Pharmacologically Active Components of Viticis Fructus (*Vitex rotundifolia*). II. The Components Having Analgesic Effects[1)]

Emi OKUYAMA,*[,a] Shigeru FUJIMORI,[a] Mikio YAMAZAKI,[a] and Takeshi DEYAMA[b]

Faculty of Pharmaceutical Sciences,[a] Chiba University, 1–33 Yayoi-cho, Inage-ku, Chiba 263–0022, Japan, and Yomeishu Seizo Co., Ltd.,[b] 2132–37, Nakaminowa, Minowa-machi, Kamiina-gun, Nagano 399–46, Japan.
Received October 8, 1997; accepted November 26, 1997

The extract of Viticis Fructus appeared to have an analgesic effect, and was subjected to activity-guided separation using acetic acid-induced writhing in mice. The active fraction gave new compounds, vitexfolin A (1A), B and C, 10-*O*-vanilloylaucubin (3), dihydrodehydrodiconiferylalcohol-β-D-(2′-*O*-*p*-hydroxybenzoyl)glucoside (4), and vanilloyl-β-D-(2′-*O*-*p*-hydroxybenzoyl)glucoside, together with agnuside (2) and *erythro*- and *threo*-guaiacylglycerols. Compounds 1A and 2—4 showed significant writhing inhibition following oral administration at doses of 15, 50, 25, and 50 mg/kg, respectively. The effect on pressure pain threshold was tested using compounds 1A and 2 at a dose of 50 mg/kg, and only the former produced the analgesia. The analgesic effect of some related iridoid glucosides is also discussed.

Key words *Vitex rotundifolia*; Verbenaceae; analgesic effect; vitexfolin; iridoid glucoside; neolignan glucoside

Viticis Fructus (Mankeishi in Japanese) is the dried fruit of *Vitex rotundifolia* L. fil. (Verbenaceae),[2)] which has been used in prescriptions in Japanese (Kampo) or Chinese traditional medicine for the treatment of colds, headache, migraine, eye-pain, *etc.*[3)] In Part I we reported that the extract of Viticis Fructus produced both a vascular relaxation effect in rat aortic strips and an analgesic effect in mice.[1)] The bioassay-oriented isolation using the former activity yielded flavonoids as the active components. The isolation procedure in Part I also showed that the vasodilatory and analgesic activities were derived from different fractions. This paper (Part II) deals with the isolation and structural elucidation of the components which produced the analgesic effect. Since iridoid glucosides were included in the active components, some other iridoids are also discussed in terms of their analgesic effects.

The methanol extract of Viticis Fructus was separated by following writhing inhibition in mice as an activity-guide, which procedure is summarized in Chart 1. By successive partition of the extract with *n*-hexane, ethyl acetate and water, the activity was found to be concentrated in the ethyl acetate fraction. This fraction was subjected to Sephadex LH-20 column chromatography after removing the insoluble part in acetone–methanol. Fraction 1-B, producing analgesia, was separated into three fractions by silica gel chromatography with a gradient of *n*-hexane–ethyl acetate as eluent. The analgesic effect was only observed in fraction 2-C, while fraction 2-A exhibited a vasodilatory effect. Separation of the latter fraction was reported in Part I.[1)] Column chromatography of fraction 2-C by silica gel with chloroform–methanol–water concentrated the activity in fraction 3-C, which was again chromatographed on Sephadex LH-20. Activity was exhibited in both fractions 4-A and 4-B with an inhibition ratio of 27% and 51%, respectively, at an oral dose of 150 mg/kg. The latter fraction was flash chromatographed on octadecyl silica gel (ODS) to give fraction 5-B, which yielded compounds **1A** and a mixture with its isomer, **1B**. Fraction 5-A showed no significant effect, but had a tendency for analgesia at a dose of 100 mg/kg. Separation of this fraction was then carried out to give compound **2**, which was also obtained from the other fractions, and compound **6**. Another active fraction, faction 4-A, was chromatographed on silica gel, and fr. 7-C, one of the two active fractions, was repeatedly chromatographed to yield compound **2** as a major component and compound **3**. Separation of fraction 7-B gave fraction 9-B, having an analgesic effect at a dose of 60 mg/kg, from which compounds **4**, **5**, **7** and **8** together with **2** and **3** were obtained.

The structure of compound **2** was examined by 2D-NMR, and was identified as agnuside, the isolation of which has been previously reported from the leaves of *V. rotundifolia*.[4)] The ^{1}H-NMR of compound **3** was very similar to that of agnuside except for the additional methyl group at δ 3.90 (3H, s). The 3″ position of the methoxyl group in **3** was determined by the nuclear Overhauser effect (NOE) of the aromatic proton at δ 7.58 (1H, d, J=2.0 Hz) caused by irradiation of this group. 2D-NMR, such as heteronuclear multiple bond correlation spectroscopy (HMBC), confirmed the structure of **3** to be 10-*O*-vanilloylaucubin.

Compound **1A** is a dark yellow powder, $[\alpha]_{589}$ −18°, and its molecular formula, $C_{25}H_{28}O_{11}$ (MW 504), was determined by high resolution (HR) FAB-MS. It gave a positive spot after spraying with ferric chloride on TLC, and showed a bathochromic shift in the UV spectrum following the addition of sodium hydroxide solution. The ^{13}C-NMR spectrum suggested a sugar moiety at δ 64.7, 72.1, 74.9, 75.7, 77.6 and 104.3. Considering their chemical shifts[4)] and the coupling constant (7.3 Hz) of the anomeric proton in the ^{1}H-NMR, the compound was indicated to be a phenolic β-D-glucoside. In the ^{1}H-NMR, there are two sets of signals at δ 6.44 (1H, dd, J=8.3 and 2.2 Hz), 7.01 (1H, d, J=8.3 Hz) and 6.65 (1H, d, J=2.2 Hz), and at δ 6.97 (1H, dd, J=8.3, 2.0 Hz), 7.07 (1H, d, J=8.3 Hz) and 6.81 (1H, d, J=2.0 Hz), corresponding to 1, 2 and 4-substituted benzenes. The olefinic protons assigned at δ 6.30 (1H, d, J=16.0 Hz) and 7.58 (1H, d, J=16.0 Hz) were *trans*-coupled to each other, and the irradiation of each proton showed NOEs in the aromatic protons at δ 6.97 and 7.07. The *trans*-caffeoyl moiety including these protons

* To whom correspondence should be addressed.

