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COUMARINS AND OTHER CONSTITUENTS OF *PRUNUS PROSTRATA*

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ABSTRACT.—From the aerial parts of *Prunus prostrata* (Rosaceae), the following new compounds were isolated: 5-(β-D-glucopyranosyloxy)-2-(3-hydroxybutyl)-1,3,3-trimethylcyclohexene [**1**], 4-(β-D-glucopyranosyloxy)-3-(3-methyl-2-butenyl)benzoic acid [**2**], fraxinol 6-β-D-galactopyranoside [**3**], and 6,7-dihydroxy-5-methoxycoumarin 6-β-D-glucopyranoside [**4**]. Their structures were established by means of fabms and a combination of homo- and hetero-nuclear 2D nmr techniques. Twelve known compounds, never previously isolated from this genus, were also found in this plant.

As a part of our phytochemical research on plants in the Rosaceae, we have investigated *Prunus prostrata* Labill. This species, a small shrub with repent stems, is typical of the Mediterranean area, and in Italy it is present only in Sardinia where it grows in garigues and rocky soils (1). The red fruits are edible and are used in traditional medicine against gastroenteric disturbances.

Plants belonging to the *Prunus* genus have a great interest for the biological actions of their extracts. Many species show sedative, antispasmodic, anti-inflammatory, anti-hyperlipidemic, and antitumor activities. For instance, *Prunus spinosa*, a species closely related to *P. prostrata,* quite recently has been shown to be endowed with strong anticancer and antidepressive actions (2–5).

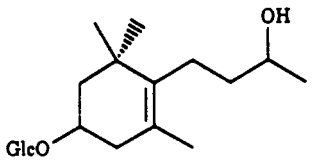
This paper deals with the isolation from *P. prostrata* of sixteen metabolites. Three compounds (two coumarins and a reduced β-ionone derivative) are new; the fourth (a benzoic acid derivative) has been described previously as a product of hydrolysis of an alkaloid. Their structural elucidations were performed by means of 1D and 2D nmr techniques including NOESY, COSY, HETCOR, and COLOC experiments.

RESULTS AND DISCUSSION

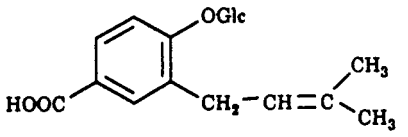
Aerial parts were defatted with petroleum ether and extracted in a Soxhlet apparatus with $CHCl_3$ and $CHCl_3$/MeOH (9:1). The last two residues were fractionated by Sephadex LH-20 gel filtration using MeOH-$CHCl_3$ (9:1) and MeOH as eluents. The $CHCl_3$ extract yielded nine known compounds: β-sitosterol 3-*O*-β-D-galactopyranoside, ampelopsionoside, boscialin 4′-β-D-glucopyranoside, mandshurin, fraxinol, herniarin, umbelliferone, kaempferol, and dihydroquercetin.

Gel filtration of the $CHCl_3$-MeOH extract gave six fractions (I–VI) from which eight compounds were isolated; four of these were new constituents. The known compounds were mandshurin, benzyl β-D-glucopyranoside, rutin, and dihydrokaempferol.

From fraction I, by Lobar RP-18 cc, compound **1** was isolated as an oil. The positive ion fabms showed an $[M+H]^+$ peak at *m/z* 375 together with a weak $[M+Na]^+$ ion at *m/z* 397 corresponding to the molecular formula $C_{19}H_{34}O_7$, deduced also by ^{13}C-nmr and DEPT analyses. The elimination of a hexosyl moiety was indicated by a weak $[M+H-162]^+$ ion at *m/z* 213. The ir spectrum showed bands at 3500–3300 and 1170 cm^{-1} due to OH functions and bands of a glycosidic linkage at 1365 and 1260 cm^{-1} (6). The ^{13}C-nmr spectrum revealed nineteen carbon signals, which were sorted by DEPT experiments into Me×4, CH_2×4, OCH×7, OCH_2×1, =C×2, and C×1 (Table 1).



