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FLAVAN DIMERS WITH LIPASE INHIBITORY ACTIVITY FROM *CASSIA NOMAME*

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Key Word Index—*Cassia nomame*; Leguminosae; flavan dimer; oligomeric flavanoid; tannin; lipase inhibitor.

Abstract—Five flavan dimers which showed lipase-inhibiting effects were isolated from fruits of *Cassia nomame* (Leguminosae). Structures of two new compounds among them were determined to be (2*S*)-3′,4′,7-trihydroxyflavan-(4$\beta \rightarrow$8)-catechin and (2*S*)-3′,4′,7-trihydroxyflavan-(4$\alpha \rightarrow$8)-catechin. Four flavan dimers structurally related to these two compounds were also synthesized for spectral comparison. Among 10 flavan dimers tested for lipase-inhibitory activity, (2*S*)-3′,4′,7-trihydroxyflavan-(4$\alpha \rightarrow$8)-catechin showed the most potent inhibitory effect. A partially purified fraction composed of oligomeric flavans with M_n 1020 also showed a noticeable inhibitory effect. © 1997 Elsevier Science Ltd

INTRODUCTION

Lipase inhibitors are expected to be candidates of medicines for prevention or treatment of obesity and/or accompanying various adult diseases [1]. Upon screening of the various plant extracts having lipase-inhibitory activity, an extract of *Cassia nomame* (= *Cassia mimosoides* var. *nomame*, Leguminosae) showed a potent inhibitory effect on lipase [2]. We previously reported that luteolin was obtained as a lipase inhibitor from its leaves, and several polyphenols of related structures also inhibited lipase [3]. Further investigation on lipase-inhibiting substances in fruits of this plant revealed that several constituents related to condensed tannins have the inhibitory activity. This paper deals with characterization of these constituents.

RESULTS AND DISCUSSION

Fractionation of the extract from *C. nomame* fruits was guided by enzyme-inhibition assay using porcine pancreatic lipase and 4-methylumbelliferyl oleate as substrate [2]. Chromatography of the ethyl acetate-soluble portion of the extract on Toyopearl HW-40 gave a fraction which showed 28% inhibition of lipase activity at 0.5 μg ml^{-1}. This fraction was further chromatographed on MCI gel CHP-20P to give two new phenolic compounds, temporarily named KA-1 (**1**) and KA-2 (**2**). Procyanidin B3 (**3**) [4, 5], (−)-fisetinidol-(4$\alpha \rightarrow$8)-catechin (**4**) [6], (+)-fisetinidol-(4$\beta \rightarrow$8)-catechin (**5**) [7], luteolin 7-*O*-β-D-glucoside and (+)-catechin were also obtained from neighboring fractions. Among them, KA-2 showed a noticeable inhibitory effect on lipase (IC_{50}, 5.5 μM). The diethyl ether-soluble portion of the extract was also separated in an analogous way, to give an oligomeric flavanoid fraction, FE-11, which showed the most potent inhibitory effect (IC_{50}, 0.20 μg ml^{-1}) among the fractions from the diethyl ether-soluble portion of the fruit extract.

Structure of KA-1 (**1**)

KA-1 (**1**) was obtained as a light-brown amorphous powder. The FAB-mass spectrum of **1** showed an $[M+Na]^+$ ion peak at *m/z* 569 and an $[M+H]^+$ ion peak at *m/z* 547 in the positive-ion mode, and an $[M-H]^-$ ion peak at *m/z* 545 in the negative-ion mode. These ion peaks and the elemental analysis indicated the molecular formula $C_{30}H_{26}O_{10}$ for this compound, which is 32 mass unit smaller than that of flavan dimers of procyanidin B series ($C_{30}H_{26}O_{12}$).

The ^{1}H NMR spectrum of **1** (in acetone-d_6+D_2O, 30°) suggested its structural similarity to dimeric procyanidins such as **3**, although the spectrum showed broad signals instead of the duplication of signals

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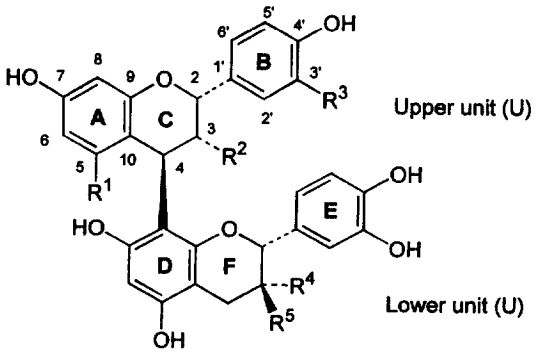
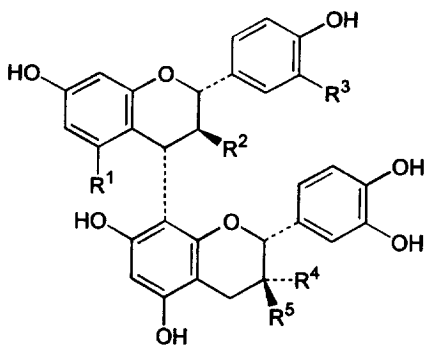


1: $R^1 = R^2 = R^4 = H$, $R^3 = R^5 = OH$
10: $R^1 = R^2 = R^3 = R^5 = OH$, $R^4 = H$
11: $R^1 = R^2 = R^3 = R^4 = OH$, $R^5 = H$

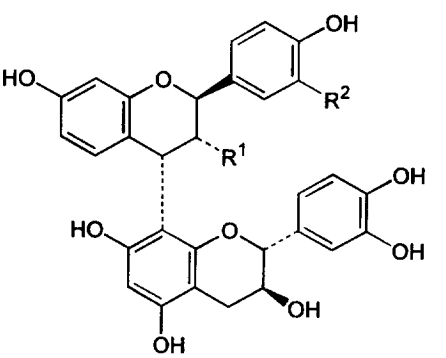


2: $R^1 = R^2 = R^4 = H$, $R^3 = R^5 = OH$
3: $R^1 = R^2 = R^3 = R^5 = OH$, $R^4 = H$
4: $R^1 = R^3 = R^5 = OH$, $R^2 = R^4 = H$
12: $R^1 = R^2 = R^3 = R^4 = OH$, $R^5 = H$