© 1998 Pharmaceutical Society of Japan

NII-Electronic Library Service

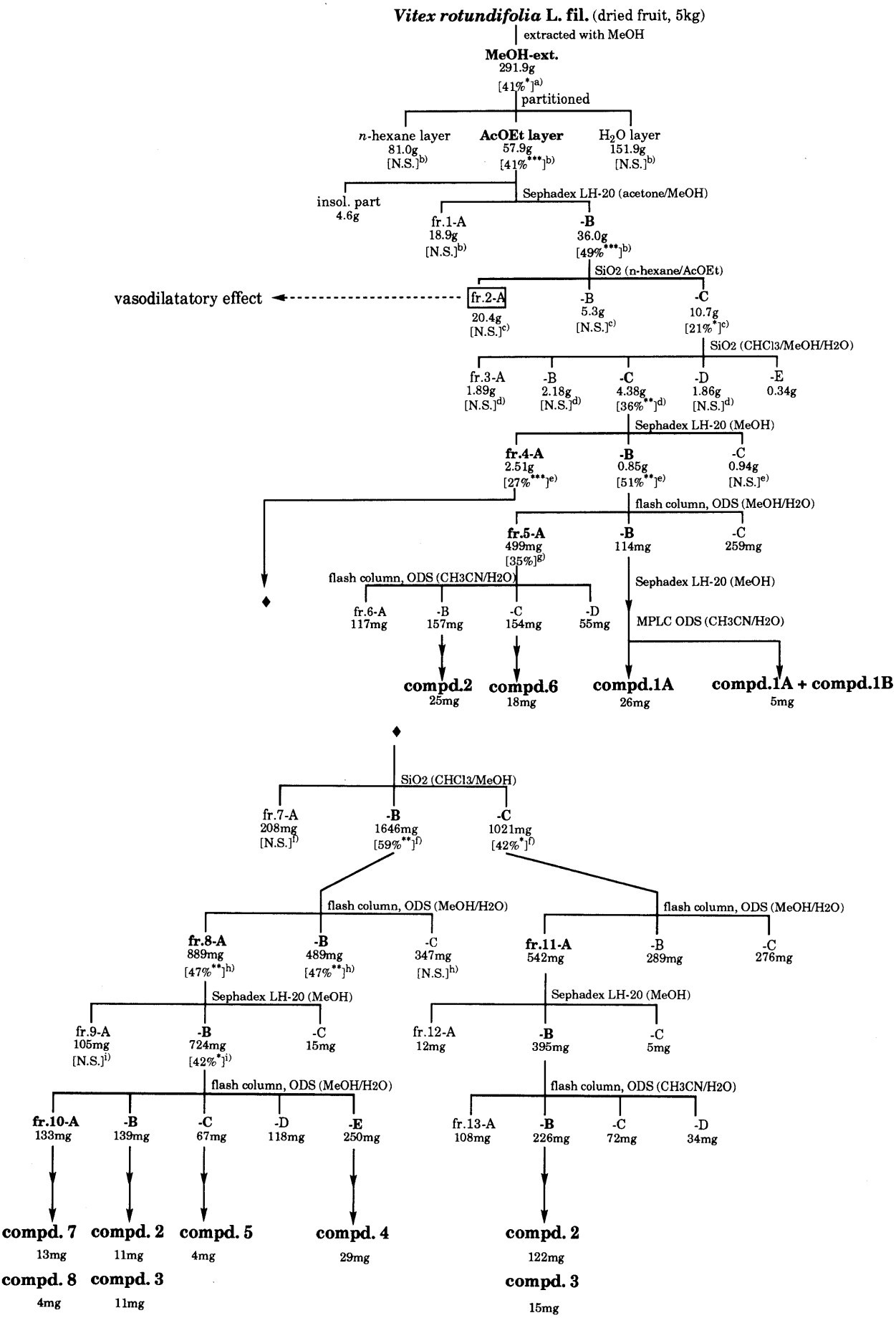

Chart 1. Isolation Procedure of the Components Having an Analgesic Effect

[ ]: writhing inhibition, % vs. control; *a*) 2 g/kg, *b*) 1 g/kg, *c*) 500 mg/kg, d*)* 200 mg/kg, *e*) 150 mg/kg, *f*) 120 mg/kg, *g*) 100 mg/kg, *h*) 80 mg/kg, *i*) 60 mg/kg, *p.o.*
$*p<0.05$, $**p<0.01$, $***p<0.001$.