1



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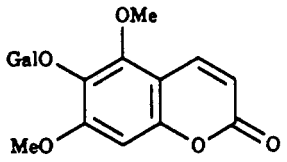
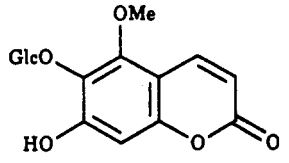


3



4

The ^{1}H-nmr spectrum of **1** confirmed the above deduction; it showed four Me groups [two tertiary at δ 0.91 and 0.95, one vinylic at δ 1.52 (br s), and one secondary at δ 1.21 (d, J=6.0 Hz)], besides signals between δ 1.60 and 2.11 attributable to eight aliphatic protons. The sugar region of the ^{1}H-nmr spectrum showed one anomeric proton signal (δ 5.10), and the large coupling constant (J=7.6 Hz) indicated a β linkage. The sugar moiety was identified as β-D-glucopyranose by comparison of the ^{13}C-nmr data with those of methyl β-D-glucopyranoside (7,8). Acid hydrolysis afforded D-glucose, identified by tlc comparison with markers, and the aglycone of **1**, identified by ^{1}H- and ^{13}C-nmr data.

The ^{1}H-nmr spectrum of the aglycone of **1** showed the Me and the aliphatic proton signals previously described for **1**. In addition, two signals of carbinolic protons were identified at δ 3.65 and 3.95. In the COSY experiment, the first signal coupled with the secondary Me group and the aliphatic protons; while the signal at δ 3.95 coupled only with the aliphatic protons. Moreover, the HETCOR experiments led to the assignments of the chemical shifts of the carbons linked to these two carbinolic protons; the resonance of the proton at δ 3.65 correlated with the C signal at δ 68.9, while the signal at δ 3.95 correlated with that at δ 64.0. All of these data were in agreement with the following isolated spin systems: -CH_2-CH(O)-CH_2- and -CH_2-CH(O)-CH_3.

The ^{13}C-nmr spectrum of the aglycone of **1** exhibited other typical signals that revealed the presence of a reduced ionone derivative (9). The identification of the aglycone of **1** as 5-hydroxy-2-(3-hydroxybutyl)-1,3,3-trimethylcyclohexene was derived by a comparative study (Table 1) of ^{13}C-nmr data with those of 3-hydroxy-7,8-dihydro-β-ionone and dihydrovomifoliol (9,10).

The location of the sugar moiety at C-5 of **1** was evidenced by the upfield shift of

TABLE 1. ^{13}C-nmr Data of the Aglycone of **1** and Related Compounds (CD_3OD, 200 MHz).

Carbon	Compound		
	Aglycone of **1**	3-Hydroxydihydro-β-ionone	Dihydrovomifoliol
C-1	125.0	125.3	164.4
C-2	136.7	136.9	73.6
C-3	37.7	38.0	41.6
C-4	49.5	49.7	50.0
C-5	64.0	64.2	197.8
C-6	42.3	42.0	126.4
C-7	23.2	22.4	23.0
C-8	35.1	44.0	35.4
C-9	68.9	207.7	68.7
C-10	23.9	29.0	23.8
C-11	29.4	28.4	24.6
C-12	22.8	29.7	19.5
C-13	19.9	19.8	23.5

this carbon (5.5 ppm) and the downfield shifts of C-4 (2.5 ppm) and C-6 (3.3 ppm), with respect to the same carbons of the aglycone. Therefore, compound **1** was identified as 5-(β-D-glucopyranosyloxy)-2-(3-hydroxybutyl)-1,3,3-trimethylcyclohexene.

From fraction II, by Si gel gravity cc, compound **2** was obtained as an oil. The positive ion fabms of **2** showed the $[M+H]^+$ peak at *m/z* 369 together with a weak $[M+Na]^+$ ion at *m/z* 391 corresponding to the molecular formula $C_{18}H_{24}O_8$, derived also by the ^{13}C-nmr and DEPT analyses. The elimination of a hexosyl moiety was indicated by a weak $[M+H-162]^+$ ion at *m/z* 207. The ir (3450–3200, 1695, 1600 cm^{-1}) and uv spectra (208 and 242 nm) indicated a sugar moiety linked to an aromatic ring (11).