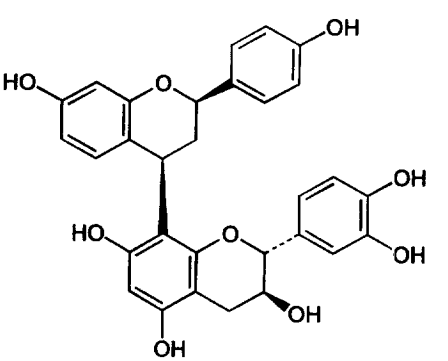
observed for **3** [5]. Signals of nine protons among ten aromatic protons in the spectrum of **1** form three sets of ABX 3-spin systems of 1,3,4-tri-substituted benzene rings {δ 6.23–6.26 [2H, *m*; H-8 of upper unit (U) and H-6 (U)], 6.62–6.66 [3H, *m*; H-5 (U), H-6′ (U) and H-6′ of lower unit (L)], 6.69, 6.74 [1H each, *d*, $J = 8$ Hz; H-5′ (U) and H-5′ (L)], 6.83 [2H, *d*, $J = 2$ Hz; H-2′ (U) and H-2′ (L)}. Two of the three tri-substituted benzene rings are attributed to B- and E-rings of dimeric flavan structure, and the other one is therefore ascribable to A-ring lacking hydroxyl group at C-5. The remaining aromatic proton at δ 6.08 is a singlet of a penta-substituted benzene ring, which is ascribed to D-ring.

Signals of four protons among eight aliphatic protons form a 4-spin system attributable to a methine-methylene-methine structure {δ 5.24 [1H, *m*; H-2 (U)], 2.17 [1H, *m*; H-3 (U)], 2.45 [1H, *br m*; H-3 (U)] and 4.47 [1H, *t*, $J = 6$ Hz; H-4 (U)]} and the remaining four aliphatic protons form a 4-spin system of a methine-methine-methylene system {δ 4.42 [1H, *br*; H-2 (L)], 4.02 [1H, *m*; H-3 (L)], 2.57 [1H, *dd*, $J = 8$, 16 Hz; H-4 (L)] and 2.92 [1H, *dd*, $J = 5.5$, 16 Hz; H-4 (L)}. The methylene protons of the latter 4-spin system are assignable to those of the terminal (lower) flavan unit, and therefore, the former 4-spin system was attributed to the upper flavan unit. The methylene protons attributed to the upper flavan unit thus indicate that this unit lacks a hydroxyl group at C-3. These assignments were consistent with the data shown by ^1H-^{13}C one-bond and long-range COSY (Table 1). Although the coupling constant between H-2 and H-3 of the lower unit was obscured by broadening due to restricted rotation around the interflavan linkage, chemical shift of C-2 of the lower unit in the ^{13}C NMR spectrum of **1** (δ 82.3) indicated the 2,3-*trans* structure [8] of the lower flavan residue.

Broadening of the signals of **1** attributable to restricted rotation around the interflavan bond suggested 4 → 8 connection which would cause more crowded structure rather than 4 → 6 connection for the interflavan bond. The location of the interflavan bond in **1** was confirmed by nuclear Overhauser effect spectroscopy (NOESY) measurement of a hepta-methylate (**1a**), which was obtained by treatment of **1** with dimethyl sulphate and potassium carbonate. If **1** has 4 → 6 linkage, the D-ring proton (H-8) of **1a** is



5: $R^1 = R^2 = OH$
8: $R^1 = R^2 = H$



9

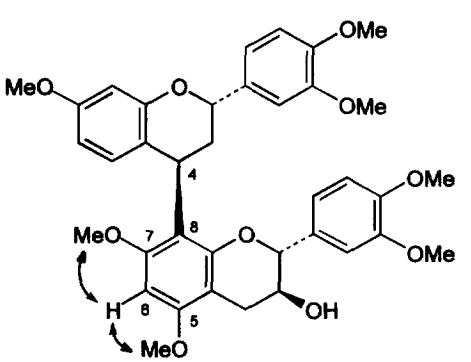
Table 1. ^{13}C and ^{1}H spectral data for KA-1 (**1**)*

Carbon	δ_c (ppm)	Correlated protons: Protons coupled *via* one bond (δ_H)	Correlated protons: Protons coupled *via* two or three bonds†
C-4 (U)‡	28.5	4.47	
C-4 (L)§	28.8	2.57, 2.92	
C-3 (U)	35.8	2.17, 2.45	
C-3 (L)	67.8	4.02	H-4 (L)
C-2 (U)	75.9	5.24	H-2′ (U), H-6′ (U)
C-2 (L)	82.3	4.42	H-4 (L), H-2′ (L)
C-6 (L)	96.6	6.08	
C-10 (L)	101.2		
C-8 (U)	103.6	6.23–6.26	H-6 (U)
C-6 (U)	108.4	6.23–6.26	H-8 (U)
C-8 (L)	110.7		H-4 (U), H-6 (L)
C-2′ (U), C-2′ (L)	114.0, 115.0	6.83	H-6′ (U), H-6′ (L)
C-5′ (U), C-5′ (L)	115.6, 115.7	6.69, 6.74	
C-6′ (U), C-6′ (L)	118.2, 119.9	6.62–6.66	H-2′ (U), H-2′ (L)
C-10 (U)	118.4		
C-5 (U)	129.5	6.62–6.66	
C-1′ (U), C-1′ (L)	131.9, 135.1		H-5′ (U), H-5′ (L)
C-4′ (U), C-4′ (L)	144.8, 145.5		H-2′ (U), H-2′ (L)
			H-6′ (U), H-6′ (L)
C-3′ (U), C-3′ (L)	145.3, 145.4		H-5′ (U), H-5′ (L)
C-5 (L)	155.0		H-4 (L), H-6 (L)
C-7 (U), C-7 (L), C-9 (U), C-9 (L)	154.7, 155.3, 156.5, 156.9		

