REVISED STRUCTURES OF BROUSSOFLAVONOLS C AND D, AND THE STRUCTURE OF BROUSSOFLAVONOL E[1]

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Abstract —— The structures of broussoflavonols C and D isolated from the root bark of Broussonetia papyrifera (L.) VENT. (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.) VENT. 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.) VENT. (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 ^{13}C 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.

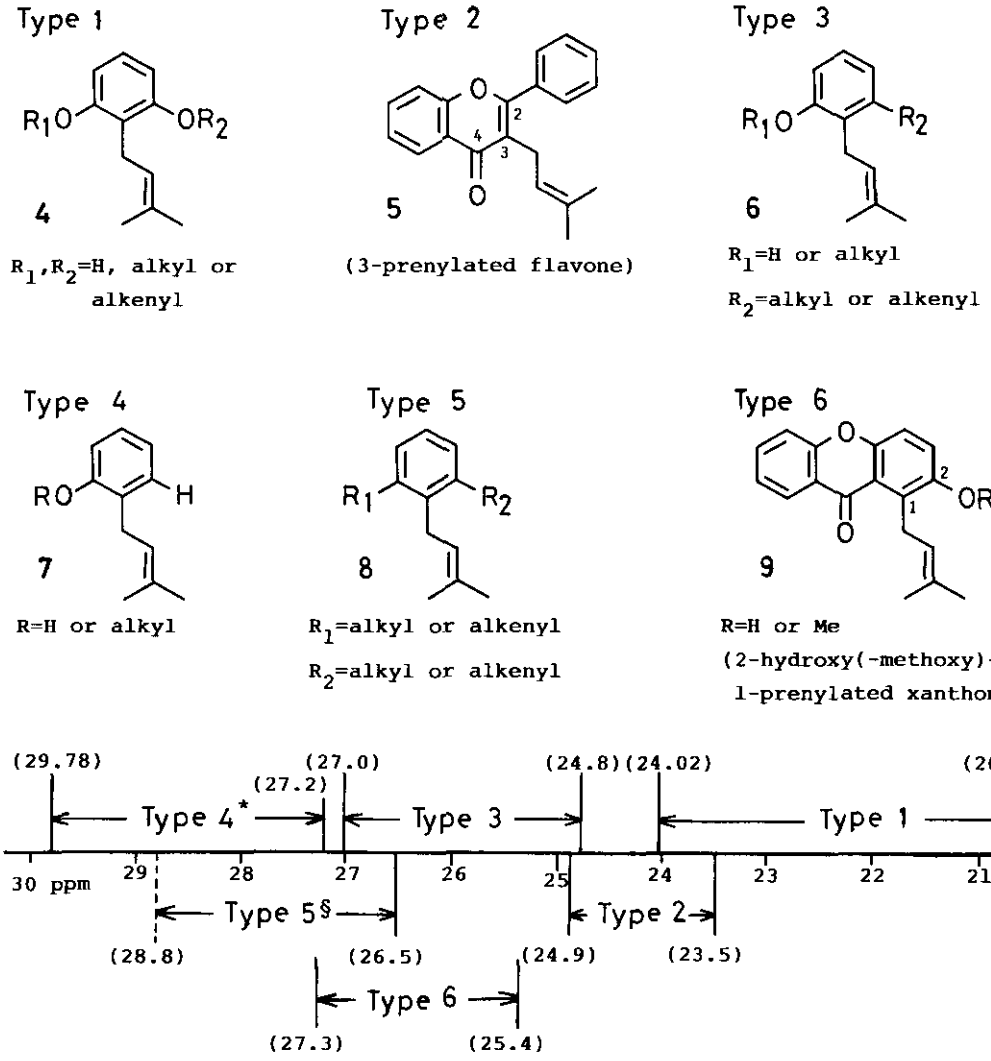


Figure 1 Chemical shifts of methylene carbons of prenyl groups

The chemical shifts were measured in $CDCl_3$, DMSO-d_6, acetone-d_6, pyridine-d_5, CD_3OD, dioxane-d_8, 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), in dioxane-d_8; 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 δ27.2-28.8, 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 δ26.5-26.8 (refs. 11, 19). The data of broussoflavonols C (**1**) and D (**2**) ($R_1 = R_2$ = 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 δ23.5 - 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 δ24.8 - 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 δ27.2 - 29.8.