NII-Electronic Library Service

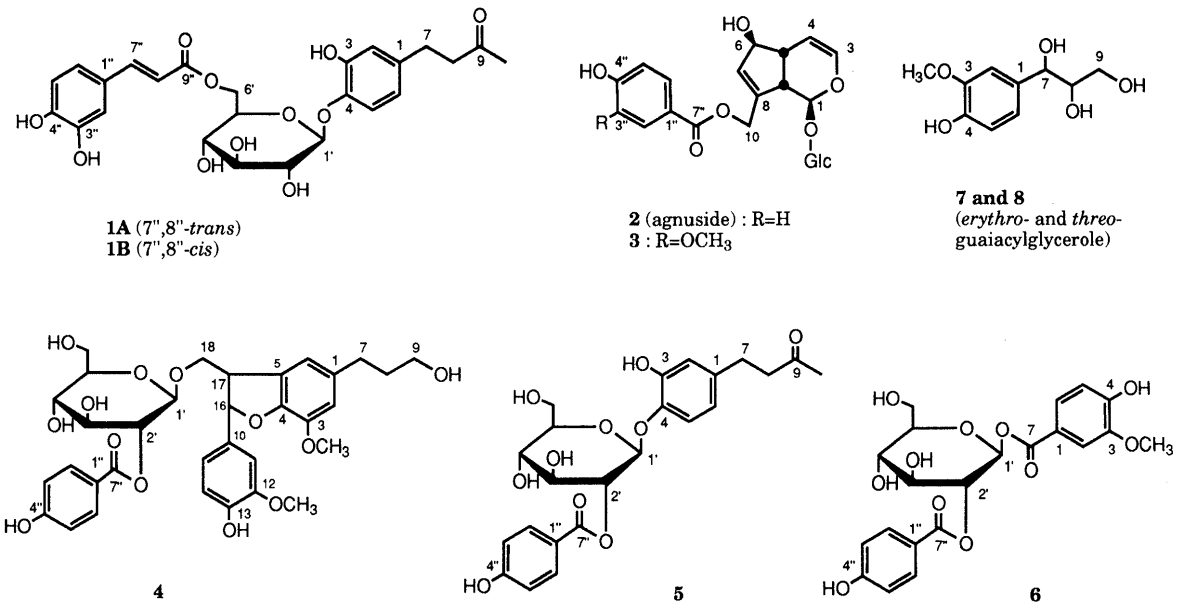

Fig. 1. Structures of the Isolated Compounds **1A**—**8**

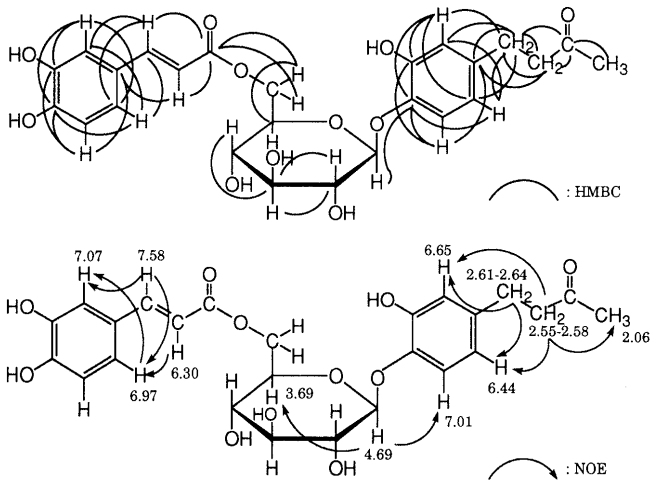

Fig. 2. HMBC and NOE Correlations of Compound **1A**

was also examined by ^1H–^1H and ^{13}C–^1H correlation spectroscopy (COSY) and HMBC. The correlations in HMBC and NOE difference (NOEDF) experiments are presented in Fig. 2. The carboxyl group of the caffeoyl moiety was connected to C-6′ in the glucose part since the carbonyl carbon at δ 168.9 (C-9″) had correlations with 6′-methylene protons at δ 4.39 and 4.54, together with δ 7.58 (H-7″) and 6.30 (H-8″). A butanone substituent in another aromatic ring (A-ring) was derived from the protons at δ 2.06 (3H, s; C-10), 2.55—2.58 (2H, m; C-8) and 2.61—2.64 (2H, m; C-7) in the ^1H-NMR, and the carbons at δ 30.0 (C-10), 211.2 (C-9), 45.7 (C-8) and 30.1 (C-7) in the ^{13}C-NMR, by COSY and HMBC. This substituent was bonded to C-1 of the A-ring, and the aromatic carbon at the 4 position to an oxygen atom at C-1′ of the glucose moiety, as suggested by the correlations of δ_H 6.44 (H-6) and 6.65 (H-2)/δ_C 30.1 (C-7), and δ_H 4.69 (H-1′)/δ_C 144.9 (C-4) in the HMBC. These are also supported by the NOEs observed between H-2 and H-6 in the A-ring and the methylenes of the butanone substituent, and between H-1′ and H-5. Although there were 14 carbons in the aromatic or olefinic region in the ^{13}C-NMR, four carbons at δ 147.0 (C-3″), 149.8 (C-4″) and 148.2 (C-3) including C-4 at δ 144.9 were estimated to have *O*-functions by their chemical shifts. From these data and the chemical formula, the structure of compound **1A** was finally elucidated as that shown in Fig. 1.

Compound **1B** was obtained in a mixture with compound **1A** because of its instability. As summarized in Table 1, the ^1H-NMR extracted from the data on the mixture was very similar to that of compound **1A**, except for the olefinic protons in the caffeoyl moiety. Compound **1B** was thought to be a *cis*-isomer at the 7″ position by the coupling constant of 12.7 Hz between the protons at δ 6.83 (H-7″) and δ 5.79 (H-8″). In the NOEDF experiment, NOEs were observed in the signals at δ 7.07 (1H, dd, J=8.3 and 1.9 Hz; H-6″), 7.38 (H, d, J=1.9 Hz; H-2″) and 5.79 (H-8″) by irradiation of the olefinic proton at δ 6.83. Irradiation of the anomeric proton at δ 4.67 (1H, d, J=7.5 Hz; H-1′) and the methylenes at δ 2.69—2.70 (4H, m; H_2-7 and H_2-8) also indicated NOEs in the signals at δ 6.96 (1H, d, J=8.3 Hz; H-5), and δ 6.49 (1H, dd, J=8.3 and 2.2 Hz; H-6) and 6.66 (1H, d, J=2.2 Hz; H-2), respectively. These data supported the position of each chromophore.

Compound **5**, a pale yellow powder, seemed to be a phenylbutanone glycoside from its spectrum. The molecular formula, $C_{23}H_{26}O_{10}$ (MW 462), was obtained from HR-FAB-MS. The presence of the same phenylbutanone part (A-ring) in compound **5** as **1A** was suggested by the corresponding signals in the ^1H- and ^{13}C-NMR. The NMR data, including 2D-NMR such as HMBC shown in Fig. 3, also indicated the glucose moiety, and the A-ring was estimated to bond to the anomeric position at δ 5.09 (1H, d, J=7.9 Hz) because of the correlation of δ 5.09 (H-1′)/d 144.7 (C-4) in the HMBC. The signals derived from the caffeoyl moiety of 1A were not observed in the ^1H-NMR of **5**, but AA′BB′ type-aromatic signals were assigned at δ 6.82—6.83 (2H, m; H-3″ and H-5″) and 7.93—7.95 (2H, m; H-2″ and H-6″). In the HMBC spec-

NII-Electronic Library Service

Table 1. ^{1}H-NMR Data (δ and J in Hz) of Compounds **1A**, B and **4**—**6** in CD_3OD

	1A	1B	5	6	4
2	6.65 d (2.2)	6.66 d (2.2)	6.63 d (2.2)	7.47 d (1.9)	6.62 br s
5	7.01 d (8.3)	6.96 d (8.3)	6.91 d (8.3)	6.78 d (8.1)	
6	6.44 dd (8.3, 2.2)	6.49 dd (8.3, 2.2)	6.55 dd (8.3, 2.2)	7.49 dd (8.1, 1.9)	6.63 br s
7	2.61—2.64 m	2.69—2.70 m	2.70 s-like		2.53 dd (7.8, 7.6)
8	2.55—2.58 m	2.69—2.70 m	2.70 s-like		1.73—1.79 m
9					3.54 t-like (6.5)
10	2.06 s	2.10 s	2.09 s		
11					6.77 d (1.6)
14					6.64 d (8.3)
15					6.60 dd (8.3, 1.6)
16					5.32 d (5.6)
17					3.43—3.47 m
18					3.62 dd (9.8, 8.5)
					4.18 dd (9.8, 4.9)
1′	4.69 d (7.3)	4.67 d (7.5)	5.09 d (7.9)	5.89 d (8.3)	4.65 d (8.0)
2′	3.52 dd (8.8, 7.3)	3.44—3.50 m	5.16 dd (9.5, 7.9)	5.23 dd (9.8, 8.3)	4.98 dd (9.8, 8.0)
3′	3.48 t-like (9.0)	3.44—3.50 m	3.75 t-like (9.2)	3.81—3.86 m	3.66 dd (9.8, 8.8)
4′	3.39 dd (9.7, 8.8)	3.36—3.39 m	3.56 dd (9.7, 9.1)	3.56—3.58 m	3.44 dd (9.8, 8.8)
5′	3.69 ddd (9.7, 7.6, 2.3)	3.64 ddd (9.5, 7.1, 2.2)	3.44—3.48 m	3.56—3.58 m	3.38 ddd (9.8, 5.6, 2.2)
6′	4.39 dd (11.8, 7.6)	4.34 dd (12.0, 7.1)	3.79 dd (12.0, 5.1)	3.75—3.78 m	3.72 dd (11.9, 5.6)
	4.54 dd (11.8, 2.3)	4.49 dd (12.0, 2.2)	3.90 dd (12.0, 2.3)	3.92 br dd (12.2, 1.5)	3.92 dd (11.9, 2.2)
2″	7.07 d (2.0)	7.38 d (1.9)	7.93—7.95 m	7.84—7.88 m	7.83—7.86 m
3″			6.82—6.83 m	6.75—6.79 m	6.77—6.80 m
5″	6.81 d (8.3)	6.69 d (8.3)	6.82—6.83 m	6.75—6.79 m	6.77—6.80 m
6″	6.97 dd (8.3, 2.0)	7.07 dd (8.3, 1.9)	7.93—7.95 m	7.84—7.88 m	7.83—7.86 m
7″	7.58 d (16.0)	6.83 d (12.7)			
8″	6.30 d (16.0)	5.79 d (12.7)			
OCH_3				3.85 s (3-OCH_3)	3.79 s (3-OCH_3)
					3.78 s (12-OCH_3)

