

REVISED STRUCTURES OF BROUSSOFLAVONOLS C AND D, AND THE STRUCTURE
OF BROUSSOFLAVONOL E¹

Toshio Fukai and Taro Nomura*

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

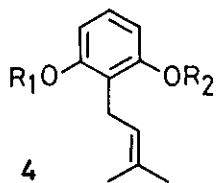
Abstract — The structures of broussoflavonols C and D isolated from the root bark of Broussonetia papyrifera (L.) VEN. (Japanese name "Kazinoki," Moraceae) were revised from the structures (1') and (2') to 1 and 2, respectively, on the basis of spectral data. Broussoflavonol E (3) was isolated from the root bark of Broussonetia sp. (Japanese name "Kōzo," a cultivated variety of paper mulberry, Broussonetia papyrifera (L.) VEN. x B. kazinoki Sieb.). The structure of broussoflavonol E was shown to be 3 on the basis of spectral evidence.

In the previous paper,² we reported the structures (1') and (2') for broussoflavonols C and D, respectively, which were isolated from the root bark of Broussonetia papyrifera (L.) VEN. (Moraceae), on the basis of spectral data.

In the course of our studies on the structure of prenylated phenols,³ we noticed that the chemical shift of the carbon atom at the C-1 position (C1) of the prenyl (3-methyl-2-butenyl) group was depended on the substituents located at the adjacent positions. To elucidate the observation, we examined the ¹³C nmr spectra of ca. 200 kinds of the known isoprenoid-substituted phenols and their derivatives, and found out that the prenyl groups in the isoprenoid-substituted phenols and their derivatives could be classified into the following six types (Types 1-6, Figure 1) according to the substituents located at the adjacent positions, and that the chemical shifts of the C1 signals of the prenyl groups were observed in the restricted range specific to each type as follows.⁴

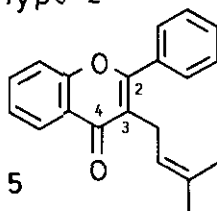
Type 1 (4): When the diortho-positions to the prenyl group were replaced by the oxygenated substituents, the chemical shift of the C1 signal of the prenyl group was observed in the range of δ20.7 - 24.0.

Type 1



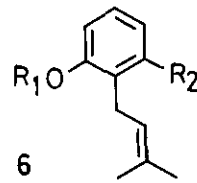
4
 $R_1, R_2 = H, \text{ alkyl or alkenyl}$

Type 2



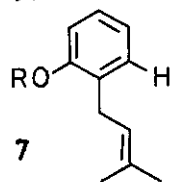
5
 (3-prenylated flavone)

Type 3



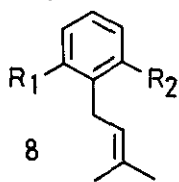
6
 $R_1 = H \text{ or alkyl}$
 $R_2 = \text{alkyl or alkenyl}$

Type 4



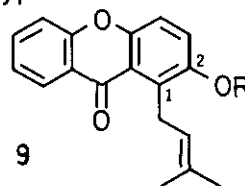
7
 $R = H \text{ or alkyl}$

Type 5



8
 $R_1 = \text{alkyl or alkenyl}$
 $R_2 = \text{alkyl or alkenyl}$

Type 6



9
 $R = H \text{ or Me}$
 (2-hydroxy(-methoxy)-
 1-prenylated xanthone)