* The spectra were measured at 30° in acetone-d_6 + D_2O.
† The average J_{CH} value for the long-range couplings was set at 8 Hz.
‡ The upper flavan residue.
§ The lower (terminal) flavan residue.

expected to show only an NOE with the methoxyl group at C-7. However, the NOESY spectrum showed cross peaks between the D-ring proton at δ 6.35 and two methoxyl groups at δ 3.67 and 3.86. Therefore, the D-ring proton must be H-6, which showed NOEs with the methoxyl groups at C-5 and C-7.

The CD spectrum of **1** (Table 2) showed a positive couplet Cotton with a large amplitude in the short wavelength region $\{[\theta]_{211} + 4.9 \times 10^4, [\theta]_{204} - 9.1 \times 10^3\}$, indicating [9, 10] β-orientation of the interflavan linkage at C-4 (U). The spectrum also showed a positive Cotton effect at around 290 nm suggesting [11] α-orientations of the phenyl groups at C-2 (U) and/or C-2 (L).



1a

In order to confirm the configurations at C-2 (U) and C-2 (L) of **1**, its CD spectrum was compared with those of synthetic compounds of related structures, **6**–**9**.

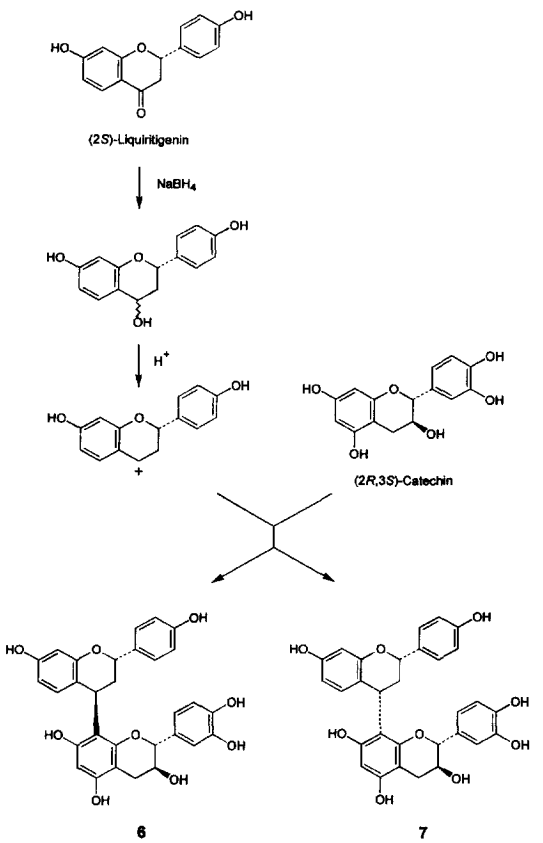
Compounds **6** and **7** were synthesized from (2*S*)-liquiritigenin and (+)-catechin with 2*R*, 3*S* structure (Scheme 1), in a way analogous to those reported for syntheses of flavan dimers [7, 9, 12, 13]. Compounds **6** and **7**, respectively, showed positive and negative Cotton effects in the short wavelength region $\{[\theta]_{211} + 9.4 \times 10^4$ (**6**); $[\theta]_{216} - 1.1 \times 10^5$ (**7**)$\}$ in their CD spectra, indicating β- and α-orientations of their interflavan bonds at C-4 (U). Chemical shifts and coupling patterns of aliphatic protons of **6** in its ^{1}H NMR spectrum are practically the same as those of **1**, indicating its 4 → 8 connection for the interflavan bond. Compound **7** also has the 4 → 8 linkage, because its ^{1}H NMR spectrum showed duplication of the signals due to restricted rotation around the interflavan linkage, in a way analogous to that shown by procyanidin B3 [4]. Therefore, **6** and **7** were regarded to have structures of (2*S*)-4′,7-dihydroxyflavan-(4β → 8)-catechin and (2*S*)-4′,7-dihydroxyflavan-(4α → 8)-catechin, respectively.

When racemic liquiritigenin, obtained upon acid hydrolysis of liquiritin, was used for the origin of the

Table 2. CD spectral data for dimeric flavans (in MeOH)

Compound	[θ] (nm)	[θ] (nm)	[θ] (nm)	[θ] (nm)	[θ] (nm)	[θ] (nm)
1	-4.6×10^3 (292)		-4.6×10^3 (273)	$+2.0\times10^4$ (234)	$+4.9\times10^4$ (211)	-9.1×10^3 (204)
2		-4.3×10^3 (286)	$+5.4\times10^3$ (274)	-2.3×10^4 (237)	-5.5×10^4 (214)	$+4.4\times10^4$ (200)*
6	-4.2×10^3 (293)		-1.9×10^4 (278)	$+5.9\times10^4$ (235)	$+9.4\times10^4$ (211)	-2.5×10^4 (200)*
7		-3.8×10^3 (286)	$+6.3\times10^3$ (276)	-4.6×10^4 (239)	-1.1×10^5 (216)	$+1.2\times10^5$ (203)
8		$+6.0\times10^3$ (286)	$+2.0\times10^3$ (278)	-1.6×10^5 (234)	-1.8×10^5 (216)	$+2.5\times10^5$ (200)*
9		$+8.3\times10^5$ (287)	-1.6×10^4 (275)	$+1.7\times10^4$ (243)	$+1.5\times10^5$ (212)	$+2.4\times10^3$ (200)*

* The lowest wavelength recorded.



