_3 × 2), 1.49 (3H, br s, C-11-CH_3), 1.70, 1.77 (each 3H, br s, C-9′-CH_3), 3.25, 3.32 (each 1H, dd, *J* = 6, 15 Hz, C-9-H), 3.43 (2H, br d, *J* = 6 Hz, C-7′-H × 2), 3.70, 3.83, 3.84, 3.86 (each 3H, s, OCH_3), 4.66 (1H, dd, *J* = 1, 11 Hz, C-16′-H, *cis*), 4.72 (1H, dd, *J* = 1, 18 Hz, C-16′-H, *trans*), 4.90 (1H, m, C-10-H), 5.06 (1H, m, C-8′-H), 6.11 (1H, dd, *J* = 11, 18 Hz, C-15′-H), 6.41 (1H, s, C-6-H), 6.75 (1H, s, C-5′-H), 13.11 (1H, s, C-5-OH). The ^{13}C-NMR (22.6 MHz) data are listed in Table I.

Broussoflavonol D (2)——Compound **2** was obtained as pale yellow plates, mp 102—110 °C (from $(CH_3)_2CO$–*n*-hexane). $FeCl_3$ test: dark green→dark brown. Mg–HCl test: orange. $ZrOCl_2$– citric acid test: positive. Na_2MoO_4 test: negative. Gibbs test: negative. High-resolution MS: Calcd for $C_{30}H_{32}O_7$ (M^+, *m/z*): 504.2147. Found: 504.2127, Calcd for $C_{26}H_{24}O_7$: 448.1522. Found: 448.1532, Calcd for $C_{12}H_{13}O_4$ (**4**): 221.0812. Found: 221.0802, Calcd for $C_8H_5O_4$: 165.0187. Found: 165.0217. UV λ_{max}^{EtOH} nm (log ε): 206 (4.78), 222 (sh 4.58), 263 (4.35), 283 (infl. 4.15), 304 (sh 3.90), 350 (3.90); $\lambda_{max}^{EtOH+AlCl_3}$: 206 (4.78), 224 (sh 4.54), 269 (4.41), 310 (sh 3.76), 405 (3.90). IR ν_{max}^{Nujol} cm^{-1}: 3300 (br), 1660, 1640 (sh), 1590, 1555. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, s, C-12′-CH_3), 1.48 (6H, br s, C-11-CH_3 and C-12′-CH_3), 1.529, 1.532 (each 3H, s, C-9′-CH_3), 1.55 (3H, br s, C-11-CH_3), *ca.* 3.3 (2H, br, C-9-H × 2), 4.97 (1H, br t, *J* = 7 Hz, C-

10-H), 5.36 (1H, br d, $J=11$ Hz, C-16′-H, *cis*), 5.44 (1H, br d, $J=18$ Hz, C-16′-H, *trans*), 5.56 (1H, br s, OH), 5.71 (1H, d, $J=10$ Hz, C-8′-H), 6.18 (1H, br s, OH), 6.31 (1H, br s, C-6-H), 6.42 (1H, dd, $J=11$, 18 Hz, C-15′-H), 6.54 (1H, d, $J=10$ Hz, C-7′-H), 6.87 (1H, s, C-5′-H), 7.33 (1H, br s, OH), 12.11. (1H, s, C-5-OH). The ^{13}C-NMR (100.4 MHz) data are listed in Table I.

Broussoflavonol D Trimethyl Ether (2a)——A mixture of broussoflavonol D (**2**, 22 mg), $(CH_3)_2SO_4$ (0.2 ml) and K_2CO_3 (10 g) in acetone (30 ml) was refluxed for 15 min, then kept at room temperature for 2 h, and treated as usual. The reaction product was purified by preparative TLC (benzene only) to give a trimethyl ether (**2a**, 12.8 mg). Compound **2a** was obtained as an oily substance. $FeCl_3$ test: green→dark green. EI-MS (75 eV) *m/z* (relative intensity): 546 (M^+, 1%), 531 (17), 515 (100), 473 (40), 235 (7), 179 (7). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, br s, C-11-CH_3), 1.47, 1.48 (each 3H, s, C-12′-CH_3), 1.51 (3H, br s, C-9′-CH_3), 1.53 (6H, br s, C-11-CH_3 and C-9′-CH_3), 3.27, 3.32 (each 1H, dd, $J=6$, 16 Hz, C-9-H), 3.68, 3.82, 3.84 (each 3H, s, OCH_3), 4.67 (1H, dd, $J=1$, 11 Hz, C-16′-H, *cis*), 4.72 (1H, dd, $J=1$, 17 Hz, C-16′-H, *trans*), 4.94 (1H, br t, $J=6$ Hz, C-10-H), 5.71 (1H, d, $J=10$ Hz, C-8′-H), 6.11 (1H, dd, $J=11$, 17 Hz, C-15′-H), 6.41 (1H, s, C-6-H), 6.52 (1H, d, $J=10$ Hz, C-7′-H), 6.76 (1H, s, C-5′-H), 13.10 (1H, s, C-5-OH). The ^{13}C-NMR (100.4 MHz) data are listed in Table I.