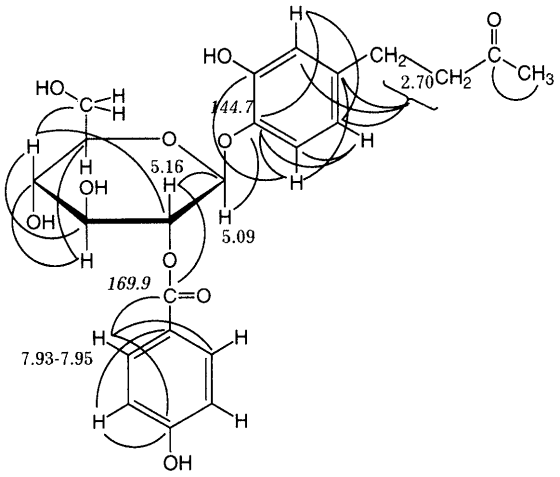

Fig. 3. HMBC Correlations of Compound **5**

trum, the latter protons exhibited a correlation with the ester carbonyl at δ 169.9 which correlated with a proton at δ 5.16 (1H, dd, J=9.5 and 7.9 Hz) assigned to H-2′ of the glucose moiety. The substituted B-ring at the 2′ position in glucose should be a *p*-hydroxybenzoyl group since, in addition to the above data, a hydroxyl-attaching carbon at δ 163.8 was assigned for C-4″ in the B-ring by HMBC and from the molecular formula. The substitution caused deshielding and shielding of the protons at the 2′ and 6′ positions, respectively, in the glucose moiety of **5**, compared with those of **1A**. The structure of compound **5** obtained from these data was also supported by the other HMBC correlations.

Compounds **1A**, **1B** and **5** are the new related compounds, and have been named vitexfolin A, B and C, respectively. From the leaves of *V. rotundifolia*, a phenylbutanone glucoside without a B-ring moiety has been isolated by Kouno *et al.*[4]

Compound **4**, $[\alpha]_{589}$ −30°, having the molecular formula of $C_{33}H_{38}O_{13}$ (MW 624), also showed the existence of 2-*p*-hydroxybenzoyl-glucose moiety in its ^{1}H- and ^{13}C-NMR by comparison with compound **5**. Besides this moiety, twelve aromatic signals were observed in the ^{13}C-NMR, corresponding to a 1, 2 and 4-substituted ring (B-ring) from the proton signals at δ 6.77 (1H, d, J=1.6 Hz; H-11), 6.64 (1H, d, J=8.3 Hz; H-14) and 6.60 (1H, dd, J=8.3 and 1.6 Hz; H-15), and four substituted ones (A-ring) with signals at δ 6.62 (1H, br s; H-2) and 6.63 (1H, br s; H-6) in the ^{1}H-NMR. The COSY and HMBC experiments suggested that the latter (A-ring) had substituents of a methoxyl group at δ 3.79 (3H, s) and a propanol group at δ 3.54 (2H, t-like, J=6.5 Hz; H_2-9), 1.73—1.79 (2H, m; H_2-8) and 2.54 (2H, dd, J=7.8 and 7.6 Hz; H_2-7) by correlations of δ_H 3.79 (3-OCH_3)/δ_C 145.1 (C-3), and δ_H 6.62 (H-2) and 6.63 (H-6)/δ_C 32.8 (C-7), respectively. The A-ring was also found to be connected to another C3 unit at δ 5.32 (1H, d, J=5.6 Hz; H-16), 3.43—3.47 (1H, m; H-17), 3.62 (1H, dd, J=9.8 and 8.5 Hz; H-18) and 4.18 (1H, dd, J=9.8 and 4.9 Hz; H-18) by δ_H 6.63 (H-6)/δ_C 53.3 (C-17) in the HMBC. This unit was attached to the B-ring since correlations of δ_H 6.60 (H-15) and 6.77 (H-11)/δ_C 88.5 (C-16) were observed. The proton at 5.32 (H-16) has a correlation with the carbon at δ 147.3 or 147.4 (C-4 or C-13), indicating a furan ring. Taken

NII-Electronic Library Service

Table 2. ^{13}C-NMR Data for Compounds **1A** and **4**—**6** in CD_3OD

	1A	5	6	4
1	138.2	138.8	121.3	137.0
2	117.2	117.2	113.9	114.3
3	148.2	148.8	148.8	145.1
4	144.9	144.7	153.5	147.3[a]
5	119.0	119.5	116.9	129.0
6	120.6	120.7	125.7	118.0
7	30.1	30.3	166.3	32.8
8	45.7	45.9		35.8
9	211.2	211.1		62.3
10	30.0	30.0		134.5
11				110.3
12				149.9
13				147.4[a]
14				116.0
15				119.4
16				88.5
17				53.3
18				72.1
1′	104.3	103.0	94.5	102.8
2′	74.9	75.7	74.5	75.4
3′	77.6	76.2	75.9	76.2
4′	72.1	71.4	71.4	71.9
5′	75.7	78.4	79.2	78.3
6′	64.7	62.3	62.3	62.8
1″	127.8	122.3	121.9	122.3
2″	115.3	133.2	133.0	133.1
3″	147.0	116.2	116.2	116.2
4″	149.8	163.8	163.7	163.5
5″	116.6	116.2	116.2	116.2
6″	123.1	133.2	133.0	133.1
7″	147.2	167.9	167.4	167.4
8″	115.1			
9″	168.9			
OCH_3			56.5 (3-OCH_3)	56.7 (3-OCH_3) 56.5 (13-OCH_3)

a) Interchangeable.

together with the molecular formula, a dihydrodehydrodiconiferylalcohol moiety was proposed, the data of which were identical with the published NMR data.[5] The relative stereochemistry at C-16 and C-17 seems to be *trans* from the proton coupling constant. The 18 position of the β-D-glucose substituent was established from the correlation of δ_H 4.65 (1H, d, J=8.0 Hz; H-1′)/δ_C 72.1 (C-18).