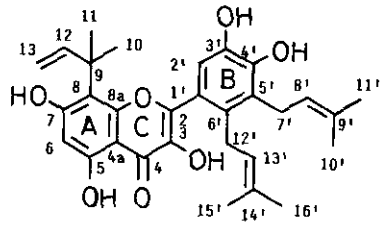
Type 5 (8): When the diortho-positions to the prenyl group were replaced by the alkyl (or alkenyl) groups, or the alkyl and alkenyl groups, the chemical shift of the C1 signal of the prenyl group was observed in the range of δ26.5 - 28.8.

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 δ25.4 -27.3.

The above results were summarized in Figure 1.

In the ^{13}C nmr spectrum of broussoflavonol C (**1**), the chemical shifts of the C1 signals of the prenyl groups were observed at δ26.2 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, broussoflavonol D (**2**) showed the chemical shift of the C1 signal of the prenyl group at δ28.0 (in $CDCl_3$).[2] From this result, the prenyl group in the broussoflavonol D seems to be of type 4 or 5. These results were inconsistent with the proposed structures **1'** and **2'** for broussoflavonols C and D, respectively, and prompted us to reinvestigate the structures of broussoflavonols C and D. In this paper, we propose the revised structures **1** and **2** for broussoflavonols C and D, respectively, and also report the structure of broussoflavonol 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]



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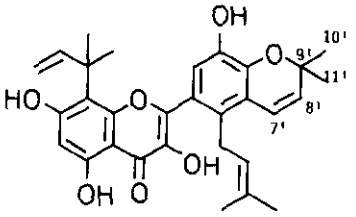

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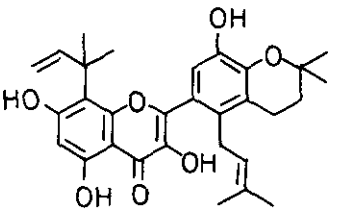

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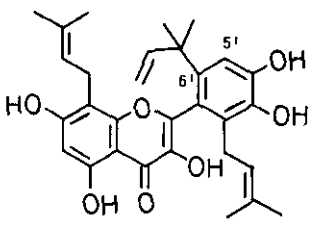

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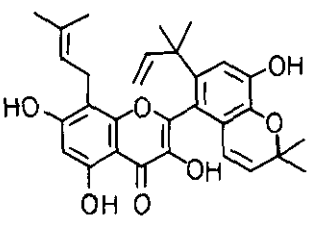

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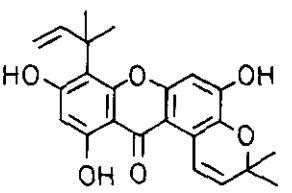

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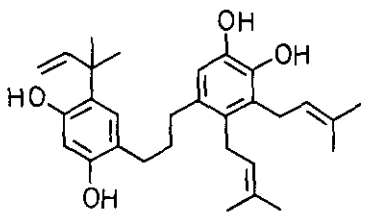

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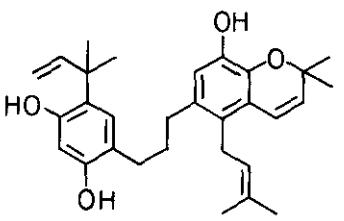

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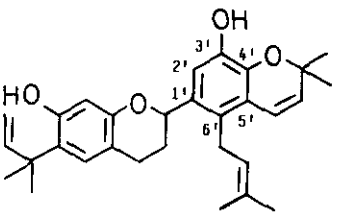