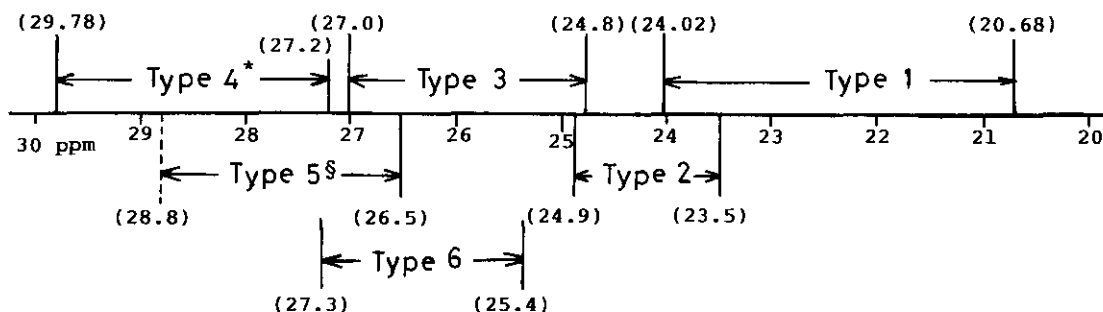


Figure 1 Chemical shifts of methylene carbons of prenyl groups

The chemical shifts were measured in $CDCl_3$, $DMSO-d_6$, $acetone-d_6$, $pyridine-d_5$, CD_3OD , $dioxane-d_6$, or $acetonitrile-d_3$. The following chemical shifts (δ) were observed in each solvent: in $CDCl_3$; type 1: 20.8–23.37 (31[†] compounds), type 2: 24.41–24.61 (3), type 3: 24.8–26.72 (16), type 4: 27.4–29.78 (9), type 5: 26.5–28.6 (15), type 6: 25.6–26.5 (8), in $DMSO-d_6$; type 1: 20.68–23.2 (60), type 2: 23.5–24.1 (10), type 3: 25.0 (1), type 4: 27.2–29.07 (10), type 6: 27.2 (1), in $acetone-d_6$; type 1: 21.4–24.02 (39), type 2: 24.4–24.61 (6), type 3: 25.1–27.0 (6), type 4: 28.1–29.1 (8), type 5: 28.1 (1), type 6: 25.4–27.3 (3), in $pyridine-d_5$; type 1: 22.2–22.5 (5), type 2: 24.3–24.9 (4), type 4: 29.0 (1), in CD_3OD ; type 1: 21.60–23.57 (4), type 2: 24.71 (1), type 3: 26.8 (2), type 4: 28.27–29.12 (3), ³ in $dioxane-d_6$; type 3: 26.3–26.4 (3), type 5: 28.8 (1), type 6: 26.3 (1), $acetonitrile-d_3$; type 1: 21.32–22.00 (3), type 2: 24.51–24.55 (2).

*: The group could be distinguished from the type 5 by nondecoupling spectrum because the long-range coupling between the methylene carbon and an aromatic proton was observed only in type 4 (observed as triplet of triplet or double doublet of triplet).

§: Generally, the group shows δ 27.2–28.8, but in the case of the prenyl group adjacent to pyrane ring in 1,3-diphenylpropane and flavane, the chemical shifts were observed in the range of δ 26.5–26.8 (refs. 11, 19). The data of brousoflavonols C (1) and D (2) ($R_1 = R_2 = \text{alkenyl}$) are not included.

Type 2 (5): In the ^{13}C nmr spectrum of 3-prenylated flavone derivatives, the chemical shift of the C1 signal of the prenyl group was observed in the range of $\delta 23.5 - 24.9$.

Type 3 (6): When one of the ortho-positions to the prenyl group was replaced by an alkyl or alkenyl group, and another by an oxygenated substituent, the chemical shift of the C1 signal of the prenyl group was observed in the range of $\delta 24.8 - 27.0$.

Type 4 (7): When one of the ortho-positions to the prenyl group was replaced by an oxygenated substituent and another by a hydrogen atom, the chemical shift of the C1 signal of the prenyl group was observed in the range of $\delta 27.2 - 29.8$.

Type 5 (8): When the diortho-positions to the prenyl group were replaced by the alkyl (or alkenyl) groups, or the alkyl and alkenyl groups, the chemical shift of the C1 signal of the prenyl group was observed in the range of $\delta 26.5 - 28.8$.

Type 6 (9): In the ^{13}C nmr spectrum of 2-oxygenated 1-prenylated xanthone derivatives, the chemical shift of the C1 signal of the prenyl group was observed in the range of $\delta 25.4 - 27.3$.

The above results were summarized in Figure 1.

In the ^{13}C nmr spectrum of brousoflavonol C (1), the chemical shifts of the C1 signals of the prenyl groups were observed at $\delta 26.2$ and 29.2 (in CDCl_3).² These chemical shifts suggest that one of the prenyl groups seems to be of type 3 or 6, and another to be of type 4 or 5. On the other hand, brousoflavonol D (2) showed the chemical shift of the C1 signal of the prenyl group at $\delta 28.0$ (in CDCl_3).² From this result, the prenyl group in the brousoflavonol D seems to be of type 4 or 5. These results were inconsistent with the proposed structures 1' and 2' for brousoflavonols C and D, respectively, and prompted us to reinvestigate the structures of brousoflavonols C and D. In this paper, we propose the revised structures 1 and 2 for brousoflavonols C and D, respectively, and also report the structure of brousoflavonol E (3)⁵ obtained from the root bark of Broussonetia sp. (a cultivated variety of paper mulberry, Broussonetia papyrifera (L.) VENT. x B. kazinoki SIEB.).⁶

The ^{13}C nmr spectra of 1 and 2 were measured, and the carbon atoms were assigned as shown in Table 1 by the off-resonance decoupling, long-range selective proton decoupling (LSPD), and deuterium induced shift techniques^{7,8} as well as by comparison of the ^{13}C nmr spectra of 1 and 2 with those of model compounds, such as, ikarisoside A (10),⁹ cudraxanthone B (11),¹⁰ kazinols C (12)¹¹ and K (13),¹¹