According to HR-FAB-MS, the molecular formula of compound **6** was determined to be $C_{21}H_{22}O_{11}$ (MW 450). The ^{1}H- and ^{13}C-NMR of **6** indicated the same *p*-hydroxybenzoyl-glucose moiety by comparison with compound **5**, although compound **6** showed downfield- and upfield-shifts in the anomeric proton at δ 5.89 (1H, d, J=8.3 Hz; H-1′) and carbon at δ 94.5 (C-1′), respectively. The remaining signals suggested the A-ring to be a hydroxy-methoxy-benzoyl moiety with the proton signals at δ 6.78 (1H, d, J=8.1 Hz; H-5), 7.47 (1H, d, J=1.9 Hz; H-2) and 7.49 (1H, dd, J=8.1 and 1.9 Hz; H-6) indicating 3,4-substitution. The vanilloyl group for the A-ring was determined by an HMBC experiment. The aromatic protons at δ 7.47 and 7.49 were correlated with the ester carbonyl at δ 166.3 (C-7) which exhibited a correlation with the anomeric proton at δ 5.89 (1H, d, J=8.3 Hz; H-1′), indicating vanilloyl-β-D-glucoside. The HMBC also supported the position of the B ring by observation of correlations of δ_H 7.84—7.88 (H-6″)/δ_C 167.4 (C-7″)/δ_H 5.23 (1H, dd, J=9.8 and 8.3 Hz; H-2′). The structure of **6** is, therefore, vanilloyl-β-D-(2′-*O*-*p*-hydroxybenzoyl)-glucoside.

Compounds **7** and **8** are apparently isomers from their ^{1}H- and ^{13}C-NMR spectra. HR-FAB-MAS afforded $C_{10}H_{14}O_5$, the molecular formula of **7**. The NMR, including HMBC and NOEDF, gave the structure of compound **7** as guaiacylglycerol. The ^{13}C-NMR was identical with the published data of an *erythro*-compound isolated from *Eucommia ulmoides*.[6] Compound **8** seems to be a *threo*-isomer, but the stereochemistry of both compounds was not completely established because of their close chemical shifts.

The analgesic effects of compounds **1A** and **2**—**4** by the acetic acid-induced writhing method in mice are shown in Fig. 4. Oral administration of **1A** produced analgesia of 23% (p<0.01) and 77% (p<0.001) at doses of 15 and 50 mg/kg, respectively. Neolignan glucoside **4** inhibited the writhing symptoms 31% (p<0.001) at a dose of 25 mg/kg, although this did not occur in a dose-dependent manner. Previously, we also observed analgesia and local anesthesia produced by a lignan such as (+)-pinoresinol.[7] The iridoids of **2** and **3** showed analgesic effects, 56% (p<0.001) and 20% (p<0.05), respectively, at a dose of 50 mg/kg. Because of the small amounts, other metabolites could not be tested. The tail pressure method for analgesia in mice was applied to compounds **1A** and **2**. (Fig. 5) The former compound only increased the pain threshold at an oral dose of 50 mg/kg. A pharmacological study of lindleyin, a phenylbutanoneglucoside isolated from *Aeonium lindleyi*, has been carried out by Darias *et al.*[8] They reported that the compound possessed an analgesic effect at a dose of 200 mg/kg using the Siegmund and Randall-Sellito tests, while it was ineffective at 250 and 500 mg/kg in a hotplate test. Lindleyin also produced anti-inflammatory and anti-pyretic effects. Considering these data, compound **1A** may have anti-inflammatory analgesia together with central activity.

As both compounds **2** and **3** exhibited an analgesic effect, some other iridoid glucosides were tested by the writhing method in mice. Their structures and inhibition ratio, including those of **2** and **3**, are summarized in Fig. 6. Acylation of a hydroxyl group at C-10 and substituents like a carboxyl group at C-4 may not be critical for the analgesia, since even aucubin itself has such activity. However, the fact that catalpol had no effect suggested that a double bond between the 7 and 8 positions was essential for the activity in this type of compound. *O*-Glycosylation at position 1 will be necessary for stabilization of the aucubin-type compounds. In the case of 4-carboxyl compounds such as geniposidic acid, genipin has been reported to exhibit writhing inhibition in spite of having a free hydroxy group at C-1.[9] The anti-inflammatory activity of iridoid glycosides was tested by using the carrageenan-induced mouse paw edema and the tetradecanoylphorbolacetate (TPA)-induced mouse ear edema models.[10] In both experiments, aucubin inhibited the edema significantly at a dose of 100 mg/kg and at 1 mg/ear, respectively, but catalpol was ineffective at the

NII-Electronic Library Service

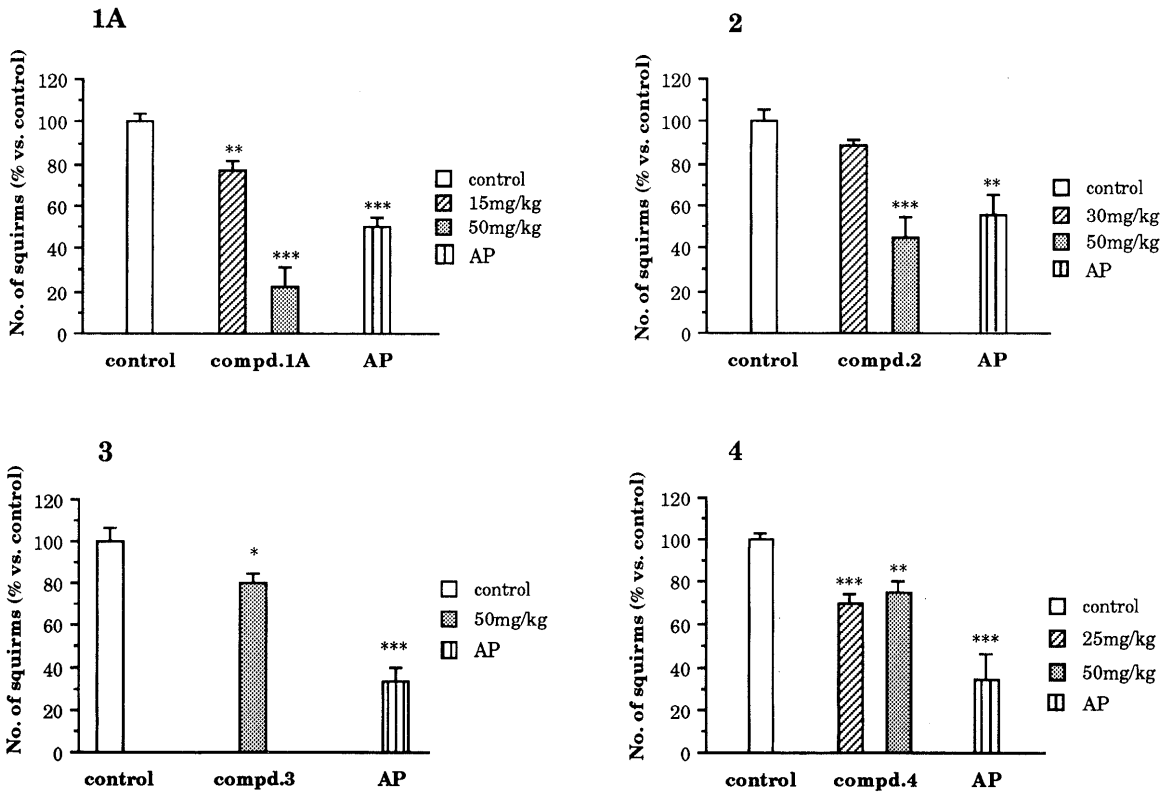

Fig. 4. Analgesic Effects of Compounds **1A**—**4** on Acetic Acid-Induced Writhing in Mice

Each bar represents the mean ± S.E.M. The writhing ratios of **1A**—**4** were shown by taking the control writhings of 21.6 ± 0.9, 23.2 ± 1.3, 22.8 ± 1.5 and 26.0 ± 0.9, respectively, as 100%. $*p<0.05$, $**p<0.01$, $***p<0.001$, $n=6$. AP: aminopyrine 50 mg/kg.