Scheme 1.

upper flavan residue, **8** and **9** in addition to **6** and **7** were obtained upon the synthetic reaction. Since **8** and **9** were not obtained when (2*S*)-liquiritigenin was used, these two are considered to be originated from (2*R*)-liquiritigenin. Compounds **8** and **9**, respectively, showed negative and positive Cotton effects in the short wavelength region $\{[\theta]_{216}-1.8\times10^5$ (**8**); $[\theta]_{212}+1.5\times10^5$ (**9**)$\}$ in their CD spectra, indicating α- and β-orientations of their interflavan bonds at C-4 (U). These structures were thus assigned to (2*R*)-4′,7-dihydroxyflavan-(4α → 8)-catechin (for **8**) and (2*R*)-4′,7-dihydroxyflavan-(4β → 8)-catechin (for **9**), respectively.

The close similarity of the signal pattern of the aliphatic protons of **1** with that of **6** among the four synthetic dimers indicated that the configurations at all of the asymmetric carbons in **1** are the same as those at the corresponding carbons of **6** or its enantiomer. The CD spectral pattern of **6** was also similar to that of **1** (Table 2). Structure of KA-1 was thus determined to be (2*S*)-3′,4′,7-trihydroxyflavan-(4β → 8)-catechin (**1**).

Structure of KA-2 (**2**)

KA-2 (**2**) was obtained as a light-brown amorphous powder. The FAB-mass spectrum of **2** showed the $[M+Na]^+$ ion peak at *m/z* 569, which corresponds to the molecular formula $C_{30}H_{26}O_{10}$.

Although the ^1H and ^{13}C NMR spectra of **2** were complicated by duplication of signals, those signals shown in Table 3 indicated that **2** also has a dimeric flavan structure composed of 3′,4′,7-trihydroxyflavan and catechin. The assignments of the ^1H signals were substantiated by ^1H-^{13}C COSY. Chemical shifts and coupling constants of aliphatic proton signals in the ^1H NMR spectrum were practically the same as those of **7**. The CD spectrum of **2** showed a positive Cotton effect $\{[\theta]_{214}-5.5\times10^4\}$ instead of the negative Cotton effect of **1** in the short-wavelength region, and the spectral pattern of **2** was analogous to that of **7** (Table 2). Therefore, KA-2 was assigned to (2*S*)-3′,4′,7-trihydroxyflavan-(4α → 8)-catechin (**2**), a stereoisomer of KA-1 concerning the configuration at C-4 (U).

Inhibitory effects of flavans on lipase

Since **2** showed a potent inhibitory effect on lipase, the inhibitory activity of dimeric flavans of related structures, **1**, **3**–**7**, and procyanidins B1 (**10**), B2 (**11**) and B4 (**12**), against lipase were examined. The results are summarized in Table 4.

Although monomeric flavans, (+)-catechin and (−)-epicatechin showed negligible inhibitory effects on lipase [3], seven dimeric flavans (**1**, **3**–**7** and **10**) showed inhibitory effects with IC_{50} values of 17–42 μM. Among dimeric flavans, those having epicatechin residue as the lower flavan unit, such as procyanidins B2 (**11**) and B4 (**12**), showed negligible inhibitory effects. Although the reason for that **2** showed the most potent inhibitory effect is unclear, stereochemistry of these dimers seems to be an important factor for the inhibitory activity.

A partially purified fraction, FE-11, showed the

Table 3. ^{13}C and ^{1}H spectral data for KA-2 (**2**)*

Carbon	δ_c (ppm)	Proton coupled *via* one bond (δ_H)
C-4 (L)†	28.8, 29.3	2.48, 2.61, 2.84, 2.91
C-4 (U)‡	32.3, 32.6	4.75–4.81
C-3 (U)	35.9, 36.0	1.84, 1.97, 2.60, 2.77
C-3 (L)	68.2, 68.8	3.56, 4.01
C-2 (U)	79.5, 79.6	4.88, 4.90
C-2 (L)	81.9, 82.8	4.43, 4.68
C-6 (L)	95.6, 97.1	6.02, 6.17
C-10 (L)	100.4, 101.5	
C-8 (U)	103.6, 103.9	6.21–6.22
C-6 (U)	108.3, 108.6	6.20, 6.30
C-8 (L)	109.2, 109.3	
C-2′ (U), C-2′ (L)	114.3, 115.0,	6.53, 6.57–6.82, 6.91, 6.96, 115.3
C-5′ (U), C-5′ (L)	115.7	6.57–6.82
C-6′ (U), C-6′ (L)	118.5, 118.7,	6.00, 6.57–6.82, 119.2, 119.6
C-10 (U)	119.5	
C-5 (U)	129.1, 129.4	6.57–6.82
C-1′ (U), C-1′ (L)	131.7, 132.3, 134.9, 135.1	
C-3′ (U), C-3′ (L), C-4′ (U), C-4′ (L)	144.5–145.7	
C-5 (L), C-7 (U), C-7 (L), C-9 (U), C-9 (L)	154.8–157.1	

* The spectra were measured at 27° in acetone-d_6 + D_2O.
† The lower (terminal) flavan residue.
‡ The upper flavan residue.

Table 4. Inhibitory effects of dimeric flavans on lipase

Compound	IC_{50} (μM)*
1	33
2	5.5
3	35
4	40
5	22
6	31
7	17
10	42
11	> 50
12	> 50

* Concentration for 50% inhibition of the activity of porcine pancreatic lipase. 4-Methylumbelliferyl oleate was used for the substrate and estimation of the enzyme activity was based on the amount of produced 4-methylumbelliferone, which was fluorometrically measured (excitation, 320 nm; emission, 450 nm).

most potent inhibitory effect (IC_{50}, 0.20 μg ml^{-1}) among the fractions from the diethyl ether-soluble portion of the fruit extract. Although composition of constituent monomers have not yet been clarified, the ^{13}C NMR spectrum [δ 26–37 (C-4, C-3), 67–82 (C-3, C-2), 95–112 (C-6, C-8, C-10), 112–122 (C-2′, C-5′, C-6′, C-10), 126–136 (C-5, C-1′), 144–146 (C-3′, C-4′), 151–158 (C-5, C-7, C-9)] which cover the signals of **1** suggested that the major monomeric constituents of the oligomers are 3′,4′,7-trihydroxyflavan and catechin. The elution profile of the fraction on gel permeation chromatography [14] (GPC) indicated M_n 1020 and M_w 1260 for this fraction.