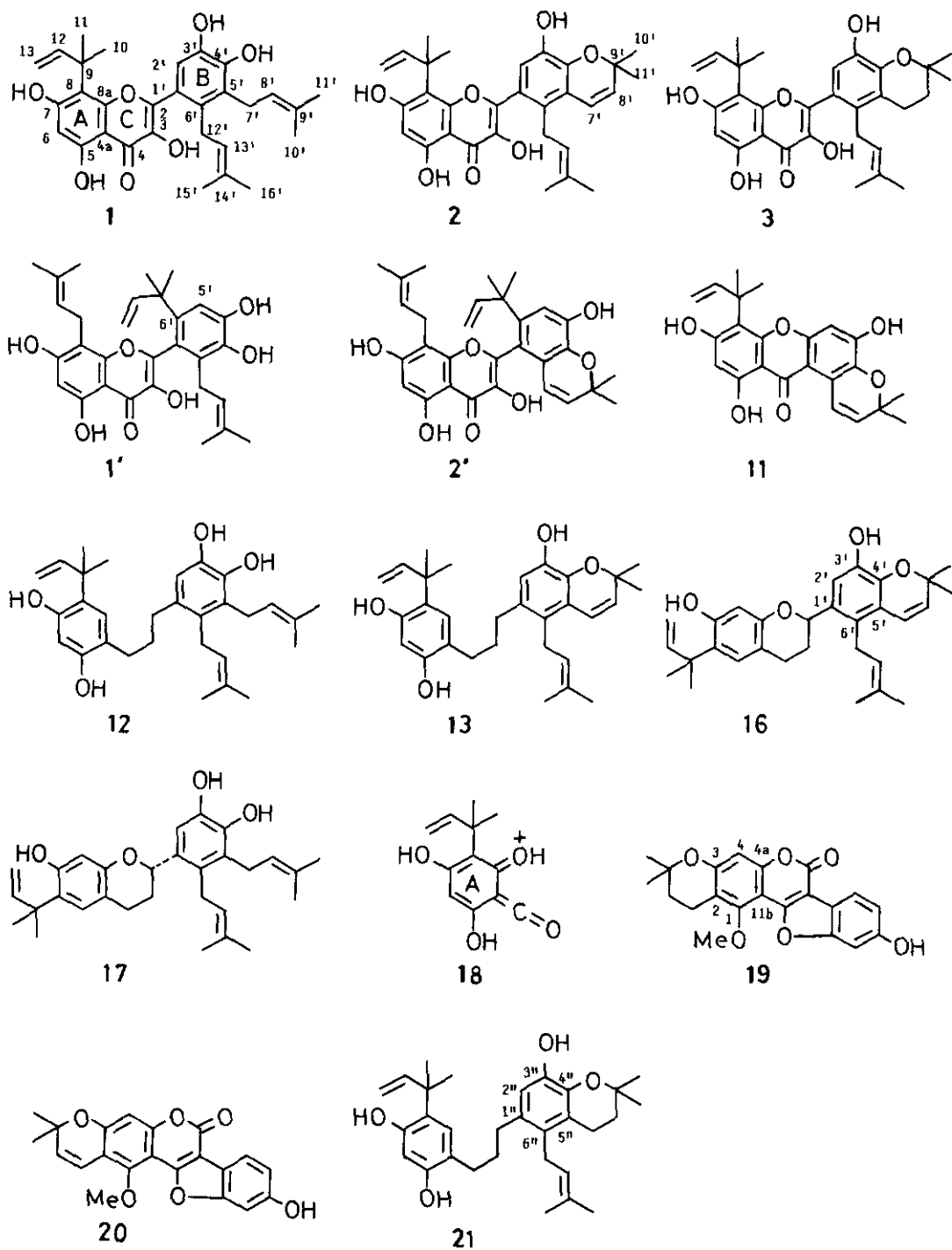


Figure 2

Table 1. ^{13}C Nmr data of brousoflavonols C (1), D (2), and E (3)