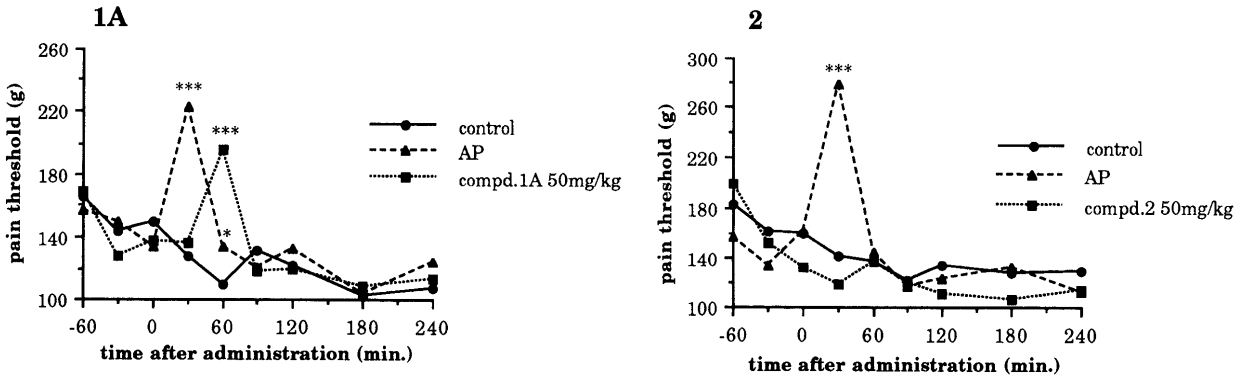

Fig. 5. Analgesic Effects of Compounds **1A** and **2** on Pressure Pain Threshold in Mice

$*p<0.05$, $***p<0.001$, $n=7$—8. AP: aminopyrine 50 mg/kg.

same doses. Probably, compounds **2** and **3** also have anti-inflammatory activity and no central analgesia.

From these findings, it seems that different kinds of compounds such as phenylbutanoneglucosides, lignans and iridoids, with different potencies, participate in the analgesic property of Viticis Fructus. However, the mechanisms for their analgesic effects may be different.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. UV spectra were measured on a Hitachi U-3400 spectrometer, and optical rotatory dispersion (ORD) for $[\alpha]_{589}$ on a JASCO J-20 polarimeter. HR-FAB-MS spectra were recorded with a JEOL HX-110 A spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL JNM GSX 500A and 400A spectrometers with tetramethylsilane or a solvent as internal standard. Column chromatography was performed on Sephadex LH-20, Wakogel C-200, Nacalai Silica Gel 60 and Chromatorex ODS (100—200 mesh). Pre-packed columns, Kusano CPO-HS-221-05 and -10, and CPS-HS-223L-05 for medium pressure liquid chromatography (MPLC); Sensyu Pak Aquasil SS-852N, ODS-4251-S and ODS-5251-N were used for HPLC.

Materials Viticis Fructus (the dried fruit of *V. rotundifolia*) was purchased in April 1994, from Uchida Wakanyaku, a commercial outlet of traditional medicines in Japan, who carried out their own identification of the plant.

Isolation Viticis Fructus 5 kg was extracted with methanol at room temperature. After evaporation of the solvent, the methanol extract (291.9 g) was fractionated using a separation-guide of writhing inhibition in mice by oral administration. The extract was partitioned successively with *n*-hexane, ethyl acetate and water. The ethyl acetate fraction only showed the activity at a dose of 1 g/kg, *p.o.* The material soluble in acetone–methanol 3/2 was applied to Sephadex LH-20 and eluted with the same solvent, to give fr. 1-A and 1-B. TLC of the insoluble part and the former fraction showed a spot at the origin. As the active fraction was fr. 1-B, it was separated by silica gel chromatography. The *n*-hexane–ethyl acetate 2/3 eluate, fr. 2-A (20.4 g), was the vasodilatation-producing fraction corresponding to the active fraction in Part I, but it did not inhibit writhing. Writhing inhibition was found in fr. 2-C (10.7 g)

NII-Electronic Library Service

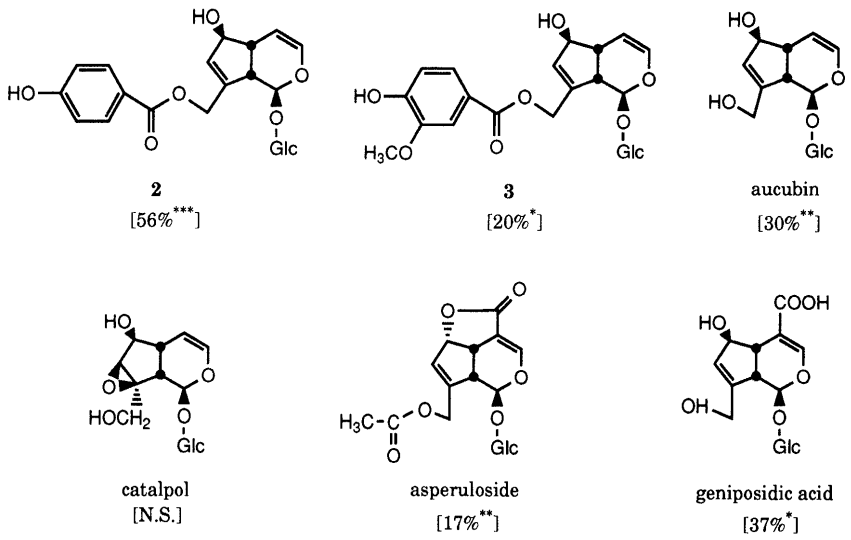

Fig. 6. Analgesic Effects of Some Iridoid Glucosides on Acetic Acid-Induced Writhing in Mice

[ ]: inhibition ratio at a dose of 50 mg/kg, *p.o.* $*p<0.05$, $**p<0.01$, $***p<0.001$, $n=6$. N.S.: no significance.

