New Glycosides from *Salvia moorcroftiana* (Lamiaceae)

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From the MeOH extract of *Salvia moorcroftiana* WALL. (Lamiaceae), four new compounds, the two flavonoid glycosides genkwanin 4′-[*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-galactopyranoside] (**1**) and genkwanin 4′-[*O*-*α*-L-arabinopyranosyl-(1 → 3)-*α*-L-rhamnopyranoside] (**2**), and the two benzene derivatives 4-hydroxy-2-isopropyl-5-methylphenyl *O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranoside (**3**) and nonyl 4-hydroxybenzoate (**4**), were isolated in addition to two known compounds. The structures of all new compounds were determined by 1D and 2D homonuclear and heteronuclear NMR spectroscopy and by comparison with published data.

1. Introduction. – Several species of the genus *Salvia* are well known for their use in medicine. A broad spectrum of biological properties of the genus have been reported ranging from antitumor activity, antifeedent, antifungal, hypoglycemic, antibabetic, antitubercular, emetic, against cough, and for hemorrhoids [1–6]. The interesting and immense medicinal importance of this genus encouraged us to undertake the phytochemical investigation of *Salvia moorcroftiana*.

Salvia moorcroftiana WALL., commonly known in Pakistan as 'kallijari' [7], is used as a folk medicine for the guinea worm and itch and is applied in the form of a poultice to wounds [8][9]. Previous phytochemical investigations of this plant revealed the presence of diterpenoids [9–12], aromatic ester [13] flavonoids, and flavonoid glycosides [14][15]. In this paper, we wish to describe the isolation and identification of the two new flavonoid glycosides **1** and **2** and of the two new benzene derivatives **3** and **4** as well as of two known compounds, apigenin 7-*O*-dirhamnoside [16] and luteolin 3′-*O*-glucoside [17].

2. Results and Discussion. – Compound **1** was obtained as a yellowish powder. Its ^{1}H- and ^{13}C-NMR data indicated the presence of a flavone skeleton [15][18][19], which was recognized by positive reaction with the *Molish* and *Shinoda* reagents [20]. The molecular-ion peak at *m/z* 593 ($[M+H]^+$) in the positive-ion fast-atom bombardment (FAB) MS suggested the molecular formula $C_{28}H_{32}O_{14}$. Moreover, the other fragment ions at *m/z* 447 ($[(M+H)-Rha]^+$) and 285 ($[(M+H)-(Gal+Rha)]^+$) were also observed, which revealed the presence of two sugar moieties in the molecule. On acid hydrolysis, compound **1** yielded rhamnose and galactose and yellow needles of the

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aglycone that was characterized as genkwanin by comparison on co-TLC with an authentic sample and of its spectral data with reported data [15][21–23]. The two sugar units were identified as rhamnopyranose and galactopyranose by comparing their chemical shifts in the ^{13}C-NMR spectra (*Table 1*) with reference data [22], and their structures were further established by co-TLC with authentic samples and by GLC analysis of their thiazolidine derivatives [24]. The ^{13}C- and ^{1}H-NMR and HMBC spectra confirmed that the structure of compound **1** is genkwanin 4′-[*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-galactopyranoside].

Table 1. *^{13}C-NMR Data of Compounds* **1** *and* **2** *in (D$_6$)DMSO*[a])

	1	**2**
C(2)	164.0	164.1
CH(3)	103.6	103.4
C=O	182.5	182.0
C(5)	161.3	161.6
CH(6)	99.4	99.2
C(7)	163.4	163.1
CH(8)	94.2	94.7
C(9)	157.6	157.2
C(10)	104.8	104.9
C(1′)	122.3	122.1
CH(2′)	128.5	128.1
CH(3′)	115.3	115.6
C(4′)	161.6	162.0
CH(5′)	115.3	115.6
CH(6′)	128.5	128.1
*Me*O–C(7)	54.7	54.5
Gal or Rha: CH(1″)	99.8	100.5
CH(2″)	75.6	69.8
CH(3″)	73.1	74.5
CH(4″)	68.5	71.9
CH(5″)	74.8	68.7
CH_2(6″) or Me(6″)	62.4	16.8
Rha or Ara: CH(1‴)	100.5	106.1
CH(2‴)	70.9	72.4
CH(3‴)	70.8	74.2
CH(4‴)	72.4	69.4
CH_2(5″)	68.7	66.7
Me(6‴)	17.2	

[a]) Multiplicities by DEPT.