C	1	1	2	2	3 ^a
2	148.70 (d) (J=4 Hz)	150.81 (d) ^b (J=4 Hz)	148.53 (d) (J=4 Hz)	149.76 (dd) ^c (J=5 and 8 Hz)	150.43 (d-like)
3	136.18 (s)	135.93 (s)	136.23 (s)	135.97 (s)	135.88 (s)
4	175.80 (s)	176.58 (s)	175.97 (s)	176.52 (s)	176.49 (s)
4a	105.15 (t) (J=5 Hz)	104.25 (t) (J=5 Hz)	105.07 (d) (J=6 Hz)	104.12 (t) ^c (J=6 Hz)	104.12 (t) (J=6 Hz)
5	159.52 (t) (J=4 Hz)	158.67 (t) (J=4 Hz)	159.04 (d) (J=4 Hz)	158.57 (t) ^c (J=4 Hz)	158.58 (dd) (J=4 and 4.4 Hz)
6	100.91 (br d) (J=164 Hz)	99.21 (dd) (J=6 and 161 Hz)	100.43 (d) (J=162 Hz)	99.10 (dd) ^c (J=7 and 161 Hz)	99.07 (dd) (J=7 and 160 Hz)
7	161.80 (s)	162.34 (s)	161.94 (s)	162.37 (s)	162.30 (s)
8	110.10 (br s)	110.78 (br s)	110.63 (br s)	110.52 (br s)	110.54 (br s)
8a	155.71 (s)	155.09 (s)	155.77 (s)	154.97 (s)	155.00 (s)
1'	121.66 (t) (J=5.5 Hz)	121.19 (t) (J=5 Hz)	122.07 (t) (J=5.5 Hz)	122.25 (t) (J=4 Hz)	120.95 (t) (J=4 Hz)
2'	114.20 (d) (J=160 Hz)	114.43 (d) (J=159 Hz)	115.95 (d) (J=160 Hz)	117.02 (dd) ^c (J=4 and 159 Hz)	114.08 (br d) (J=158 Hz)
3'	142.04 (d) (J=4 Hz)	142.48 (d) (J=3 Hz)	142.89 (br s) (J=4 Hz)	143.14 (br s)	143.71 (d)
4'	144.80 (td) (J=4 and 7 Hz)	144.85 (td) (J=4 and 7 Hz)	141.62 (dd) (J=ca.4 and 6 Hz)	141.68 (dd) (J=ca.4 and 6 Hz)	143.38 (br s)
5'	127.13 (m)	127.08 (m)	120.00 (m)	119.73 (m)	119.87 (br s)
6'	132.73 (br s)	130.88 (br s)	129.12 (br s)	127.40 (br s)	129.82 (br s)
9	40.50 (m)		40.72 (m)		
10	27.48 (mq) (J=128 Hz)	29.02 (mq) (J=126 Hz)	27.85	28.74 (mq) ^d (J=124 Hz)	29.28
11	28.05 (mq) (J=128 Hz)	29.23 (mq) (J=126 Hz)	27.85	29.18 (mq) ^d (J=124 Hz)	28.96
12	149.12 (md) (J=156 Hz)	149.38 (md) (J=156 Hz)	149.23 (md)	149.27 (md) (J=156 Hz)	149.32 (md) (J=156 Hz)
13	113.55 (dd) (J=156 and 161 Hz)	107.84 (dd) (J=153 and 158 Hz)	111.98 (br dd) (J=154 and 159 Hz)	107.82 (dd) (J=153 and 157 Hz)	107.89 (dd) (J=152 and 156 Hz)
7'	26.15 (dt) (J=4 and 127 Hz)	25.16 (dt) (J=3 and 126 Hz)	119.52 (d) (J=164 Hz)	119.10 (d) (J=163 Hz)	19.79 (br t) (J=128 Hz)
8'	121.87	124.12 (md) (J=154 Hz)	130.79 (md) (J=163 Hz)	131.23 (md) (J=160 Hz)	32.18 (mt) (J=124 Hz)
9'	134.35 (m)	130.03 (m)		75.12	73.37 (br s)
10'	18.03 (mq) (J=125 Hz)	17.73 (mq) (J=125 Hz)	26.72 (mq) (J=126 Hz)	27.07 (mq) ^d (J=126 Hz)	25.85
11'	25.77 (mq) (J=126 Hz)	25.37 (mq) (J=125 Hz)	26.72 (mq) (J=126 Hz)	27.51 (mq) ^d (J=125 Hz)	26.97
12'	29.20 (dt) (J=4 and 126 Hz)	28.40 (dt) (J=3 and 126 Hz)	28.11 (dt) (J=4 and 127 Hz)	27.31 (br t) (J=ca. 128 Hz)	27.92 (dt) (J=4 and 128 Hz)
13'	123.47	123.47 (md) (J=154 Hz)	123.49 (md) (J=154 Hz)	123.60 (md) (J=156 Hz)	123.11 (md) (J=154 Hz)
14'	131.36 (m)	129.44 (m)	131.25 (m)	129.83 (m)	129.91 (m)
15'	17.77 (mq) (J=125 Hz)	17.28 (mq) (J=126 Hz)	17.75 (mq) (J=125 Hz)	17.26 (mq) (J=125 Hz)	17.28 (mq) (J=124 Hz)
16'	25.43 (mq) (J=126 Hz)	25.16 (mq) (J=124 Hz)	25.50	25.21 (mq) (J=124 Hz)	25.21 (mq) (J=124 Hz)
solvent					
	$\text{CDCl}_3 + \text{CD}_3\text{OD}^e$	$\text{DMSO}-d_6$ at 60 °C	$\text{CDCl}_3 + \text{CD}_3\text{OD}^f$	$\text{DMSO}-d_6$ at 31 °C	$\text{DMSO}-d_6$ at 31 °C

a: The multiplicities were measured at 60 °C. b: The signal was observed as broad singlet when measured at 31 °C. c: addition of D_2O ; C2(dd → d, J=5 Hz), C4a(t → d, J=6 Hz), C5(t → d, J=4 Hz), C6(dd → d, J=161 Hz), C2'(dd → d, J=159 Hz). d: measured at 70 °C; 29.11 ppm (2C, br, C10 and C11), 27.35 ppm (2C, br, C10' and C11'). e: The sample (60 mg) was subjected to measurement in $\text{CDCl}_3 + \text{CD}_3\text{OD}$ (0.6 ml:3 drops) at 31 °C: The protons of the hydroxyl groups were exchanged for deuterium except 5-hydroxyl group. f: The sample (15 mg) was subjected to measurement in $\text{CDCl}_3 + \text{CD}_3\text{OD}$ (0.6 ml:3 drops) at 31 °C: The proton of hydroxyl groups was exchanged for deuterium. Abbreviations: mq = multiplet of quartet (the splitting pattern with long-range couplings is not clear), md = multiplet of doublet, mt = multiplet of triplet.

and quercetin (14).¹² From these results and the spectral data described in the previous paper,² brousoflavonol C seems to be a 5,7,3',4'-tetrahydroxyflavonol having two prenyl groups¹³ and a 1,1-dimethylallyl group in the structure, and brousoflavonol D to be a 5,7,3'-trihydroxyflavonol or 5,7,4'-trihydroxyflavonol having a prenyl and a 1,1-dimethylallyl group as well as a 2,2-dimethylpyrane ring system.