which was eluted with a solvent ratio of 1/2. Fraction 2-C was further separated by silica gel with chloroform–methanol–water as an eluent. The active fraction, fr. 3-C, eluting at a ratio of 100/20/1, was then applied to the Sephadex LH-20 column with methanol. As activity was observed in fr. 4-A and fr. 4-B, both were separated independently. The latter fraction gave a major component, compound **1A** (26 mg), and a mixture of **1A** and **1B** (5 mg) by Sephadex LH-20 chromatography and ODS-MPLC eluting with acetonitrile–water 1/2. Fraction 5-A, separated from fr. 4-B, did not show any significant effect, but had a tendency for inhibition at a dose of 100 mg/kg. Flash chromatography of this fraction on ODS with acetonitrile–water 1/4 yielded compound **2** (25 mg) and **6** (18 mg). Fraction 4-A, another active fraction, was separated by silica gel chromatography using chloroform–methanol 5/1—1/1, and both fractions 7-B and 7-C possessed activity. Fr. 7-C was subjected to repeated chromatography using a flash column on ODS and Sephadex LH-20, and then MPLC on ODS (acetonitrile–water 1/4) and HPLC on Aquasil (chloroform–methanol–water 100/10/0.1) to give compound **2** (122 mg) as a major component and compound **3** (15 mg). Separation of fr. 7-B by ODS-flash chromatography with methanol–water 1/1 gave the active fraction 8-A at a dose of 80 mg/kg. Although fr. 8-B also showed activity, some spots on TLC overlapped with those of fr. 8-A. Fraction 8-A, therefore, was further separated using Sephadex LH-20 and ODS-flash chromatography followed by repeated HPLC (ODS, methanol–water 1/5 or acetonitrile–water 2/5; Aquasil, chloroform–methanol–water 100/10/0.1 or 160/20/1). Compounds **2**—**5**, and **7** and **8** were obtained in yields of 11, 11, 29, 4, 13 and 4 mg, respectively.

Compound **1A**: Dark yellow amorphous powder, $[\alpha]_{589}^{27}$ −18° ($c=0.13$, methanol). HR-FAB-MS (glycerol+PEG 600) *m/z*: 505.1693 $(M+H)^+$ (err. −1.7 mmu for $C_{25}H_{29}O_{11}$). UV λ_{max} (methanol) nm (log ε): 217 (4.32), 246 (4.01), 287 (4.04), 304 sh (4.08), 330 (4.20).

Compound **2**: Colorless powder, mp 149—151 °C (lit.[12] mp 145—146 °C), $[\alpha]_{589}^{22}$ −91° ($c=0.11$, ethanol) (lit.[12] $[\alpha]_D^{20}$ −91.5°, ethanol). FAB-MS (glycerol+NaCl) *m/z*: 489 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 2.70 (1H, dddd, $J=7.8$, 5.8, 3.9, 2.0 Hz; H-5), 2.99 (1H, br dd, $J=7.8$, 7.3 Hz; H-9), 3.24 (1H, dd, $J=9.1$, 7.9 Hz; H-2′), 3.27—3.29 (1H, m; H-5′), 3.31 (1H, t, $J=8.9$ Hz; H-4′), 3.37 (1H, dd, $J=9.1$, 8.7 Hz; H-3′), 3.65 (1H, dd, $J=12.0$, 5.6 Hz; H-6), 3.85 (1H, dd, $J=12.0$, 2.2 Hz; H-6), 4.47 (1H, dt, $J=5.8$, 2.0 Hz; H-6), 4.69 (1H, d, $J=7.9$ Hz; H-1′), 4.91 (1H, dtd, $J=14.9$ 1.7, 0.7 Hz; H-10), 4.99 (1H, d, $J=7.3$ Hz; H-1), 5.09 (1H, dddd, $J=14.9$, 2.1, 1.8, 0.8 Hz; H-10), 5.12 (1H, dd, $J=6.1$, 3.9 Hz; H-4), 5.82—5.83 (1H, m; H-7), 6.34 (1H, dd, $J=6.1$, 2.0 Hz; H-3), 6.83—6.85 (2H, m; H-3″, 5″), 7.90—7.93 (2H, m; H-2″, 6″). The IR and ^{13}C-NMR were identical with the published data except for the opposite assignment of C-3′ and C-5′ in the latter spectrum.[11,12]

Compound **3**: Colorless amorphous powder, mp 108—110 °C, $[\alpha]_{589}^{22}$ −73° ($c=0.11$, methanol). HR-FAB-MS (glycerol+PEG 600+NaCl) *m/z*: 519.1470 $(M+Na)^+$ (err. −0.8 mmu for $C_{23}H_{28}NaO_{12}$). UV λ_{max} (methanol) nm (log ε): 219 (4.30), 264 (4.07), 291 (3.82). ^{1}H-NMR (CD_3OD) δ: 2.69—2.73 (1H, m; H-5), 3.00 (1H, br t, $J=7.3$ Hz; H-9), 3.24 (1H, dd, $J=9.0$, 7.8 Hz; H-2′), 3.27—3.33 (2H, m; H-4′, 5′), 3.37 (1H, dd, $J=9.0$, 8.6 Hz; H-3′), 3.65 (1H, dd, $J=12.0$, 5.6 Hz; H-6′), 3.84 (1H, dd, $J=12.0$, 2.1 Hz; H-6′), 3.90 (1H, 3H, s; 3″-OCH_3), 4.46—4.48 (1H, m; H-6), 4.69 (1H, d, $J=7.8$ Hz; H-1′), 4.93 (1H, br d, $J=14.9$ Hz; H-10), 5.09 (1H, br d, $J=14.9$ Hz; H-10), 5.11 (1H, dd, $J=6.1$, 3.9 Hz; H-4), 5.83 (1H, br t, $J=1.5$ Hz; H-7), 6.34 (1H, dd, $J=6.1$, 1.9 Hz; H-3), 6.86 (1H, d, $J=8.3$ Hz; H-5″), 7.58 (1H, d, $J=2.0$ Hz; H-2″), 7.60 (1H, dd, $J=8.3$, 2.0 Hz; H-6″). ^{13}C-NMR (CD_3OD) δ: 46.25 (C-5), 48.41 (C-9), 56.55 (3′-OCH_3), 62.83 (C-6′), 63.82 (C-10), 71.56 (C-4′), 74.97 (C-2′), 78.04 (C-3′), 78.33 (C-5′), 82.88 (C-6), 97.90 (C-1), 100.28 (C-1′), 105.60 (C-4), 113.75 (C-2″), 116.08 (C-5″), 122.49 (C-1″), 125.26 (C-6″), 132.66 (C-7), 141.76 (C-3), 142.96 (C-8), 148.89 (C-3″), 153.12 (C-4″), 167.85 (C-7″).

Compound **4**: Colorless amorphous powder, $[\alpha]_{589}^{25}$ −30° ($c=0.11$, methanol). HR-FAB-MS (glycerol+PEG 600) *m/z*: 624.2342 $(M)^+$ (err. +3.0 mmu for $C_{33}H_{38}O_{13}$). UV λ_{max} (ethanol) nm (log ε): 238 (4.26), 260 (4.23), 276 sh (4.10).