The ^{13}C-nmr spectrum of **2** revealed eighteen carbon signals which were sorted by DEPT experiments into Me×2, CH_2×1, OCH×5, OCH_2×1, =C×4, =CH×4, and COOH×1. The chemical shift at δ 173.9 of the COOH was in agreement with that reported in the literature for benzoic acid derivatives (12). In the ^{1}H-nmr spectrum of **2**, the aromatic region was defined by two signals that correlated in the COSY experiment, one at δ 6.97 (1H, d, J=9.0 Hz) and the other at δ 7.65 (2H, m), indicating a trisubstituted aromatic ring. The signal at δ 6.97 was attributed to an aromatic proton ortho to an oxygen (13). The sugar region of the ^{1}H-nmr spectrum showed a multiplet (δ 3.18–3.68) due to six carbinolic protons and one anomeric proton signal (δ 4.85) with a large coupling constant (J=7.6 Hz) that indicated a β linkage. The sugar moiety was again identified as β-D-glucopyranose by comparison of the ^{13}C-nmr data with those of methyl β-D-glucopyranoside (7,8). Acid hydrolysis of **2** afforded D-glucose and 4-hydroxy-3-(3-methyl-2-butenyl) benzoic acid, identified by the ^{1}H- and ^{13}C-nmr data (14).

Therefore, **2** was identified as 4-(β-D-glucopyranosyloxy)-3-(3-methyl-2-butenyl) benzoic acid, a compound new from a natural source but previously described as a hydrolysis product of the alkaloid malaxine (15). The ir and ^{1}H-nmr data of **2** were concident with those reported for malaxinic acid (15).

From fractions III and IV, by low pressure Lobar RP8 cc and Si gel gravity cc, compounds **3** and **4** were isolated as amorphous white powders. Both compounds produced violet colorations on treatment with hydroxylamine and $FeCl_3$, a reaction typical for coumarins (16).

The ir spectra of **3** and **4** showed bands at 1720 cm^{-1} (lactone C=O), 1640 cm^{-1} (3,4-double bond), and 1265 cm^{-1} (ether), while their glycosidic nature was evidenced

by bands at 3250 and 1060 cm^{-1}. Their uv spectra exhibited absorptions between 325 and 330 nm typical of 6,7- or 5,7-dioxygenated and 5,6,7-trioxygenated coumarins (17,18).

The positive ion fabms of **3** showed an $[M+H]^+$ peak at *m/z* 381, corresponding to the molecular formula $C_{17}H_{20}O_{10}$, which was confirmed also by the ^{13}C-nmr and DEPT spectra. The elimination of a hexosyl moiety was indicated by a weak $[M+H-162]^+$ ion at *m/z* 219. The ^{13}C-nmr spectrum of **3** revealed seventeen carbon signals that were sorted by DEPT into MeO×2, CH_2O×1, CHO×5, =CH×3, C×5, and C=O×1 (Table 2). The complete structural elucidation was established on the basis of the chemical shifts and *J* values of the ^1H-nmr spectrum and from detailed spectral analyses of NOESY, HETCOR, and COLOC.