The locations of the isoprenoid moieties in the structure of brousoflavonol C were confirmed by the following evidences (1 - 5).

1. The presence of an isoprenoid moiety at the C-8 position was confirmed by the following LSPD experiments. In the ^{13}C nmr spectrum (in DMSO-d_6 at 60°C , gated decoupling with NOE), the C6 signal at $\delta 99.21$ was observed as doublet of doublet ($^1J = 161$ Hz, $^3J = 6$ Hz). The assignment of the signal was confirmed as follow: When the proton signal at $\delta 12.78$ (C-5-OH) was irradiated, the signal at $\delta 99.21$ changed to doublet ($^1J = 161$ Hz) (Table 1).

2. The signal at $\delta 6.28$ (in DMSO-d_6 at 60°C) was assigned to that of the C-6-H as follows: When the signal at $\delta 6.28$ was irradiated, the signal at $\delta 158.67$ (C5, t, $J = 4$ Hz) changed to doublet ($J = 4$ Hz), and the signal at $\delta 104.25$ (C4a, t, $J = 5$ Hz) to doublet ($J = 5$ Hz) (Table 1). Furthermore, as reported in the previous paper,² the NOE was observed at the C-6-H ($\delta 6.30$, in CDCl_3) by the irradiation of the hydrogen-bonded hydroxyl group ($\delta 12.12$, in CDCl_3). From these results, one of the two aromatic proton signals at $\delta 6.80$ (in DMSO-d_6 at 60°C) was assigned to that of the B-ring proton.

3. In the ^{13}C nmr spectrum (in $\text{CDCl}_3 + \text{CD}_3\text{OD}$, gated decoupling with NOE), both of the C1 signals of the prenyl groups were observed as doublet of triplet at $\delta 26.15$ ($^2J_{\text{C1,H2}} = 4$ Hz, $^1J = 127$ Hz) and 29.20 ($^2J_{\text{C1,H2}} = 4$ Hz, $^1J = 126$ Hz) (Table 1). This result suggests that no hydrogen atom is located at the adjacent positions to the prenyl groups.

4. In the ^{13}C nmr spectrum (in DMSO-d_6 , gated decoupling with NOE), the C8a signal was observed as singlet (Figure 3). This result suggested that the isoprenoid moiety located at the C-8 position was a 1,1-dimethylallyl group. The above suggestion was further supported by the following evidence. In the ^{13}C nmr spectrum of ikarisosides A (10)⁹ and E (15)⁹ (in DMSO-d_6 , gated decoupling with NOE), the signals at $\delta 153.59$ (C8a of 10) and $\delta 149.62$ (C8a of 15) were observed as a triplet ($^3J = 4$ Hz) and a doublet ($^3J = 2$ Hz), respectively (Figure 3).¹⁴

5. In the ^{13}C nmr spectrum (in DMSO-d_6 at 31°C , gated decoupling with NOE), the C2

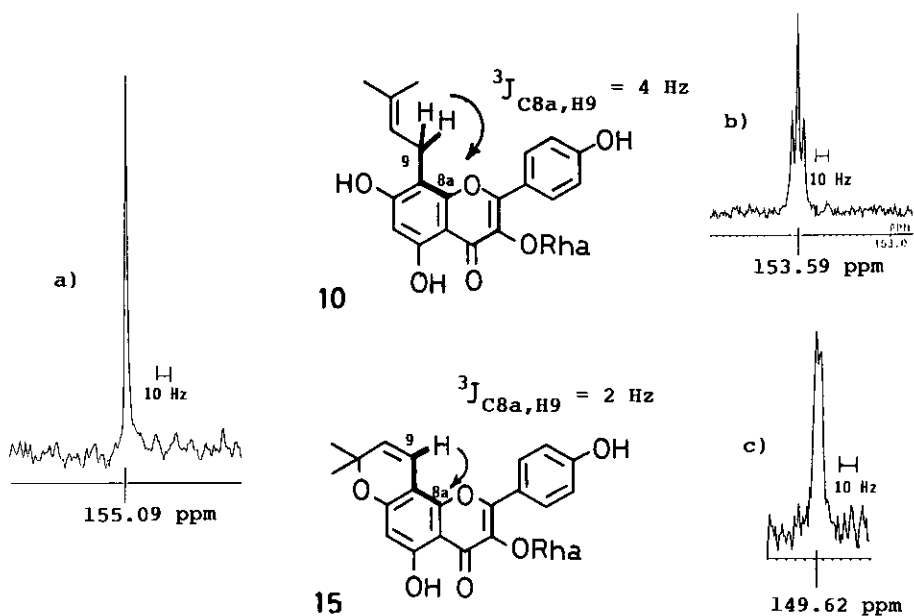


Figure 3 The signals of C8a of 1, 10, and 15

- a): The C8a signal (δ 155.09) of brousoflavonol C (1) in $\text{DMSO}-d_6$ at 60°C .
 b): The C8a signal (δ 153.59) of ikarisoside A (10) in $\text{DMSO}-d_6$ at 60°C .
 c): The C8a signal (δ 149.62) of ikarisoside E (15) in $\text{DMSO}-d_6$ at 60°C .