Compound **5**: Yellow amorphous powder. HR-FAB-MS (NBA+NaCl) *m/z*: 485.1432 $(M+Na)^+$ (err. +0.9 mmu for $C_{23}H_{26}NaO_{10}$). UV λ_{max} (ethanol) nm (log ε): 260, 272 sh, 325 sh.

Compound **6**: Colorless amorphous powder. HR-FAB-MS (glycerol+PEG 600+KI) *m/z*: 489.0184 $(M+K)^+$ (err. +1.5 mmu for $C_{21}H_{22}KO_{11}$). UV λ_{max} (methanol) nm: 217 sh, 225 sh, 261, 299 sh.

Compound **7**: Colorless amorphous powder. HR-FAB-MS (glycerol+PEG 200, 400+NaCl) *m/z*: 237.0740 $(M+Na)^+$ (err. +0.1 mmu for $C_{10}H_{14}NaO_5$). UV λ_{max} (methanol) nm: 230, 281, 286 sh. ^{1}H-NMR (CD_3OD) δ: 3.35 (1H, dd, $J=11.2$, 6.4 Hz; H-9), 3.47 (1H, dd, $J=11.2$, 3.9 Hz; H-9), 3.66 (1H, td, $J=6.3$, 3.9 Hz; H-8), 3.85 (1H, s; 3-OCH_3), 4.51 (1H, d, $J=6.3$ Hz; H-7), 6.75 (1H, d, $J=8.1$ Hz; H-5), 6.80 (1H, ddd, $J=8.1$, 1.7, 0.5 Hz; H-6), 6.99 (1H, d, $J=1.7$ Hz; H-2). ^{13}C-NMR (CD_3OD) δ: 56.42 (3-OCH_3), 64.32 (C-9), 75.52 (C-7), 77.66 (C-8), 111.63 (C-2), 115.94 (C-5), 120.73 (C-6), 134.89 (C-1), 147.18 (C-4), 148.94 (C-3). The ^{13}C-NMR was almost identical with those of *erythro*-guaiacylglycerol in acetone-d_6 except the assignment of C-7 and 8.[6]

Compound **8**: Colorless amorphous powder. ^{1}H-NMR (CD_3OD) δ: 3.58 ((1H, dd, $J=11.3$, 6.3 Hz; H-9), 3.66 (1H, dd, $J=11.3$, 3.9 Hz; H-9), 3.73 (1H, td, $J=6.6$, 3.9 Hz; H-8), 3.86 (1H, s; 3-OCH_3), 4.52 (1H, d, $J=6.1$ Hz; H-7), 6.76 (1H, d, $J=8.1$ Hz; H-5), 6.82 (1H, ddd, $J=8.1$, 1.9, 0.5 Hz; H-6), 7.01 (1H, d, $J=1.9$ Hz; H-2). ^{13}C-NMR (CD_3OD)δ: 56.37 (3-OCH_3), 64.57 (C-9), 76.13 (C-7 or 8), 76.68 (C-8 or 7), 111.91 (C-2), 115.74 (C-5), 121.03 (C-6), 134.82 (C-1), 147.00 (C-4), 148.78 (C-3).

Pharmacological Assay Male ddY strain mice weighing 23—33 g were used. The animals (4 weeks old) bred at Japan SLC, Hamamatsu, Japan, were housed for about a week in a controlled 12-h light-dark environment at 22±1 °C, water and food being provided *ad libitum*. Pharmacological assay was carried out as previously described.[13] Samples were dissolved or suspended in saline with 1—5% gum arabic and/or 5% Tween 80. Aminopyrine 50 mg/kg was used as a positive control. The number of

NII-Electronic Library Service

squirms (mean ± S.E.M. for six mice) for each iridoid in Fig. 6 was as follows: control (22.3 ± 1.1), aminopyrine (9.8 ± 2.0, $p < 0.001$), aucubin (15.7 ± 1.4, $p < 0.01$), catalpol (21.3 ± 2.1); control (19.5 ± 1.3), aminopyrine (12.3 ± 1.5, $p < 0.001$), asperuloside (16.2 ± 0.8, $p < 0.05$), geniposidic acid (12.3 ± 1.3, $p < 0.01$).

Statistical significance was evaluated by Student's *t*-test.

Acknowledgements We thank the staff of the Analytical Center of Chiba University for measurement of FAB-MS.

References and Notes

1) Part I: Okuyama E., Suzumura K., Yamazaki M., *Natural Medicines*, **46**, accepted. The results of this paper were reported in the following meetings: Okuyama E., Suzumura H., Fugimori S., Yamazaki M., Abstracts of Papers, The 41st Annual Meeting of the Japanese Society of Pharmacognosy, Sapporo, September 1994, p. 89; Okuyama E., Fujimori S, Yamazaki M., Abstracts of Papers, The 42nd Annual Meeting of the Japanese Society of Pharmacognosy, Fukuyama, September 1995, p. 64.
2) "Standards for Non-pharmacopoeial Crude Drugs in Japan," Yakujinippo, Tokyo, 1989.
3) Kimura T., But P. P. H., Guo J.-X., Sung C. K. (eds.), "International Collation of Traditional and Folk Medicine," World Scientific, Singapore, 1996, pp. 141—142; Zhuang Z.-X., Li N.-H. (chief eds.), "Chinese Medicinal Herbs of Hong Kong," Vol. 2, Hong Kong, 1985, pp. 120—121.
4) Kouno I., Inoue M., Onizuka Y., Fujisaki T., Kawano N., *Phytochemistry*, **27**, 611—612 (1988).
5) Agrawal P. K., Rastogi R. P., Osterdahl B.-G., *Org. Magn. Res.*, **21**, 119—121 (1983).
6) Hattori M., Che Q.-M., Gewali M. B., Nomura Y., Tezuka Y., Kikuchi T., Namba T., *Shoyakugaku Zasshi*, **42**, 76—80 (1988).
7) Okuyama E., Suzumura K., Yamazaki M., *Chem. Pharm. Bull.*, **43**, 2200—2204 (1995).
8) Darias V., Gonzáles A. G., Boada J. N., Feria M., Martorell F., *Il Farmaco Edizione Scientifica*, **33**, 460—467 (1978).
9) Harada M., Tenmyo N., Aburada M., Endo T., *Yakugaku Zasshi*, **94**, 157—162 (1974).
10) Recio M. del C., Giner R. M., Máñez S., Ríos J. L., *Planta Medica*, **60**, 232—234 (1994).
11) Dutta P.K., Chowdhury U. S., Chakravarty A. K., Achari B, Pakrashi C., *Tetrahedron*, **39**, 3067—3072 (1983).
12) Winde E., Hänsel R., *Arch. Pharm.*, **293**, 556—567 (1960).
13) Okuyama E., Okamoto Y., Yamazaki M., Satake M., *Chem. Pharm. Bull.*, **44**, 333—336 (1996); Okuyama E., Umeyama K., Ohmori S., Yamazaki M., Satake M., *ibid.*, **42**, 2183—2186 (1994).

NII-Electronic Library Service