In the ^1H-nmr spectrum of **3**, the pair of doublets at δ 6.35 and 8.30, with $J=9.7$ Hz, were attributable to H-3 and H-4, and the latter chemical shift indicated that an oxygen was linked to C-5 (17); in the aromatic region, a signal at δ 6.93 (br s) due to H-8 was also present, and the H-4/H-8 coupling ($J=1.0$ Hz) confirmed that C-8 was unsubstituted (19). The oxygen substitutions at C-5, C-6, and C-7 were deduced from the absence of other signals in the aromatic region of the ^1H-nmr spectrum. The ^1H-nmr spectrum showed also two MeO singlets at δ 3.92 and 3.97, and the 2D ^1H-^{13}C nmr direct chemical shift correlation experiments gave the unambiguous assignments of their resonances: the protons at δ 3.92 correlated with the carbon resonance at 62.4 ppm (one MeO with substituents at the two ortho positions), while the protons at δ 3.97 correlated with the carbon resonance at 57.4 ppm (one MeO with at least one free ortho position). NOESY experiments revealed correlations between the MeO at δ 3.92 with the signal at δ 8.30 (H-4) and between the MeO at δ 3.97 with the signal at δ 6.93 (H-8). These data led to the conclusion that the MeO giving a signal at δ 3.92 was linked to C-5 and the MeO group at δ 3.97 was bound to C-7. Finally, the resonances of the C-5 and C-7 were deduced by COLOC experiments from the ^1H-^{13}C three-bond couplings of the proton resonances of the two MeO groups (Table 2).

TABLE 2. ^1H and ^{13}C nmr of the Aglycone Moieties of **3** and **4** (CD_3OD, 200 MHz).

Proton	δ (ppm)	H-H COSY correlations	NOESY correlations	Carbon	δ (ppm)	HETCOR correlations	COLOC correlations
Aglycone moiety of **3**							
				C-2	164.8 (C)		
H-3	6.35, d, $J=9.7$	H-4		C-3	109.4 (CH)	H-3	
H-4	8.30, br d, $J=9.7$	H-3,H-8	OMe 3.92	C-4	142.3 (CH)	H-4	
				C-4a	112.5 (C)		
				C-5	146.2 (C)		OMe 3.92
				C-6	139.2 (C)		
				C-7	158.0 (C)		OMe 3.97,H-8
H-8	6.93, br s	H-4	OMe 3.97	C-8	98.2 (CH)	H-8	
				C-8a	151.9 (C)		
OMe	3.97, s		H-8	OMe	57.4 (Me)	OMe 3.97	
OMe	3.92, s		H-4	OMe	62.4 (Me)	OMe 3.92	
Aglycone moiety of **4**							
				C-2	164.8 (C)		
H-3	6.04, d, $J=9.6$	H-4		C-3	109.4 (CH)	H-3	
H-4	8.23, br d, $J=9.6$	H-3,H-8	OMe 3.89	C-4	142.3 (CH)	H-4	
				C-4a	112.4 (C)		
				C-5	146.1 (C)		OMe 3.89
				C-6	139.2 (C)		
				C-7	152.7 (C)		H-8
H-8	6.51, br s	H-4		C-8	99.0 (CH)	H-8	
				C-8a	151.8 (C)		
OMe	3.89, s		H-4	OMe	62.3 (Me)	OMe 3.89	

In the sugar region, ^{1}H-nmr signals of a hexose in the β-pyranose form in **3** were present (doublet of the anomeric proton at δ 5.11 with J=7.3 Hz); the sugar was identified as β-D-galactopyranose by comparison of the ^{13}C-nmr data with those of methyl β-D-galactopyranoside (7,8). Finally, acid hydrolysis of **3** afforded D-galactose and fraxinol, both identified by chromatographic comparisons with markers. Therefore, compound **3** is fraxinol 6-β-D-galactopyranoside.