signal was observed at δ 150.71 as broad singlet, while in the spectrum measured at 60°C , the signal was observed at δ 150.81 as doublet ($^3J = 4 \text{ Hz}$). When the signal at δ 6.80 (B-ring proton) was irradiated, both signals at δ 150.81 (C2) and at δ 142.48 (C3', d, $^2J = 3 \text{ Hz}$) changed to the singlet as well as the signal at δ 144.85 (C4', td, $^3J_{\text{C4}',\text{H2}'} = 7 \text{ Hz}$, $^3J_{\text{C4}',\text{H7}'} = 4 \text{ Hz}$) to the triplet ($^3J_{\text{C4}',\text{H7}'} = 4 \text{ Hz}$) signal (Table 1). On the other hand, we reported in the previous paper² that the NOE was observed at the C-5'-H (δ 6.81, in CDCl_3 , on formula 1') by the irradiation of the methyl protons (δ 1.49) in the 1,1-dimethylallyl group. This fact was one of the grounds of locating the 1,1-dimethylallyl group at the C-6' position. While the fact could be explained on the following revised structure (1) as follow: On the examination with Dreiding model, it was suggested that the 1,1-dimethylallyl group at the C-8 position could take a possible conformation of expecting the observation of the NOE between the methyl protons in the 1,1-dimethylallyl group and the C-2'-H. The above results taken into consideration, the structure of brousoflavonol C should be revised from the formula 1' to 1.

The ^{13}C nmr spectrum of brousoflavonol D (2) being compared with that of 1, the chemical shifts of all the carbon atoms of 2, except those of the carbon atoms at

Table 2. ^{13}C Nmr data of the B-ring of 1, 2, 16, and 17

C	1*	2*	$\Delta\delta(1-2)^+$	17 §	16 §	$\Delta\delta(17-16)^+$
1'	121.4	121.5	(-0.1)	131.0	131.3	(-0.3)
2'	113.8	115.3	(-1.5)	111.3	112.9	(-1.6)
3'	141.5	142.3	(-0.8)	141.7	143.3	(-1.6)
4'	144.0	140.9	(+3.1)	142.4	139.3	(+3.1)
5'	126.4	119.4	(+7.0)	127.0	119.8	(+7.2)
6'	130.8	128.7	(+2.1)	130.2	126.4	(+3.8)

*: measured in CDCl_3 , data from ref. 2.

§ : measured in CDCl_3 , data from ref. 11.

+ : differences of chemical shifts between 1 (17) and 2 (16).

the C-2', -4', -5', and -6' positions and those of the carbon atoms of an isoprenoid moiety, were in good agreement with those of the relevant carbon atoms of 1 (Table 1). We reported the 2,2-dimethylpyrane ring system to be located in the B-ring.² Furthermore the location of the three isoprenoid moieties was supported by the following evidences (1 - 3).

1. In the ^{13}C nmr spectrum (in $\text{CDCl}_3 + \text{CD}_3\text{OD}$, gated decoupling with NOE), the C8a signal at δ 155.77 was observed as singlet (Table 1). This result supports the 1,1-dimethylallyl group to be located at the C-8 position.

2. In the ^{13}C nmr spectrum, the C2 signal at δ 148.53 and the C4' signal at δ 141.62 were observed as the doublet ($^3J = 4$ Hz) and the doublet of doublet ($^3J_{\text{C4}',\text{H2}'} = \text{ca. } 6$ Hz, $^3J_{\text{C4}',\text{H7}'} = \text{ca. } 4$ Hz), respectively (Table 1). When the signal at δ 6.86 (C-2'-H) was irradiated, the signals at δ 148.53 and 141.62 changed to the singlet and the doublet ($^3J = 4$ Hz), respectively. These results confirmed the presence of a hydrogen atom at the C-2' position.

3. The result of the comparison of the chemical shifts of the B-ring carbon atoms of 2 with those of the relevant carbon atoms of 1 is shown in Table 2. The similar result was obtained by the comparison of the ^{13}C nmr spectra between kazinols H (16)¹¹ and E (17)¹¹ (Table 2).

From these results, the structure of brousoflavonol D should be revised from the formula 2' to 2.