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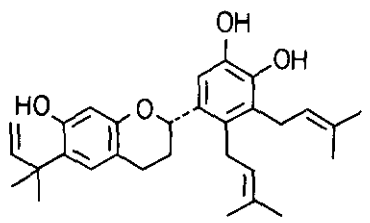

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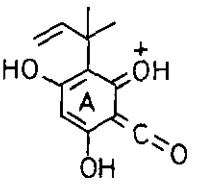

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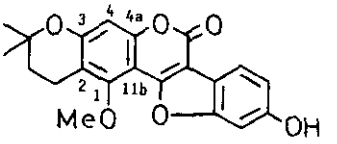

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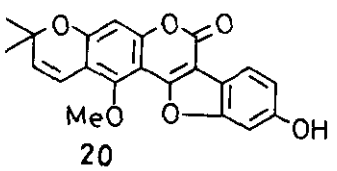

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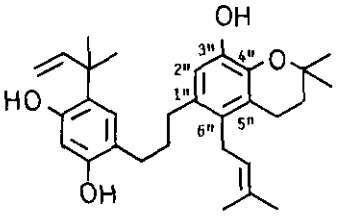

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Figure 2

Table 1. ^{13}C Nmr data of broussoflavonols 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) (^{1}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) (^{1}J=128 Hz)	29.02 (mq) (^{1}J=126 Hz)	27.85	28.74 (mq)[d] (^{1}J=124 Hz)	29.28
11	28.05 (mq) (^{1}J=128 Hz)	29.23 (mq) (^{1}J=126 Hz)	27.85	29.18 (mq)[d] (^{1}J=124 Hz)	28.96
12	149.12 (md) (^{1}J=156 Hz)	149.38 (md) (^{1}J=156 Hz)	149.23 (md)	149.27 (md) (^{1}J=156 Hz)	149.32 (md) (^{1}J=156 Hz)
13	113.55 (dd) (J=156 and 161 Hz)	107.84 (dd) (J=153 and 158 Hz)	111.98 (br dd) (^{1}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) (^{1}J=154 Hz)	130.79 (md) (^{1}J=163 Hz)	131.23 (md) (^{1}J=160 Hz)	32.18 (mt) (^{1}J=124 Hz)
9'	134.35 (m)	130.03 (m)	------	75.12	73.37 (br s)
10'	18.03 (mq) (^{1}J=125 Hz)	17.73 (mq) (^{1}J=125 Hz)	26.72 (mq) (^{1}J=126 Hz)	27.07 (mq)[d] (^{1}J=126 Hz)	25.85
11'	25.77 (mq) (^{1}J=126 Hz)	25.37 (mq) (^{1}J=125 Hz)	26.72 (mq) (^{1}J=126 Hz)	27.51 (mq)[d] (^{1}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) (^{1}J=ca. 128 Hz)	27.92 (dt) (J=4 and 128 Hz)
13'	123.47	123.47 (md) (^{1}J=154 Hz)	123.49 (md) (^{1}J=154 Hz)	123.60 (md) (^{1}J=156 Hz)	123.11 (md) (^{1}J=154 Hz)
14'	131.36 (m)	129.44 (m)	131.25 (m)	129.83 (m)	129.91 (m)
15'	17.77 (mq) (^{1}J=125 Hz)	17.28 (mq) (^{1}J=126 Hz)	17.75 (mq) (^{1}J=125 Hz)	17.26 (mq) (^{1}J=125 Hz)	17.28 (mq) (^{1}J=124 Hz)
16'	25.43 (mq) (^{1}J=126 Hz)	25.16 (mq) (^{1}J=124 Hz)	25.50	25.21 (mq) (^{1}J=124 Hz)	25.21 (mq) (^{1}J=124 Hz)
solvent	$CDCl_3$+CD_3OD[e]	DMSO-d_6 at 60 °C	$CDCl_3$+CD_3OD[f]	DMSO-d_6 at 31 °C	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 $CDCl_3$+CD_3OD(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 $CDCl_3$+CD_3OD (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] broussoflavonol C seems to be a 5,7,3',4'-tetrahydroxyflavonol having two prenyl groups[13] and a 1,1-dimethylallyl group in the structure, and broussoflavonol 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 broussoflavonol 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 δ99.21 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 δ12.78 (C-5-OH) was irradiated, the signal at δ99.21 changed to doublet (^1J = 161 Hz) (Table 1).