EXPERIMENTAL

General. *m*-Nitrobenzyl alcohol was used as a matrix reagent for FAB-MS. ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 126 MHz, respectively, in acetone-d_6 containing *ca* 3% of D_2O, unless mentioned otherwise. Chemical shifts are given in δ values (ppm), and solvent signals (δ 2.04 for ^{1}H and δ 29.8 for ^{13}C) were used as int. standards for the chemical shifts. A YMC-pack A324 (ODS, 5 μm) column (10 mm × 30 cm), and two solvent systems 0.01 M H_3PO_4–0.01 M KH_2PO_4–MeCN (41:41:18 and 43:43:14) were used for prep. HPLC.

Estimation of lipase inhibitory activity. A mixt. of porcine pancreatic lipase (Type II, 2.2 μg), 4-methylumbelliferyl oleate (0.05 mM) and a tested compound in McIlvaine buffer (pH 7.4, 200 μl) containing 1.25% THF was incubated for 20 min at 37°. The enzyme reaction was then terminated by addition of 0.1 M HCl (1 ml), and the pH of the soln was adjusted to pH 4.3 with 0.1 M sodium citrate (2 ml). The inhibitory activity of the tested sample was estimated based on the amount of produced 4-methylumbelliferone in the soln which was fluorometrically measured (excitation, 320 nm; emission, 450 nm).

High-performance GPC. Oligomeric flavan frs were acetylated with acetic anhydride and pyridine, and then analysed by GPC using a Shimadzu HSG-15 column (7.9 mm × 50 cm) developed with THF. Column temp. and flow rate were set at 40° and 1.0 ml min^{-1}, respectively. Peracetates of (−)-epicatechin (monomer), procyanidin B2 (dimer) and procyanidin C1 (trimer) were used as standards. The M_n and M_w values for the acetates of oligomer frs were then con-

verted for the free phenolic forms of multiplication by 0.58.

Isolation of phenolic components from Cassia nomame. Dried fruits (100 g) of *Cassia nomame*, collected at Naka-kubiki-gun, Niigata Prefecture, Japan, in October, were homogenized in Me_2CO–H_2O (7:3, 2.3 L), and concd filtrate from the homogenate was extracted with Et_2O, AcOEt and *n*-BuOH, successively. Each solvent was evapd off, to give the Et_2O (4.1 g), EtOAc (4.3 g), *n*-BuOH (4.9 g) and aq. extracts. The Et_2O extract (3.8 g) was subjected to CC over Toyopearl HW-40 (coarse grade) with 70% EtOH (for FE-1–FE-12) and then with 70% Me_2CO (for FE-13) as eluants, to separate FE-1–FE-13. The EtOAc extract was treated in an analogous way, to give FA-1–FA-11 (70% EtOH eluate) and FA-12 (70% Me_2CO eluate). FA-3 was further purified by CC on MCI-gel CHP-20P with aq. MeOH to give luteolin 7-*O*-β-D-glucoside (5.1 mg). FA-6 was chromatographed over MCI-gel CHP-20P with aq. MeOH to give (+)-catechin (1.8 mg), KA-1 (**1**) (79.4 mg), KA-2 (**2**) (39.3 mg), (−)-fisetinidol-(4α → 8)-catechin (**4**) (9.9 mg) and (+)-fisetinidol-(4β → 8)-catechin (**5**) (4.8 mg). FA-7 was purified by CC on MCI-gel CHP-20P and then on Toyopearl HW-40 (coarse grade) to give procyanidin B3 (**3**) (0.2 mg).

KA-1 (**1**). $[\alpha]_D$: +59° (MeOH, *c* 1.0). (Found: C, 61.3; H, 5.1. $C_{30}H_{26}O_{10}\cdot 2H_2O$ requires: C, 61.8; H, 5.2%.) FAB-MS *m/z*: 569 ($[M+Na]^+$), 547 ($[M+H]^+$) (positive-ion mode); 545 ($[M-H]^-$) (negative-ion mode). UV λ^{MeOH} nm (log ε): 209 (4.82), 232 (sh) (4.45), 282 (3.97). ^{1}H NMR (30°): δ 2.17 [1H, *m*; H-3 of upper unit (U)], 2.45 [1H, *br m*; H-3 (U)], 2.57 [1H, *dd*, *J* = 8, 16 Hz; H-4 of lower unit (L)], 2.92 [1H, *dd*, *J* = 5.5, 16 Hz; H-4 (L)], 4.02 [1H, *m*; H-3 (L)], 4.42 [1H, *br*; H-2 (L)], 4.47 [1H, *t*, *J* = 6 Hz; H-4 (U)], 5.24 [1H, *m*; H-2 (U)], 6.08 [1H, *s*; H-6 (L)], 6.23–6.26 [2H, *m*; H-8 (U), H-6 (U)], 6.62–6.66 [3H, *m*; H-6′ (U), H-6′ (L), H-5 (U)], 6.69, 6.74 [1H each, *d*, *J* = 8 Hz; H-5′ (U), H-5′ (L)], 6.83 [2H, *d*, *J* = 2 Hz; H-2′ (U), H-2′ (L)]. ^{13}C NMR: see Table 1.