Brousoflavonol E (3) was obtained as pale yellow prisms, mp 168 - 170 °C, $M^+ = 506.2279$, $\text{C}_{30}\text{H}_{34}\text{O}_7$, exhibiting a positive ferric chloride test, magnesium-hydrochloric acid test, and zirconium oxychloride test,¹⁵ but a negative sodium molybdate test¹⁶ and Gibbs test. The ir spectrum of 3 disclosed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties. The uv spectrum of 3 showed absorption maxima at 209, 260, 306, and 351 nm, and was similar to those

Table 3. Difference in chemical shift of the B-ring carbons between brousoflavonols D (2) and E (3) [$\Delta\delta(2-3)$]⁺ and related compounds (between 20 and 19, 13 and 21)

C	$\Delta\delta(2-3)^a$	C	$\Delta\delta(20-19)^b$	C	$\Delta\delta(2-3)^c$	C	$\Delta\delta(13-21)^c$
1'	+1.30	(11b) [§]	+0.94	1'	+0.8	(1'') [§]	+0.3
2'	+2.94	(4a)	+1.28	2'	+2.4	(2'')	+1.9
3'	-0.57	(4)	+0.07	3'	-1.1	(3'')	-1.1
4'	-1.70	(3)	-1.23	4'	-2.1	(4'')	-2.2
5'	-0.14	(2)	-0.88	5'	-0.9	(5'')	-0.6
6'	-2.42	(1)	-2.70	6'	-2.8	(6'')	-2.7

+: The chemical shift of 2 minus the chemical shift of 3.

a: measured in DMSO-d₆ at 31°C.

b: measured in DMSO-d₆ at 60°C.

c: measured in CDCl₃ at 35°C.

§: The carbon numbers correspond to those of 2 and 3.

of brousoflavonols C (1) and D (2).² The spectrum showed a bathochromic shift in the presence of aluminium chloride as follows: 210, 268, 317, and 404 nm. The ¹H nmr spectrum of 3 indicated the presence of a 3,3-dimethylallyl group [δ 1.56 (6H, br s), 3.23, 3.37 (each 1H, br dd, $J = \text{ca.}$ 7 and 14 Hz), and 4.97 (1H, br t, $J = 7$ Hz)], a 1,1-dimethylallyl group [δ 1.46, 1.50 (each 3H, s), 4.77 (1H, dd, $J = 1$ and 10.5 Hz), 4.85 (1H, dd, $J = 1$ and 17 Hz), and 6.24 (1H, dd, $J = 10.5$ and 17 Hz)], and 3,4-dihydro-2,2-dimethylpyrane ring system [δ 1.35, 1.38 (each 3H, br s), 1.90 (2H, t, $J = 6$ Hz), and 2.80 (2H, br td-like signal)]. The spectrum also indicated the presence of two aromatic protons [δ 6.33 (1H, s) and 6.83 (1H, s)] and a hydrogen-bonded hydroxyl group (δ 12.61), while no proton signal was observed at the C-3 position of a flavone skeleton. The EI-ms of 3 showed the fragments at m/z 450 ($M^+ - C_4H_8$)² and 221 (18)² as observed in 1 and 2. In the ¹³C nmr spectrum of 3, the carbon atoms were assigned as shown in Table 1 by the off-resonance decoupling and LSPD techniques as well as by comparison of the spectrum with those of 1, 2, and other model compounds.⁹⁻¹² Two oxygenated carbon signals were observed at δ 143.38 and 143.71, suggesting that 3 has a 3',4'-dioxxygenated phenyl partial structure.¹² Comparison of the ¹³C nmr spectra between 2 and 3 revealed that the chemical shifts of all the carbon atoms of 3, except those of the carbon atoms at the C-1', -2', -4', and -6' positions along with those of the carbon atoms of a 3,4-dihydro-2,2-dimethylpyrane ring system, were in good agreement with those of the relevant carbon atoms of 2. From the above results, brousoflavonol E is a 5,7-dihydroxy-3',4'-dioxxygenated flavonol having an isoprenoid moiety in the A-ring and two isoprenoid moieties in the B-ring. The locations of the isoprenoid moieties were supported by the following evidences (1 and 2).

1. In the ¹³C nmr spectrum of 3 (in DMSO-d₆, gated decoupling with NOE), the

chemical shifts and the coupling patterns of the C8a and C2 signals were similar to those of the relevant carbon atoms of **1** and **2** (Table 1), suggesting that a 1,1-dimethylallyl group is located at the C-8 position and a hydrogen atom at the C-2' position.

2. The results of the comparison of the chemical shifts of the B-ring carbons of **3** with the relevant carbon atoms of **2** were obtained as described in Table 3. The similar results were obtained by the comparisons of the ^{13}C nmr spectra of isoglycyrol (**19**),¹⁷ gancaonin F (**20**),¹⁷ kazinols D (**21**)¹¹ and K (**13**)¹¹ (Table 3).

From the above results, the structure of brousoflavonol E was shown to be the formula 3.

EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, td = triplet of doublet, m = multiplet, br = broad. The general procedures followed those described in the previous papers.^{2,9,18} The following instruments were used: melting point; Yazawa micromelting point apparatus, uv spectra; Shimadzu UV-265 spectrophotometer, ir spectra; Hitachi 260-30 IR spectrophotometer, ms: JEOL JMS OISG-2 and Hitachi RMU-7M mass spectrometers, ^1H and ^{13}C nmr spectra; JEOL JNM GX-400 FT NMR spectrometer.