2. The signal at δ6.28 (in DMSO-d_6 at 60 °C) was assigned to that of the C-6-H as follows: When the signal at δ6.28 was irradiated, the signal at δ158.67 (C5, t, J = 4 Hz) changed to doublet (J = 4 Hz), and the signal at δ104.25 (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 (δ6.30, in $CDCl_3$) by the irradiation of the hydrogen-bonded hydroxyl group (δ12.12, in $CDCl_3$). From these results, one of the two aromatic proton signals at δ6.80 (in DMSO-d_6 at 60 °C) was assigned to that of the B-ring proton.

3. In the ^{13}C nmr spectrum (in $CDCl_3$ + CD_3OD, gated decoupling with NOE), both of the C1 signals of the prenyl groups were observed as doublet of triplet at δ26.15 ($^2J_{C1,H2}$ = 4 Hz, ^1J = 127 Hz) and 29.20 ($^2J_{C1,H2}$ = 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 δ153.59 (C8a of **10**) and δ149.62 (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

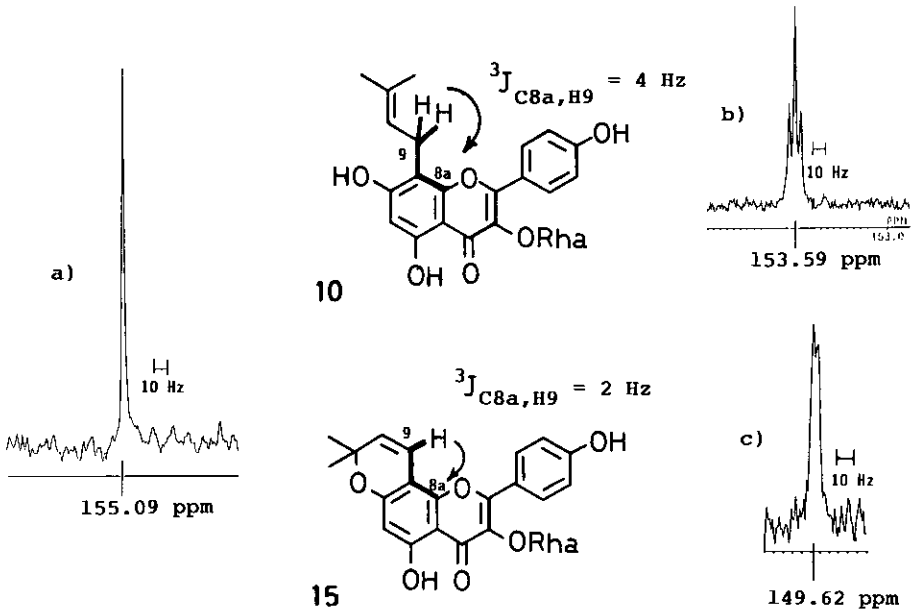

Figure 3 The signals of C8a of **1**, **10**, and **15**

a): The C8a signal (δ155.09) of broussoflavonol C (**1**) in DMSO-d_6 at 60 °C.

b): The C8a signal (δ153.59) of ikarisoside A (**10**) in DMSO-d_6 at 60 °C.

c): The C8a signal (δ149.62) of ikarisoside E (**15**) in DMSO-d_6 at 60 °C.

signal was observed at δ150.71 as broad singlet, while in the spectrum measured at 60 °C, the signal was observed at δ150.81 as doublet (^3J = 4 Hz). When the signal at δ6.80 (B-ring proton) was irradiated, both signals at δ150.81 (C2) and at δ142.48 (C3', d, ^2J = 3 Hz) changed to the singlet as well as the signal at δ144.85 (C4', td, $^3J_{C4',H2'}$ = 7 Hz, $^3J_{C4',H7'}$ = 4 Hz) to the triplet ($^3J_{C4',H7'}$ = 4 Hz) signal (Table 1). On the other hand, we reported in the previous paper[2] that the NOE was observed at the C-5'-H (δ6.81, in $CDCl_3$, on formula **1'**) by the irradiation of the methyl protons (δ1.49) 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 broussoflavonol C should be revised from the formula **1'** to **1**.