The two anomeric signals of **1** at δ 5.53 (d, J = 7.9 Hz) and 5.45 (d, J = 3.1 Hz) in the ^{1}H-NMR spectrum and their correlated C-atoms in the ^{13}C-NMR spectrum at δ 99.8 and 100.5, respectively, indicated the presence of two sugar moieties in the compound. The downfield chemical shift of C(2″) of the galactose moiety established the site of attachment of the rhamnose unit [18][25]. This was also confirmed by the HMBC interaction of the anomeric proton of rhamnose at δ 5.45 to C(2) (δ 75.6) of galactose. The 1.8 ppm upfield shift of C(4′) in the ^{13}C-NMR spectrum indicated the attachment of the sugar residue at C(4′) instead of a free OH group as the presence of a MeO group at C(7) had already been established [25]. This was further confirmed by the HMBC spectrum in which the anomeric proton H–C(1″) of the galactopyranose unit at δ 5.53 showed the connectivity with C(4′) (δ 161.6).

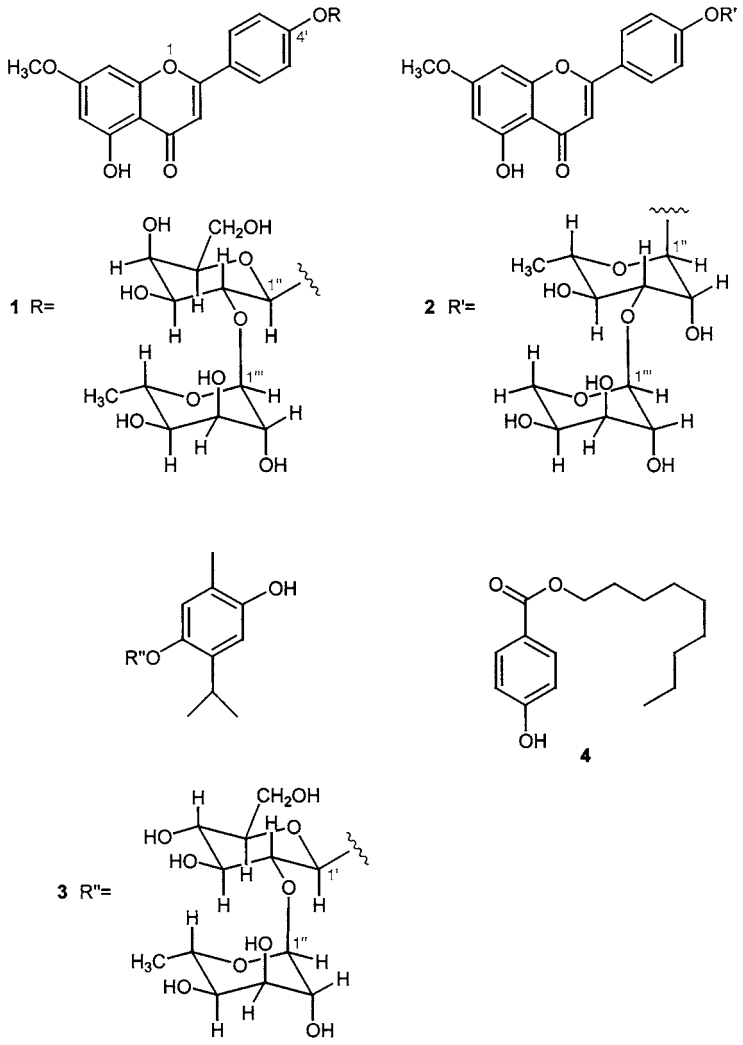
Compound **2** was obtained as yellow powder. The positive-ion FAB-MS showed a molecular-ion peak at *m/z* 563 ($[M+H]^+$) corresponding to the molecular formula $C_{27}H_{30}O_{13}$. The peaks of fragment ions at *m/z* 431 ($[(M+H)-Ara]^+$) and 285 ($[(M+H)-(Ara+Rha)]^+$) revealed the presence of two sugar moieties in compound **2**. The spectral data of the aglycone of **2** were similar to that of compound **1**, whereas the sugar signals were not identical. On acid hydrolysis, **2** yielded genkwanin as aglycone [15][21–23] and rhamnose and arabinose as sugar moieties, which were identified by co-TLC with authentic samples in different solvent systems and by GLC analysis of their thiazolidine derivatives [24]. From these results and the spectral data, the structure of compound **2** was established as genkwanin 4′-[*O*-*α*-L-arabinopyranosyl-(1 →)-*α*-L-rhamnopyranoside].