Broussoflavonol D Triacetate (2b)——Compound **2** (17 mg) was acetylated with Ac_2O (0.2 ml) and pyridine (0.1 ml) at room temperature for 10 min. The reaction product was treated as usual, and purified by preparative TLC (benzene only) to give the triacetate (**2b**, 9 mg). Broussoflavonol D triacetate (**2b**) was obtained as an oily substance. $FeCl_3$ test: brown. EI-MS (20 eV) *m/z* (relative intensity): 630 (M^+, 1%), 572 (75), 571 (100), 529 (29); (75 eV) *m/z*: 588 (9), 572 (40), 571 (100), 529 (36), 487 (11), 263 (6), 221 (43), 43 (86). ^{1}H-NMR ($CDCl_3$) δ: 1.44, 1.52, 1.59 (each 3H, br s, C-11-CH_3, C-9′-CH_3, or C-12′-CH_3), 1.46 (9H, br s, C-11-CH_3, C-9′-CH_3, or C-12′-CH_3), 2.18, 2.23, 2.30 (each 3H, s, $OCOCH_3$), 3.26 (1H, dd, $J=5$, 16 Hz, C-9-H), 3.33 (1H, dd, $J=7$, 16 Hz, C-9-H), 4.81 (1H, br d, $J=11$ Hz, C-16′-H, *cis*), 4.84 (1H, br d, $J=18$ Hz, C-16′-H, *trans*), 4.94 (1H, br triplet like, C-10-H), 5.75 (1H, d, $J=10$ Hz, C-8′-H), 6.11 (1H, dd, $J=11$, 18 Hz, C-15′-H), 6.45 (1H, s, C-6-H), 6.49 (1H, d, $J=10$ Hz, C-7′-H), 6.85 (1H, s, C-5′-H), 12.40 (1H, s, C-5-OH).

Acknowledgement We are grateful to Emeritus Prof. H. Hara, University of Tokyo, and to Prof. K. Iwatsuki, the head of the Botanical Gardens, Faculty of Science, University of Tokyo, for the gift of the root bark of *Broussonetia papyrifera* (L.) VENT. We are also grateful to Emeritus Prof. M. Ikuse, Toho University, for valuable advice on the identification of the material, and to Prof. S. Sakai, Faculty of Pharmaceutical Sciences, Chiba University, for high-resolution MS measurements.