The ^{13}C nmr spectrum of broussoflavonol 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 $CDCl_3$ + CD_3OD, gated decoupling with NOE), the C8a signal at δ155.77 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 δ148.53 and the C4' signal at δ141.62 were observed as the doublet (^3J = 4 Hz) and the doublet of doublet ($^3J_{C4',H2'}$ = ca. 6 Hz, $^3J_{C4',H7'}$ = ca. 4 Hz), respectively (Table 1). When the signal at δ6.86 (C-2'-H) was irradiated, the signals at δ148.53 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 broussoflavonol D should be revised from the formula **2'** to **2**.

Broussoflavonol E (**3**) was obtained as pale yellow prisms, mp 168 - 170 °C, M^+ = 506.2279, $C_{30}H_{34}O_7$, exhibiting a positive ferric chloride test, magnesium-hydrochloric acid test, and zirconium oxychloride test,[15] but a negative sodium molybdate test[16] 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 broussoflavonols 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-d_6 at 31°C.
b: measured in DMSO-d_6 at 60°C.
c: measured in $CDCl_3$ at 35°C.
§: The carbon numbers correspond to those of **2** and **3**.

of broussoflavonols 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 ^{1}H nmr spectrum of **3** indicated the presence of a 3,3-dimethylallyl group [δ1.56 (6H, br s), 3.23, 3.37 (each 1H, br dd, J = ca. 7 and 14 Hz), and 4.97 (1H, br t, J = 7 Hz)], a 1,1-dimethylallyl group [δ1.46, 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 [δ1.35, 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 [δ6.33 (1H, s) and 6.83 (1H, s)] and a hydrogen-bonded hydroxyl group (δ12.61), 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 ^{13}C 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 δ143.38 and 143.71, suggesting that **3** has a 3',4'-dioxygenated phenyl partial structure.[12] Comparison of the ^{13}C 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, broussoflavonol E is a 5,7-dihydroxy-3',4'-dioxygenated 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 ^{13}C nmr spectrum of **3** (in DMSO-d_6, 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 broussoflavonol 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 O1SG-2 and Hitachi RMU-7M mass spectrometers, ^{1}H and ^{13}C nmr spectra; JEOL JNM GX-400 FT NMR spectrometer.

Plant Materials

The root bark of Broussonetia sp. (Japanese name "Közo," a cultivated variety of paper mulberry, Broussonetia papyrifera (L.) VENT. 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 Broussoflavonol 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 broussoflavonol E (**3**, 5 mg).

Broussoflavonol 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. Uv λ_{max}^{EtOH} nm (log ε): 209 (4.79), 260 (4.49), 306 (3.98), 351 (4.05). Uv $\lambda_{max}^{EtOH+AlCl_3}$: 210 (4.79), 268 (4.60), 317 (3.85), 404 (4.13). Ir ν_{max}^{KBr} cm^{-1}: 3600, 3300, 1660, 1640, 1590, 1510. EI-Ms (probe) 75 eV, m/z (relative intensity): 507 $[M+H]^+$ (37%), 506 $[M]^+$ (100), 451 (25), 450 (79), 437 (12), 284 (64), 257 (65), 221 (73). High-resolution-Ms, M/z: 506.2279 $[M]^+$ ($C_{30}H_{34}O_7$