In the ^{1}H- and ^{13}C-NMR spectrum (*Table 1*) of **2**, signals of anomeric protons were observed at δ 5.40 ($d, J = 3.5$ Hz) and 5.45 ($d, J = 3.1$ Hz) and their correlated C-signals at δ 100.5 and 106.1, respectively. This information along with other resonances for sugar protons and C-atoms also inferred the presence of two sugar moieties in **2**. The downfield chemical shifts of C(3″) of the rhamnopyranose unit established the site of attachment of the arabinose to the rhamnose unit [25]. In the HMBC spectrum, correlation peaks were observed at δ 5.45 (H–C(1‴) of arabinopyranose) to δ 74.5 (C(3″) of the substituted rhamnose). Thus the arabinosyl-(1 → 3)-rhamnose structure was revealed. Finally, with the help of the HMBC spectrum, the linkage of the diglycoside moiety with the aglycone was found at C(4′).

Compound **3** was isolated as gummy residue. The molecular-ion peak was observed in the FD-MS at *m/z* 474 corresponding to the molecular formula $C_{22}H_{33}H_{11}$, which was confirmed by HR-MS, with six degrees of unsaturation. The positive-ion FAB-MS also exhibited the $[M+H]^+$ peak at *m/z* 475. In the same spectrum, fragment ions at *m/z* 329 ($[M+H-Rha]^+$) and *m/z* 167 ($[M+H-Rha-Glc]^+$) revealed the presence of two sugar moieties in the molecule. On acid hydrolysis, compound **3** yielded an aglycone, glucose, and rhamnose. The aglycone was identified as 2-isopropyl-5-methylbenzene-1,4-diol on the basis of IR and ^{1}H- and ^{13}C-NMR spectra and cochromatography with an authentic sample [28]. The sugar residues were identified by co-TLC with the authentic samples in three solvent systems and by GLC analysis of their thiazolidine derivatives. Thus, the structure of **3** was elucidated as 4-hydroxy-2-isopropyl-5-methylphenyl *O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranoside.

In the ^{1}H-NMR spectrum of **3**, two anomeric-proton signals at δ 4.52 ($J = 7.8$ Hz, H–C(1′)) and δ 5.05 ($J = 1.6$ Hz, H–C(1″)) corresponding to the signals in the ^{13}C-NMR spectrum (*Table 2*) at δ 101.8 and 103.2, respectively, confirmed the presence of two sugar residues. A combination of all ^{1}H,^{1}H COSY, HMQC, HMBC, and NOESY experiments allowed unambiguous assignments of all ^{1}H- and ^{13}C-NMR signals of the sugar moieties, identified as a *β*-D-glucopyranoside and a *α*-L-rhamnose unit. The ^{1}H- and ^{13}C-NMR signals of the aglycone moiety of **3** showed good general agreement with those of the same aglycone of a glycoside whose structure was determined by X-ray crystallography [26], but the signals due to the sugar moiety were not identical. Moreover, the positive-ion FAB-MS of **3** gave a peak at *m/z* 475 ($[M+H]^+$), which was 146 units higher than that of 2-isopropyl-5-methyl-1,4-benzenediol 1-(*O*-*β*-D-glucopyranoside) [26][27]. Furthermore, the ^{13}C-NMR spectrum also indicated the presence of a rhamnose unit, which was linked to C(2) of the glucopyranoside moiety as suggested by the glycosylation shift [23]; this was confirmed by a HMBC interaction of H–C(1″) of the rhamnose moiety with C(2′) of the glucose unit.

Compound **4** was obtained as a colorless gum from the AcOEt-soluble part of the MeOH extract. Its molecular-ion peak in the CI-MS was observed at *m/z* 264, corresponding to the molecular formula $C_{16}H_{24}O_3$, which was confirmed by HR-MS,



showing the presence of five degrees of unsaturation. The ^{1}H- and ^{13}C-NMR (*Table 2*), ^{1}H,^{1}H-COSY, and HBMC data of **4** were consistent with the structure of nonyl 4-hydroxybenzoate.

The ^{1}H-NMR spectrum of **4** showed an *AA′BB′* system at δ 7.05 (H–C(2), H–C(6)) and δ 6.68 (H–C(3), H–C(5)), which was further confirmed by ^{1}H,^{1}H COSY indicating *para* substitution of the ring. The ^{1}H-NMR spectrum also showed 2 *t*, one with a 2 H integration at δ 4.19 (t, J = 7.3 Hz, CH_2(1′)) and the other with a 3 H integration at δ 0.88 (t, J = 7.1 Hz, Me(9′)) corresponding to the presence of CH_2 and Me groups, respectively. A broad *s* of twelve protons in the upfield region at δ 1.16–1.25 and its cross-peaks with the signals at δ 4.19 (CH_2(1′)) and 0.88 (Me(9′)) in the ^{1}H,^{1}H COSY were consistent with a C_9 chain. This chain was found to be part of an ester, which was determined with the aid of a HMBC correlation of the downfield CH_2 signal at δ 4.19 to the ester carbonyl (δ 173.8). All the chemical shifts were confirmed by HMBC and ^{1}H,^{1}H COSY experiments.