Plant Materials

The root bark of Broussonetia sp. (Japanese name "Kōzo," a cultivated variety of paper mulberry, Broussonetia papyrifera (L.) VENI. x B. kazinoki SIEB.) was collected in Hanazono Village, Saitama Prefecture, Japan, in August 1982. The material was identified by Dr. N. Sahashi, Faculty of Pharmaceutical Sciences, Toho University.

Isolation of Brousoflavonol E (3)

The dried root bark of Broussonetia sp. (2.5 kg) was extracted with *n*-hexane (10 l x 2) at room temperature for 7 days. Evaporation of the extract to dryness yielded 47 g of the residue. This residue (30 g) was chromatographed on silica gel (200 g) with *n*-hexane containing different concentrations of acetone as eluents, each fraction being monitored by tlc. The fraction eluted with *n*-hexane containing 7% acetone was evaporated to give a residue (0.9 g), which was fractionated sequentially by preparative tlc (solvent system, *n*-hexane:ether = 1:1, *n*-hexane:acetone = 1:1) and by recrystallization from benzene to give brousoflavonol E (**3**, 5 mg).

Brousoflavonol E (3)

Compound **3** was recrystallized from benzene to give pale yellow prisms, mp 168-170 °C. FeCl_3 test: dark green. Mg-HCl test: orange. ZrOCl_2 -citric acid test: positive. Na_2MoO_4 test: negative. Gibbs test: negative. $\text{Uv}\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 209 (4.79), 260 (4.49), 306 (3.98), 351 (4.05). $\text{Uv}\lambda_{\text{max}}^{\text{EtOH}+\text{AlCl}_3}$: 210 (4.79), 268 (4.60), 317 (3.85), 404 (4.13). $\text{Ir}\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600, 3300, 1660, 1640, 1590, 1510. EI-MS (probe) 75 eV, m/z (relative intensity): 507 $[\text{M}+\text{H}]^+$ (37%), 506 $[\text{M}]^+$ (100), 451 (25), 450 (79), 437 (12), 284 (64), 257 (65), 221 (73). High-resolution-MS, M/z : 506.2279 $[\text{M}]^+$ ($\text{C}_{30}\text{H}_{34}\text{O}_7$

Japan. In the abstract, brousoflavonol E was described as compound 4 and the formula 3' was suggested for the compound.

6. S. Kitamura and G. Murata, 'Gensyoku Nihon Shokubutsu Zukan, Mokuhon-hen (Coloured Illustrations of Woody Plants of Japan),' Vol. II, p. 244, Osaka, Hoikusha Publishing Co., 1979.
7. Y. Shirataki, I. Yokoe, M. Noguchi, T. Tomimori, and M. Komatsu, Chem. Pharm. Bull., 1988, **36**, 2220 and references cited therein.
8. The sample (1, 18 mg) was subjected to measurement in DMSO-d₆, D₂O, and H₂O (0.6, 0.06, and 0.06 ml, respectively) at 5°C.
9. T. Fukai and T. Nomura, Phytochemistry, 1988, **27**, 259.
10. T. Nomura, Y. Hano, and T. Fujimoto, Heterocycles, 1983, **20**, 213.
11. J. Ikuta (née Matsumoto), Y. Hano, T. Nomura, Y. Kawakami, and T. Sato, Chem. Pharm. Bull., 1986, **34**, 1968.
12. K.R. Markham, V.M. Chari, and T.J. Mabry, 'The Flavonoids: Advances in Research,' eds. by J.B. Harborne and T.J. Mabry, Chapman and Hall, London, 1982, pp. 19-134.
13. ¹H Nmr spectrum (in CDCl₃) of 1: δ1.77, 1.83 (each 3H, br s, C-9'-CH₃), 3.43 (2H, br d, J = 7 Hz, C-7'-Hx2), 5.20 (1H, br t, J = 7 Hz, C-8'-H), 1.45 (3H, br s, C-14'-CH₃), 1.53 (6H, br s, C-9-CH₃ and C-14'-CH₃), 3.23, 3.26 (each 1H, br dd, J = 7 and 14 Hz, C-12'-H), 4.95 (1H, br t, J = 7 Hz, C-13'-H).
14. T. Fukai, unpublished data. The assignments of the signals at δ153.59 (C8a of 10) and 149.62 (C8a of 15) were confirmed by LSPD technique.
15. L. Hörhammer and R. Hänsel, Arch. Pharm. Ber. Dtsch. Pharm. Ges., 1953, **286**, 425.
16. K. Takeda and K. Hayashi, 'Shokubutu Shikiso (Coloring Matters of Plants)', ed. by K. Hayashi, Yokendo, Tokyo, 1980, p. 179.
17. T. Fukai, Q.-H. Wang, T. Kitagawa, K. Kusano, T. Nomura, and Y. Iitaka, Heterocycles, 1989, **29**, 1761.
18. J. Matsumoto, T. Fujimoto, C. Takino, M. Saitoh, Y. Hano, T. Fukai, and T. Nomura, Chem. Pharm. Bull., 1985, **33**, 3250.
19. S. Kato, T. Fukai, J. Ikuta (née Matsumoto), and T. Nomura, Chem. Pharm. Bull., 1986, **34**, 2448.

Received, 7th September, 1989