requires: 506.2303). ^{1}H Nmr (in acetone-d_6): δ 1.35, 1.38 (each 3H, br s, C-9'-CH_3), 1.46, 1.50 (each 3H, s, C-9-CH_3), 1.56 (6H, br s, C-14'-CH_3x2), 1.90 (2H, t, J = 6 Hz, C-8'-Hx2), 2.80 (2H, br, td-like, C-7'-Hx2), 3.23, 3.37 (each 1H, br dd, J = ca. 7 and 14 Hz, C-12'-H), 4.77 (1H, dd, J = 1 and 10.5 Hz, C-13-H), 4.85 (1H, dd, J = 1 and 17 Hz, C-13-H), 4.97 (1H, br t, J = 7 Hz, C-13'-H), 6.24 (1H, dd, J = 10.5 and 17 Hz, C-12-H), 6.33 (1H, s, C-6-H), 6.83 (1H, s, C-2'-H), 12.61 (1H, s, C-5-OH). ^{1}H Nmr (in $CDCl_3$): δ 1.38, 1.42 (each 3H, br s, C-9'-CH_3), 1.47, 1.49 (each 3H, br s, C-9-CH_3), 1.53 (6H, br s, C-14'-CH_3x2), 1.89 (2H, t, J = 7 Hz, C-8'-Hx2), 2.74 (2H, br td-like, C-7'-Hx2), 3.22, 3.29 (each 1H, br dd, J = 7 and 14 Hz, C-12'-H), 4.91 (1H, br t, J = 7 Hz, C-13'-H), 5.40 (1H, d, J = 10 Hz, C-13-H), 5.48 (1H, d, J = 18 Hz,C-13-H), 5.66, 6.15 (each 1H, br s, OH), 6.30 (1H, s, C-6-H), 6.42 (1H, dd, J = 10 and 18 Hz, C-12-H), 6.83 (1H, s, C-2'-H), 7.35 (1H, br s, OH), 12.13 (1H, s, C-5-OH).

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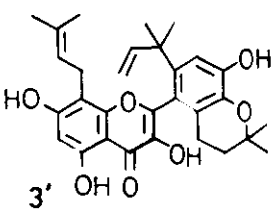
REFERENCES AND NOTES

1. Part 5 in the series 'Constituents of the Moraceae Plants.' For Part 4 see Y. Hano, M. Aida, and T. Nomura, J. Nat. Prod., submitted.
2. T. Fukai, J. Ikuta (née Matsumoto), and T. Nomura, Chem. Pharm. Bull., 1986, **34**, 1987.
3. T. Nomura, 'Progress in the Chemistry of Organic Natural Products,' Vol. 53, eds. W. Herz, H. Grisebach, G.W. Kirby, and Ch. Tamm, Springer, Wien, 1988, pp. 87-201 and references cited therein.
4. a) T. Fukai and T. Nomura, 109th Annual Meeting of Pharmaceutical Society of Japan, 'Abstract Papers,' Vol. 3, p. 162, April, 1989, Nagoya, Japan. While abstract of the work was reported at the Annual Meeting, the details will be reported as a review article in the near furture; b) Furukawa et al. reported that in the case of acridone alkaloids, the methylene signal (Cl') of the prenyl groups at C-2 when two oxygenated substituents are present at the ortho-positions appears in the region of δ 21.1-22.5. On the other hand, the methylene signals (Cl') of the prenyl group at C-4 in the N-methyl compounds move downfield to δ 26.0 - 27.1 (H. Furukawa, M. Yogo, and T.-S. Wu, Chem. Pharm. Bull., 1983, **31**, 3084).
5. S. Kato, Y. Hano, T. Fukai, T. Nomura, and J.-Y. Sun, 107th Annual Meeting of Pharmaceutical Society of Japan, 'Abstract Papers,' p. 348, 1987, Kyoto,



Japan. In the abstract, broussoflavonol 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-d_6, D_2O, and H_2O (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 $CDCl_3$) of **1**: δ1.77, 1.83 (each 3H, br s, C-9'-CH_3), 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'-CH_3), 1.53 (6H, br s, C-9-CH_3 and C-14'-CH_3), 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.

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