Methylation of KA-1 (**1**). A mixt. of **1** (28 mg), dimethyl sulphate (45 μl) and K_2CO_3 (45 mg) in Me_2CO (9 ml) was stirred overnight, and then refluxed for 4 hr. Insoluble material in the mixt. was removed by centrifugation, and the supernatant was evapd. The residue was subjected to prep. TLC developed with toluene–Me_2CO (4:1), to give a heptamethyl derivative (**1a**), ^{1}H NMR (in acetone-d_6, 40°): δ 2.26 [1H, *dt*, *J* = 7, 13 Hz; H-3 (U)], 2.41 [1H, *ddd*, *J* = 3.5, 7, 13 Hz; H-3 (U)], 2.53 [1H, *dd*, *J* = 9, 16.5 Hz; H-4 (L)], 3.00 [1H, *dd*, *J* = 6, 16.5 Hz; H-4 (L)], 3.63, 3.67, 3.71, 3.74, 3.79, 3.86 (3H each, *s*; $OCH_3\times 7$), 4.01 [1H, *m*; H-3 (L)], 4.26 [1H, *br m*; H-2 (L)], 4.51 [1H, *t*, *J* = 7 Hz; H-4 (U)], 5.34 [1H, *dd*, *J* = 3.5, 7 Hz; H-2 (U)], 6.14 [1H, *br s*; H-8 (U)], 6.25 [1H, *dd*, *J* = 2.5, 8 Hz; H-6 (U)], 6.35 [1H, *s*; H-6 (L)], 6.59 [1H, *d*, *J* = 8 Hz; H-5 (U)], 6.74, 6.86 [1H each, *dd*, *J* = 2, 8 Hz; H-6′ (U), H-6′ (L)], 6.78, 6.83 [1H each, *d*, *J* = 8 Hz; H-5′ (U), H-5′ (L)], 6.84, 6.95 [1H each, *d*, *J* = 3 Hz; H-2′ (U), H-2′ (L)].

KA-2 (**2**). $[\alpha]_D$: −85° (MeOH, *c* 1.0). (Found: C, 62.1; H, 5.0. $C_{30}H_{26}O_{10}\cdot 2H_2O$ requires: C, 61.9; H, 5.2%.) FAB-MS *m/z*: 569 ($[M+Na]^+$) (positive-ion mode). UV λ^{MeOH} nm (log ε): 210 (4.84), 231 (sh, 4.46), 281 (3.99). ^{1}H NMR (27°): δ 1.84, 1.97 [1H in total, *ddd*, *J* = 1.5, 5.5, 12.5 Hz; H-3 (U)], 2.48, 2.61 [1H in total, *dd*, *J* = 9, 16 Hz; H-4 (L)], 2.60, 2.77 [1H in total, *m*; H-3 (U)], 2.84, 2.91 [1H in total, *dd*, *J* = 5.5, 16 Hz; H-4 (L)], 3.56, 4.01 [1H in total, *m*; H-3 (L)], 4.43, 4.68 [1H in total, *d*, *J* = 7.5 Hz; H-2 (L)], 4.75–4.81 [1H in total, *m*; H-4 (U)], 4.88, 4.90 [1H in total, *br d*, *J* = 12.5 Hz; H-2 (U)], 6.00 [0.5H, *dd*, *J* = 2, 8 Hz; H-6′ (L)], 6.02, 6.17 [1H in total, *s*; H-6 (L)], 6.20, 6.30 [1H in total, *dd*, *J* = 2.5, 8.5 Hz; H-6 (U)], 6.21–6.22 [1H in total, *d*, *J* = 2.5 Hz; H-8 (U)], 6.53 [0.5H, *d*, *J* = 2 Hz; H-2′ (L)], 6.57–6.82 [5H in total, *m*; H-2′ (L), H-5′ (L), H-6′ (L), H-5 (U), H-5′ (U), H-6′ (U)], 6.91, 6.96 [1H in total, *br s*, H-2′ (U)]. ^{13}C NMR: see Table 3.

(−)-*Fisetinidol*-(4α → 8)-*catechin* (**4**). $[\alpha]_D$: −67° (MeOH, *c* 1.0). FAB-MS *m/z*: 585 ($[M+Na]^+$), 563 ($[M+H]^+$) (positive-ion mode). UV λ^{MeOH} nm (log ε): 208 (4.81), 230 (sh, 4.47), 280 (4.03). CD (MeOH) [θ] (nm): -4.4×10^3 (285), $+4.4\times 10^3$ (273), -2.8×10^4 (234), -5.5×10^4 (214), $+6.1\times 10^4$ (202). ^{1}H NMR (28°): δ 2.49, 2.61 [1H in total, *dd*, *J* = 8.5, 16 Hz; H-4 (L)], 2.80, 2.87 [1H in total, *dd*, *J* = 5.5, 16 Hz; H-4 (L)], 3.65, 3.98 [1H in total, *m*; H-3 (L)], 4.45–4.65 [4H in total, *m*; H-2 (L), H-2 (U), H-3 (U) and H-4 (U)], 6.02, 6.17 [1H in total, *s*; H-6 (L)], 6.06 [0.5H, *dd*, *J* = 2, 8 Hz; H-6′ (L)], 6.21, 6.31 [1H in total, *dd*, *J* = 2.5, 8.5 Hz; H-6 (U)], 6.19 [1H in total, *br d*, *J* = 8 Hz; H-8 (U)], 6.54–6.59 [2H in total, *m*; H-2′ (L), H-6′ (L), H-5 (U)], 6.61, 6.68, 6.73, 6.78 [2H in total, *d*, *J* = 8 Hz; H-5′ (L), H-5′ (U)], 6.76–6.83 [1H in total, *dd*, *J* = 2, 8 Hz; H-6′ (L), H-6′ (U)], 6.97, 7.00 [1H in total, *d*, *J* = 2 Hz; H-2′ (L), H-2′ (U)]. ^{13}C NMR (40°) δ: 28.8, 29.0 [C-4 (L)], 41.4, 41.7 [C-4 (U)], 68.4, 68.5 [C-3 (L)], 70.5 [C-3 (U)], 82.0, 82.7 [C-2 (L)], 84.0, 84.1 [C-2 (U)], 96.1, 97.1 [C-6 (L)], 100.3, 101.4 [C-10 (L)], 103.1, 103.4 [C-8 (U)], 107.7 [C-8 (L)], 108.8, 109.0 [C-6 (U)], 114.9, 115.2, 115.5 [C-2′ (U), C-2′ (L)], 115.6, 115.7, 116.0, 116.3 [C-5′ (U), C-5′ (L)], 118.6, 119.1 [C-10 (U)], 119.2, 119.9, 120.5, 120.7 [C-6′ (U), C-6′ (L)], 129.5, 129.7 [C-5 (U)], 131.8, 132.3, 132.5, 132.7 [C-1′ (U), C-1′ (L)], 144.8–145.7 [C-3′ (U), C-3′ (L), C-4′ (U), C-4′ (L)], 154.7, 156.9 [C-5 (L), C-7 (U), C-7 (L), C-9 (U), C-9 (L)].