The positive ion fabms of compound **4** showed an $[M+H]^+$ peak at *m/z* 365 corresponding to the molecular formula $C_{16}H_{18}O_{10}$; the elimination of a hexosyl moiety was indicated by a weak $[M+H-162]^+$ ion at *m/z* 203. The ^{13}C-nmr spectrum of **4** revealed sixteen carbon signals, which were sorted by DEPT into MeO×1, CH_2O×1, CHO×5, =CH×3, C×5, and C=O×1. The complete structural elucidation of this coumarin was deduced by comparison of the chemical shifts and J values of the signals in the ^{1}H- and ^{13}C-nmr spectra with those of compound **3**. The ^{1}H-nmr spectrum showed the same signals except for the lack of one MeO. The location of the OH group at C-7 and the presence of the sugar moiety at C-6 and of the MeO at C-5 were deduced from the NOESY spectrum, which showed a strong correlation between the resonance of H-4 and that of the MeO group but none between the anomeric proton and H-8, as expected for 7-*O*-glycosyl coumarin derivatives. These substitutions were also confirmed by an analysis of the ^{13}C-nmr resonances. The C-7 showed an upfield shift of 5.9 ppm with respect to C-7 of compound **3** (Table 2) owing to the absence of the ethereal linkage. The sugar moiety was identified as β-D-glucopyranose by comparison of the ^{13}C-nmr data with those of methyl β-D-glucopyranoside (7,8). The 2D experiments (HETCOR and COLOC), compared with those of **3**, confirmed that **4** is 6,7-dihydroxy-5-methoxycoumarin 6-β-D-glucopyranoside. Among coumarins, glycoside derivatives, particularly galactosyl coumarins, are rather rare.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Bruker AC-200 Spectrospin spectrometer; fabms spectra in positive ion mode in a glycerol matrix, VG ZAB instrument; optical rotation, Perkin-Elmer 241 polarimeter; lpc, Duramat pump; ir and uv spectra, Perkin-Elmer spectrophotometers models, 684 and 330, respectively; Lobar RP8 and RP18 (40-63 μm, Merck), Si gel 60 (70–230 mesh and 230–400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for cc. Tlc was performed on Si gel 60 and RP8 precoated layers (Merck).

One- and two-dimensional nmr spectra were measured in CD_3OD or D_2O and were run as described previously (20). In the case of 2D ^{13}C-^{1}H shift correlations by long range coupling (COLOC), delays were adjusted to an average CH coupling of 7 Hz to obtain the maximum polarization transfer.

PLANT MATERIAL.—The aerial parts (branches, leaves, and flowers, 800 g) of the plant were collected in May 1991, near Nuoro, Sardegna, Italy. A voucher specimen is maintained in Pisa, at the Dipartimento di Chimica Bioorganica.

EXTRACTION AND ISOLATION.—Powdered, air-dried, aerial parts (800 g) were defatted with petroleum ether and extracted in a Soxhlet apparatus with $CHCl_3$ and $CHCl_3$-MeOH (9:1). The residues from the last two extracts (18.2 g and 23.7 g) were chromatographed on Sephadex LH-20 column, using MeOH-$CHCl_3$ (9:1) and MeOH, respectively, as mobile phases. The fractions were collected after monitoring by tlc on Si gel using $CHCl_3$-MeOH (4:1) and $CHCl_3$-MeOH-H_2O (7:3:0.3), respectively, as eluents, and detected by $Ce(SO_4)_2/H_2SO_4$ spray reagent.

A part of the $CHCl_3$ residue (7.0 g) gave five fractions (I–V). Fraction I (463 mg) was further purified by flash cc on Si gel (eluents: mixtures of $CHCl_3$/MeOH of increasing polarity, from 90 to 87% $CHCl_3$), to yield β-sitosterol 3-β-D-galactopyranoside (16 mg). Fraction II (850 mg), by flash cc on Si gel [solvent $CHCl_3$-MeOH (9:1)], gave ampelopsionoside (16 mg) and boscialin 4′-β-D-glucopyranoside (22 mg). Fraction III (2100 mg) was crystallized from MeOH and gave mandshurin (1730 mg); the mother layers were purified by flash cc on Si gel ($CHCl_3$ as eluent) and yielded herniarin (24 mg). Fraction IV (2400 mg), by flash cc on Si gel ($CHCl_3$), gave fraxinol (1140 mg) and umbelliferone (20 mg). Fraction V (450 mg), by gravity cc on Si gel followed by preparative Si gel tlc [$CHCl_3$-MeOH (4:1) as eluents], gave kaempferol (10 mg) and dihydroquercetin (8 mg).