Table 2. *^{13}C-NMR Data of Compounds* **3** *and* **4** *in CD_3OD and $CDCl_3$*[a])

	3		**4**
C(1)	149.3	C(1)	128.5
C(2)	137.6	CH(2)	129.9
CH(3)	113.0	CH(3)	115.9
C(4)	151.6	C(4)	156.6
C(5)	122.6	CH(5)	115.9
CH(6)	120.8	CH(6)	129.9
Me_2CH	27.0	C=O	173.8
*Me_2*CH	23.0, 23.1	CH_2(1′)	64.8
		CH_2(2′)	29.3
Me–C(5)	16.5	CH_2(3′) to CH_2(8′)	29.2
		Me(9′)	15.6
Glu: CH(1′)	103.2		
CH(2′)	76.9		
CH(3′)	77.7		
CH(4′)	71.8		
CH(5′)	77.2		
CH_2(6′)	62.5		
Rha: CH(1″)	101.8		
CH(2″)	71.1		
CH(3″)	72.1		
CH(4″)	73.5		
CH(5″)	69.6		
Me(6″)	16.8		

[a]) Multiplicities by DEPT.

The molecular formula of a further compound was determined with the help of HR-MS as $C_7H_6O_3$ with five degrees of unsaturation. On the basis of spectral information, the structure of 4-hydroxybenzoic acid was deduced for this compound, which is a very common metabolite and has been isolated from various plants [28]. This compound has not been reported earlier from our investigation source.

The ^{1}H-NMR of **5** showed only two 2 H *d* in the aromatic region at δ 6.75 and 7.05 with same coupling constant ($J = 6.9$ Hz, H–C(2) and H–C(6) and H–C(3) and H–C(5), resp.).

Experimental Part

General. Purity of the compounds were checked by HPLC: *Zorbax ODS-C_{18}* column (25 × 4.6 mm); flow rate 1.7 ml min^{-1}; UV detection at 280 nm; isocratic MeOH/H_2O/AcOEt 20 : 4 : 1. GLC: *GC-18A* equipped with FID; t_R in min. IR and UV Spectra: *Shimadzu IR-46* and *Shimadzu UV-240*, resp.; in cm^{-1} and nm, resp. ^{1}H- and ^{13}C-NMR Spectra: at 500 and 125 MHz, resp.; *Bruker AM-500* spectrometer; δ in ppm, J in Hz. Fast-atom-bombardment mass spectra (FAB-MS): double-focusing *Varian MAT-312* spectrometer in a positive-ion mode; lactic acid as solvent; in *m/z*.

Collection, Identification, and Extraction. The plant material (whole parts, 6 kg) was collected from Manshera, Peshawar, Pakistan, in June 2000 and identified by Dr. *Abdd-ur-Rashid* (Department of Botany, University of Peshawar, Peshawar, Pakistan). A voucher specimen (No. 854) was deposited in the herbarium of the same department. The shade-dried plant material was extracted repeatedly with MeOH at r.t. The combined MeOH extracts were evaporated to afford a gummy residue (345 g). This residue was partitioned between H_2O and hexane. The aq. layer was then extracted 3 × each with AcOEt (118 g) and then with BuOH 76.8 g).

Isolation, Purification, and Characterization. The BuOH extract was subjected to column chromatography (CC; silica gel, gradient MeOH/$CHCl_3$). The fractions eluted with 20–25% $CHCl_3$/MeOH contained **1**, were combined, and evaporated. The residue was applied to a *Sephadex-LH-20* column, and eluted with 75% MeOH and allowed to stand at 5° for two days: *2-[4-{[*O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-β-*D*-galactopyranosyl]-oxy}phenyl]-5-hydroxy-7-methoxy-4*H*-1-benzopyran-4-one* (**1**; 10.8 mg). Yellowish powder. M.p. 212–214°. R_f 0.70 (BuOH/AcOH/H_2O 12:3:5), 0.59 (BuOH/EtOH/H_2O 12:3:5), 0.41 (15% aq. AcOH). UV (MeOH): 269, 320. UV (MeOH + NaOMe): 287, 360. UV (MeOH + $AlCl_3$·HCl): 279, 297, 333, 380. UV (MeOH + NaOAc): 270, 314. UV (MeOH + NaOAc/H_3BO_3): 269, 320. IR (KBr): 3510–3485, 2950, 2868, 1690, 1130–1015, 825. ^{1}H-NMR ((D$_6$)DMSO, 500 MHz): 6.92 (*s*, H–C(3)); 6.43 (*d*, *J* = 2, H–C(6)); 6.83 (*d*, *J* = 2, H–C(8)); 8.02 (*d*, *J* = 8.0, H–C(2′), H–C(6′)); 7.20 (*d*, *J* = 8.0, H–C(3′), H–C(5′)); 5.53 (*d*, *J* = 7.9, H–C(1″)); 5.45 (*d*, *J* = 3.1, H–C(1‴)); 3.80 (*s*, MeO); 1.02 (*d*, *J* = 5.7, Me(6‴). ^{13}C-NMR: *Table 1*. FAB-MS: 593 ([*M* + H]$^+$), 447 ([(*M* + H) – Rha]$^+$), 285 ([(*M* + H) – (Gal + Rha)]$^+$). HR-MS: 593.5191 ($C_{28}H_{32}O_{14}^+$; calc. 593.5198).