(+)-*Fisetinidol*-(4β → 8)-*catechin* (**5**). $[\alpha]_D$: −41° (MeOH, *c* 1.0). FAB-MS *m/z*: 585 ($[M+Na]^+$), 563 ($[M+H]^+$) (positive-ion mode). UV λ^{MeOH} nm (log ε): 209 (4.80), 230 (sh, 4.38), 280 (3.88). CD (MeOH) [θ] (nm): -5.5×10^3 (292), -5.5×10^3 (279), -1.1×10^4 (230), -2.7×10^4 (214), $+6.6\times 10^4$ (200, lowest wavelength measured). ^{1}H NMR (28°): δ 2.55 [1H, *dd*, *J* = 7.5, 16 Hz; H-4 (L)], 2.88 [1H, *br d*, *J* = 16 Hz; H-4 (L)], 3.95 [1H, *m*; H-3 (L)], 4.33 [1H, *br m*; H-3 (U)], 4.48 [1H, *br s*; H-2 (L)], 4.76 [1H, *br s*; H-4 (U)],

5.24 [1H, *s*; H-2 (U)], 6.01 [1H, *br s*, H-6 (L)], 6.25–6.74 [9H, *m*, H-2′ (L), H-5′ (L), H-6′ (L), H-5 (U), H-2′ (U), H-5′ (U), H-6′ (U)]. ^{13}C NMR (40°): δ 28.6 [C-4 (L)], 31.9 [C-4 (U)], 67.8 [C-3 (L)], 72.3 [C-3 (U)], 81.6 [C-2 (L)], 82.3 [C-2 (U)], 97.8 [C-6 (L)], 100.5 [C-10 (L)], 103.2 [C-8 (U)], 105.7 [C-6 (U)], 108.6 [C-8 (L)], 114.0, 115.0 [C-2′ (U), C-2′ (L)], 115.6, 115.7 [C-5′ (U), C-5′ (L)], 118.2, 119.9 [C-6′ (U), C-6′ (L)], 118.4 [C-10 (U)], 129.5 [C-5 (U)], 131.8, 132.7 [C-1′ (U), C-1′ (L)], 144.9, 145.3, 145.6 [C-3′ (U), C-3′ (L), C-4′ (U), C-4′ (L)], 155.0, 155.4, 155.9, 156.8, 157.6 [C-5 (L), C-7 (U), C-7 (L), C-9 (U), C-9 (L)].

FE-11. (Found: C, 64.96; H, 5.01. Calc. for $(C_{15}H_{12}O_{4.2}\cdot H_2O)_n$: C, 64.93; H, 5.09.) UV λ^{MeOH} nm: 224, 282. IR ν^{KBr} cm^{-1}: 1600, 1500, 1440, 1280–1180, 1150, 1110, 1070, 1020, 970, 840, 800, 770. CD [θ] (nm) (in MeOH): -1.5×10^3 (289), $+4.8\times10^3$ (276), $+2.7\times10^4$ (235), $+6.3\times10^3$ (223), $+1.8\times10^4$ (215), -4.0×10^4 (200, shortest wavelength measured). The [θ] values were calculated based on the M_n value of 1020, which was estimated with high-performance GPC. ^{13}C NMR (in acetone-d_6+D_2O, 1:1, 40°): see text.

Syntheses of dimeric flavans **6–9**. (2*S*)-Liquiritigenin (30 mg) in EtOH (6 ml) was treated with sodium borohydride (250 mg) in the presence of (+)-catechin (90 mg) under N_2 stream for 30 min at pH 6–7 which was adjusted with 1.5 M AcOH. After adding water (6 ml) to the reaction mixt., the soln was adjusted to pH 5 with 1.5 M AcOH, and was kept at 45° for 8.5 hr under N_2 stream. The soln was then diluted with H_2O (6 ml), and extracted with EtOAc (8 ml × 5). The organic phase was evapd off, and the residue was subjected to CC over MCI-gel CHP-20P, to give **6** (9.3 mg) and **7** (4.8 mg).

(2*RS*)-liquiritigenin (30 mg) was treated in an analogous way. A mixt. of products was chromatographed over MCI-gel CHP-20P and further purified by prep. HPLC, to give **6** (1.1 mg), **7** (1.5 mg), **8** (2.6 mg) and **9** (2.5 mg).