A part of the $CHCl_3$/MeOH residue (8.0 g) gave six fractions (I–VI). Fraction I (324 mg) by Lobar RP-18 cc [solvent MeOH-H_2O (1:1)] yielded compound **1** (26 mg). From fraction II (2300 mg), by Si gel gravity cc (eluents: mixtures of $CHCl_3$/MeOH of increasing polarity, from 80 to 70%), compound **2** (28 mg) and benzyl β-D-glucopyranoside (85 mg) were obtained. Fraction III (112 mg), by Si gel gravity cc [eluent $CHCl_3$-MeOH (17:3)], gave compound **3** (26 mg). Fraction IV (2600 mg), by low pressure Lobar RP8 cc [solvent MeOH-H_2O (3:2)], gave mandshurin (1800 mg) and compound **4** (21 mg). Fraction V (210 mg), by low pressure Lobar RP8 cc [solvent MeOH-H_2O (3:2)], yielded rutin (18 mg). Fraction VI (214 mg), by preparative Si gel tlc [eluent $CHCl_3$-MeOH (4:1)], gave dihydrokaempferol (12 mg).

KNOWN COMPOUNDS.—Coumarins and flavonoids were identified by comparison of their ^{1}H-nmr and uv data with those in the literature (21–29) and by co-tlc with authentic samples. β-Sitosterol 3-β-D-galactopyranoside, ampelopsionoside, boscialin 4′-β-D-glucopyranoside, and benzyl β-D-glucopyranoside were identified by comparing their ^{1}H- and ^{13}C-nmr data with those from the literature (30–33).

5-(β-D-GLUCOPYRANOSYLOXY)-2-(3-HYDROXYBUTYL)-1,3,3-TRIMETHYLCYCLOHEXENE [**1**].—Ir ν max (NaCl) 3500–3300, 1375, 1365, 1260, 1170 cm^{-1}; $[\alpha]^{20}D$ −83.6 (MeOH, c=0.5); fabms m/z $[M+Na]^+$ 397, $[M+H]^+$ 375, $[M+H-hexose]^+$ 213; ^{1}H nmr (CD_3OD) δ 0.91 and 0.95 (6H, Me-11 and Me-12), 1.21 (3H, d, J=6.2 Hz, Me-10), 1.52 (3H, br s, Me-13), 1.60–2.11 (8H, m, H_2-4, H_2-6, H_2-7, H_2-8), 3.31–4.03 (8H, m, carbinolic protons), 5.10 (1H, d, J=7.6 Hz, H-1′); ^{13}C nmr (CD_3OD) δ 20.0 (C-13), 24.0 (C-10), 29.6 (C-11), 22.9 (C-12), 23.0 (C-7), 35.5 (C-8), 38.0 (C-3), 39.3 (C-6), 47.0 (C-4), 61.4 (C-6′), 68.8 (C-9), 70.2 (C-4′), 71.9 (C-3), 77.8 (C-5), 78.1 (C-5′), 125.4 (C-1), 136.9 (C-2).

ACID HYDROLYSIS OF **1**.—A mixture containing 1.0 ml 1 N HCl, 2.0 ml dioxane, and **1** (15 mg) was heated in a sealed tube at 80° for 4 h; then 5.0 ml of H_2O was added. The mixture was extracted with 10.0 ml of $CHCl_3$, and the organic layers gave the aglycone by evaporation. The aqueous layer was neutralized with Amberlite IRA 400 (OH- type) and evaporated to dryness. The sugar residue was directly analyzed by co-tlc with authentic samples on Si gel 60 F_{254} (Merck) using EtOAc-H_2O-MeOH-HOAc (13:3:3:4) as eluent; the detection was made with *p*-anisidine phtalate and naphthoresorcinol reagents. Glucose was identified by chromatographic comparisons with an authentic sample.