References and Notes

1) Part XXXIII on Constituents of the Cultivated Mulberry Tree. Part XXXII: reference 2. Part II of Components of *Broussonetia papyrifera* (L.) VENT.: reference 4.
2) J. Ikuta (née Matsumoto), Y. Hano, T. Nomura, Y. Kawakami, and T. Sato, *Chem. Pharm. Bull.*, **34**, 1968 (1986).
3) J. Matsumoto, T. Fujimoto, C. Takino, M. Saitoh, Y. Hano, T. Fukai, and T. Nomura, *Chem. Pharm. Bull.*, **33**, 3250 (1985).
4) J. Ikuta (née Matsumoto), Y. Hano, and T. Nomura, *Heterocycles*, **23**, 2835 (1985).
5) The crystals of **1** and **2** were kept at room temperature for 24 h, then analyzed by TLC. Unknown compounds were detected. If the crystals (**1** and **2**) were kept in an ice box, such compounds were not obtained.
6) T. Swain and J. L. Goldstein, "Methods in Polyphenol Chemistry," ed. by J. B. Pridham, Pergamon Press, Oxford, 1964. [K. Takeda and K. Hayashi, "Syokubutsu Shikiso," ed. by K. Hayashi, Yokendo, Tokyo, 1980, p. 179].
7) *a*) T. Okamoto and T. Murakami, "Tennenbutsu Kagaku," Hirokawa Shoten, Tokyo, 1970, p. 249; *b*) L. Hörhammer and R. Hänsel, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **286**, 425 (1953).
8) T. J. Mabry, K. R. Markham, and M. B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970, Chapter V or VIII.
9) T. J. Mabry and K. R. Markham, "The Flavonoids," Part 1, ed. by J. B. Harborne, T. J. Mabry, and H. Mabry, Academic Press Inc., New York, 1975, pp. 79—126.
10) K. R. Markham and V. M. Chari, "The Flavonoids: Advances in Research," ed. by J. B. Harborne and J. J. Mabry, Chapman and Hall, New York, 1982, pp. 19—134.
11) *a*) E. Wenkert and H. E. Gottlieb, *Phytochemistry*, **16**, 1811 (1977); *b*) K. R. Markham and B. Ternai, *Tetrahedron*, **32**, 2067 (1976).
12) The position of the 1,1-dimethylallyl group was supported by the following NOE findings in **1**. When the C-11 methyl protons (δ 1.451) were irradiated, NOE was not observed anywhere. When the C-12′-CH_3 protons (δ 1.489) were irradiated, the intensities of the methyl signals at δ 1.451 (C-11-CH_3) and 1.534 (C-11- and C-12′-CH_3) decreased to *ca.* 70 and 60%, respectively, but NOEs were only observed at the C-5′, C-15′, and C-16′ (*trans*) protons. When the C-11 and C-12′ methyl protons (δ 1.534) were irradiated, the intensity of the methyl

signal at δ 1.489 (C-12′-CH_3) decreased to *ca.* 65%, and NOEs were only observed at the C-10, C-5′, C-15′, C-16′ (*trans*) protons.

13) K. S. Dhami and J. B. Stothers, *Can. J. Chem.*, **44**, 2855 (1966).
14) J. N. Roitman and L. F. James, *Phytochemistry*, **24**, 835 (1985).
15) *a*) V. H. Deshpande, P. C. Parthasarathy, and K. Venkataraman, *Tetrahedron Lett.*, **1968**, 1715; *b*) T. Nomura, T. Fukai, S. Yamada, and M. Katayanagi, *Chem. Pharm. Bull.*, **26**, 1394 (1978).
16) The position of the 1,1-dimethylallyl group was supported by the following NOE findings in **2a**. When the C-11 methyl protons (δ 1.464) were irradiated, the intensity of the C-12′ methyl signal (δ 1.471) decreased to *ca.* two-thirds and NOEs were observed at the C-5′ (less than 1%), C-15′ (3%), and C-16′ (*trans*) (3%) protons. When the C-12′ methyl protons (δ 1.471) were irradiated, the intensities of the methyl signals at δ 1.464 (C-11-CH_3), 1.482 (C-12′-CH_3), 1.514 (C-9′-CH_3), and 1.534 (C-11- and C-9′-CH_3) decreased to *ca.* 60, 60, 75, and 85%, respectively, and NOEs were only observed at the C-10 (less than 5%), C-5′, C-15′, C-16′ (*trans*) protons. When the C-12′ methyl protons (δ 1.482) were irradiated, the intensities of the methyl signals decreased to *ca.* 80 (δ 1.534, C-11- and C-9′-CH_3), 70 (δ 1.514, C-9′-CH_3), 60 (δ 1.471, C-12′-CH_3), and 85% (δ 1.464, C-11-CH_3), and NOEs were observed only at the C-10 (less than 2%), C-5′, C-15′, and C-16′ (*trans*) protons. When the C-9′ methyl protons (δ 1.514) were irradiated, the intensity of the methyl signal at δ 1.534 (C-11- and C-9′-CH_3) decreased to *ca.* 90%, and NOE was observed only at the C-8′ proton. When the C-11- and C-9′-CH_3 protons (δ 1.534) were irradiated, the intensity of the signal at δ 1.534 (C-11- and C-9′-CH_3) decreased to *ca.* 10%. The intensity of the methyl signal at δ 1.514 (C-9′-CH_3) also decreased to *ca.* 90%, but NOEs were observed only at the C-10 and C-8′ protons.
17) K. Tachibana, P. J. Scheuer, Y. Tsukitani, H. Kikuchi, D. Van Engen, J. Clardy, Y. Copichand, and F. J. Schmitz, *J. Am. Chem. Soc.*, **103**, 2469 (1981).