Compound **6**. $[\alpha]_D$: +57° (MeOH, *c* 1.3). UV λ^{MeOH} nm (log ε): 207 (4.75), 228 (sh, 4.45), 282 (3.78). ^{1}H NMR δ: 2.18 [1H, *m*; H-3 (U)], 2.41 [1H, *br m*; H-3 (U)], 2.53 [1H, *dd*, J = 8, 16 Hz; H-4 (L)], 2.90 [1H, *dd*, J = 5.5, 16 Hz; H-4 (L)], 3.99 [1H, *m*; H-3 (L)], 4.39 [1H, *br*; H-2 (L)], 4.46 [1H, *t*, J = 6.5 Hz; H-4 (U)], 5.27 [1H, *m*; H-2 (U)], 6.07 [1H, *s*; H-6 (L)], 6.21–6.24 [2H, *m*; H-8 (U) and H-6 (U)], 6.62 [1H, *m*; H-6′ (L)], 6.70 [2H, *d*, J = 9 Hz; H-3′ (U), H-5′ (U)], 6.65–6.74 [2H, *m*; H-5 (U), H-5′ (L)], 6.83 [1H, *br s*; H-2′ (L)], 7.11 [2H, *d*, J = 8 Hz; H-2′ (U), H-6′ (U)].

Compound **7**. $[\alpha]_D$: −87° (MeOH, *c* 1.0). UV λ^{MeOH} nm (log ε): 207 (4.75), 228 (sh, 4.45), 282 (3.78). ^{1}H NMR δ: 1.81, 1.94 [1H in total, *ddd*, J = 2, 6, 12 Hz; H-3 (U)], 2.47, 2.61 [1H in total, *dd*, J = 9, 16 Hz; H-4 (L)], 2.64, 2.80 [1H in total, *m*; H-3 (U)], 2.87, 2.90 [1H in total, *dd*, J = 5.5, 16.5 Hz; H-4 (L)], 3.54, 4.00 [1H in total, *m*; H-3 (L)], 4.38, 4.67 [1H in total, *d*, J = 8 Hz; H-2 (L)], 4.76, 4.81 [1H in total, *dd*, J = 6, 12 Hz; H-4 (U)], 4.92, 4.96 [1H in total, *dd*, J = 2, 12.5 Hz; H-2 (U)], 6.02, 6.17 [1H in total, *s*; H-6 (L)], 6.04 [0.5H, *dd*, J = 2, 8 Hz; H-6′ (L)], 6.20, 6.29 [1H in total, *dd*, J = 2.5, 8 Hz; H-6 (U)], 6.21–6.22 [1H in total, *d*, J = 2.5 Hz; H-8 (U)], 6.55 [0.5H, *d*, J = 2 Hz; H-2′ (L)], 6.58–6.77 [2.5H in total, *m*; H-5′ (L), H-6 (L), H-5 (U)], 6.71, 6.82 [1H in total, *d*, J = 8.5 Hz; H-3′ (U), H-5′ (U)], 6.90 [0.5H, *d*, J = 2 Hz; H-2′ (L)], 7.04, 7.28 [1H in total, *d*, J = 8.5 Hz; H-2′ (U), H-6′ (U)].

Compound **8**. $[\alpha]_D$: −135° (MeOH, *c* 1.2). UV λ^{MeOH} nm (log ε): 207 (4.76), 228 (sh, 4.47), 282 (3.81). ^{1}H NMR δ: 2.20 [1H, *m*; H-3 (U)], 2.50 [1H, *br m*; H-3 (U)], 2.52 [1H, *dd*, J = 8, 16 Hz; H-4 (L)], 2.91 [1H, *dd*, J = 5.5, 16 Hz; H-4 (L)], 3.72 [1H, *br m*; H-3 (L)], 4.42 [1H, *t*, J = 6 Hz; H-4 (U)], 4.48 [1H, *d*, J = 8 Hz; H-2 (L)], 5.29 [1H, *m*; H-2 (U)], 6.07 [1H, *s*; H-6 (L)], 6.24 [1H, *dd*, J = 2, 8 Hz; H-6 (U)], 6.30 [1H, *d*, J = 2 Hz; H-8 (U)], 6.58–6.73 [4H, *m*; H-2′ (L), H-5′ (L), H-6′ (L), H-5 (U)], 6.71 [2H, *d*, J = 8.5 Hz; H-3′ (U), H-5′ (U)], 7.11 [2H, *d*, J = 8.5 Hz; H-2′ (U), H-6′ (U)].

Compound **9**. $[\alpha]_D$: +67° (MeOH, *c* 0.5). UV λ^{MeOH} nm (log ε): 208 (4.75), 228 (sh, 4.46), 282 (3.78). ^{1}H NMR δ: 1.82, 1.86 [1H in total, *ddd*, J = 2, 5.5, 12.5 Hz; H-3 (U)], 2.43, 2.57 [1H in total, *dd*, J = 9, 16 Hz; H-4 (L)], 2.80, 2.85 [1H in total, *m*; H-3 (U)], 2.94, 2.97 [1H in total, *dd*, J = 5.5, 16 Hz; H-4 (L)], 3.83, 3.98 [1H in total, *dt*, J = 5.5, 9 Hz; H-3 (L)], 4.02, 4.62 [1H in total, *d*, J = 8 Hz; H-2 (L)], 4.69, 4.77 [1H in total, *dd*, J = 5.5, 12.5 Hz; H-4 (U)], 4.89, 4.93 [1H in total, *dd*, J = 2, 12.5 Hz; H-2 (U)], 5.99 [0.5H, *d*, J = 2.5 Hz; H-8 (U)], 6.02, 6.18 [1H in total, *s*; H-6 (L)], 6.14 [0.5H, *dd*, J = 2.5, 8.5 Hz; H-6 (U)], 6.23–6.26 [1H in total, *m*; H-6 (U), H-8 (U)], 6.47, 6.52 [1H in total, *dd*, J = 2, 8 Hz; H-6′ (L)], 6.66–6.79 [5H in total, *m*; H-2′ (L), H-5′ (L), H-5 (U)], 6.80, 6.82 [1H in total, *d*, J = 8.5 Hz; H-3′ (U), H-5′ (U)], 6.91 [0.5H, *br s*; H-2′ (L)], 7.16, 7.25 [1H in total, *d*, J = 8.5 Hz; H-2′ (U), H-6′ (U)].

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