5-HYDROXY-2-(3-HYDROXYBUTYL)-1,3,3-TRIMETHYLCYCLOHEXENE.—$[\alpha]^{20}D$ −10.5 (MeOH, c=0.4); ^{1}H nmr (CD_3OD) δ 0.90 and 0.98 (6H, Me-11 and Me-12), 1.25 (3H, d, J=6.3 Hz, Me-10), 1.56 (3H, br s, Me-13), 1.58–2.15 (8H, m, H_2-4, H_2-6, H_2-7, H_2-8), 3.70 (1H, m, H-9), 3.99 (1H, m, H-5); ^{13}C nmr (CD_3OD) see Table 1.

4-(β-D-GLUCOPYRANOSYLOXY)-3-(3-METHYL-2-BUTENYL)BENZOIC ACID [**2**].—Ir ν max cm^{-1} 3500–3300, 1670, 1605, 1380, 1280, 1110; $[\alpha]^{20}D$ −44.0 (MeOH, c=1.2); uv λ max (MeOH) 246, 250 nm; fabms m/z $[M+Na]^-$ 391, $[M+H]^+$ 369, $[M+H-hexose]^-$ 207; ^{1}H nmr (CD_3OD) δ 1.73 (6H, s, Me-4′, Me-5′), 3.18–3.68 (6H, m, sugar protons), 3.45 (2H, br d, J=7.5 Hz, H_2-1′), 4.85 (1H, d, J=6.9 Hz, H-1″), 5.35 (1H, br t, J=7.5 Hz, H-3′), 6.97 (1H, d, J=9.0 Hz, H-5), 7.65 (2H, m, H-2, H-6); ^{13}C nmr (CD_3OD) δ 17.9 (C-5′), 26.0 (C-4′), 29.3 (C-1′), 62.4 (C-6″), 71.2 (C-2″), 74.9 (C-4″), 78.1 (C-3′), 78.2 (C-5′), 101.9 (C-1′), 114.6 (C-5), 123.7 (C-2′), 128.9 (C-2), 131.3 (C-4), 131.8 (C-6), 173.9 (C-1).

FRAXINOL 6-β-D-GALACTOPYRANOSIDE [**3**].—$[\alpha]^{20}D$ −24.5 (MeOH, c=0.8); uv λ max (MeOH) 236, 256 (sh), 314, 340 nm; fabms m/z $[M+H]^+$ 381, $[M+H-hexose]^+$ 219. Galactosyl moiety: ^{1}H-nmr (CD_3OD) δ 3.03–3.68 (6H, m, H-2′–H-6′), 5.11 (1H, d, J=7.3 Hz, H-1′); ^{13}C nmr (CD_3OD) δ 61.4 (C-6′), 68.8 (C-4′), 70.9 (C-2′), 73.3 (C-3′), 75.6 (C-5′). For ^{1}H- and ^{13}C-nmr data of aglycone moiety, see Table 2.

6,7-DIHYDROXY-5-METHOXYCOUMARIN 6-β-D-GLUCOPYRANOSIDE [**4**].—$[\alpha]^{20}D$ −32.9 (MeOH, c=1.4); uv λ max (MeOH) 236, 254 (sh), 310, 342 nm; fabms m/z $[M+Na]^+$ 387, $[M+H]^+$ 365, $[M+H-hexose]^+$ 203. Glucosyl moiety: ^{1}H-nmr (CD_3OD) δ 3.03–3.71 (6H, m, H-2′–H-6′), 5.07 (1H, d, J=7.8 Hz, H-1′); ^{13}C nmr (CD_3OD) 62.0 (C-6′), 70.5 (C-4′), 74.0 (C-2′), 76.6 (C-3′), 76.8 (C-5′). For ^{1}H- and ^{13}C-nmr data of aglycone moiety, see Table 2.

ACID HYDROLYSES OF COMPOUNDS **2**–**4**.—Each compound (5 mg) was hydrolyzed with 5% aqueous MeOH/HCl under reflux for 3 h and worked up in the usual way. The residue yielded aglycones, identified by spectroscopic methods, and the sugars, detected as described for hydrolysis of **1**.

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