With 30% MeOH/$CHCl_3$, the main CC (silica gel) yielded fractions containing **2**, which were further subjected to chromatography with a *Sephadex-LH-20* column and eluted with 70% MeOH. Purification by prep. TLC (purple band under UV) yielded *2-[4-{[*O*-α-*L*-arabinopyranosyl-(1 → 3)-α-*L*-rhamnopyranosyl]oxy}-phenyl]-5-hydroxy-7-methoxy-4*H*-1-benzopyran-4-one* (**2**; 9.50 mg). M.p. 223–225°. R_f 0.54 (BuOH/AcOH/H_2O 12:3:5), 0.41 (BuOH/EtOH/H_2O 12:3:5), 0.36 (15% aq. AcOH). UV (MeOH): 270, 320. UV (MeOH + NaOMe): 285, 358. UV (MeOH + $AlCl_3$·HCl): 275, 299, 336, 380. UV (MeOH + NaOAc): 271, 316. UV (MeOH + NaOAc/H_3BO_3): 270, 320. IR (KBr): 3510–3470, 2955, 2875, 1695, 1130–1010, 825. ^{1}H-NMR ((D$_6$)DMSO, 500 MHz): 6.94 (*s*, H–C(3)); 6.45 (*d*, *J* = 2, H–C(6)); 6.86 (*d*, *J* = 2.2, H–C(8)); 7.98 (*d*, *J* = 7.8, H–C(2′), H–C(6′)); 7.19 (*d*, *J* = 7.8, H–C(3′), H–C(5′)); 5.40 (*d*, *J* = 3.5, H–C(1″)); 5.45 (*d*, *J* = 3.1, H–C(1‴)); 1.05 (*d*, *J* = 5.5, Me(6″)); 0.89 (*d*, *J* = 5.5, Me‴); 3.80 (*s*, MeO). ^{13}C-NMR: *Table 1*. FAB-MS: 563 ([*M* + H]$^+$), 431 ([(*M* + H) – Ara]$^+$), 285 ([(*M* + H) – (Ara + Rha)]$^+$). HR-MS: 563.6870 ($C_{27}H_{30}O_{13}^+$; calc. 563.6878).

Repeated CC of the fraction obtained from the main CC (silica gel) with 20% MeOH gave *4-hydroxy-2-isopropyl-5-methylphenyl* O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-β-*D*-glucopyranoside* (**3**; 14.3 mg). Gummy residue. $[\alpha]_D^{24} = -23.6$ (*c* = 1.35, MeOH). ^{1}H-NMR (CD_3OD, 500 MHz): 0.98 (*J* = 6.5, Me(6″)); 1.14, 1.16 (2*d*, *J* = 7.0, each, *Me*$_2$CH); 2.13 (*s*, Me–C(5)); 2.64 (*m*, H–C(5′)); 3.11 (*t*, *J* = 9.2, H–C(4′)); 3.25 (*ddd*, *J* = 2.5, 5.5, 9.1, H–C(5′)); 3.25 (*m*, Me$_2$C*H*); 3.28 (*t*, *J* = 9.1, H–C(4′)); 3.43 (*m*, H–C(3′)); 3.51 (*m*, H–C(2′)); 3.65 (*t*, *J* = 9.1, H–C(3′)); 3.68 (*dd*, *J* = 5.2, 11.8, 1 H–C(6′)); 3.82 (*dd*, *J* = 2.5, 11.8, 1 H–C(6′)); 4.05 (*dd*, *J* = 7.6, 9.1, H–C(2′)); 4.52 (*d*, *J* = 7.8, H–C(1′)); 5.05 (*d*, *J* = 1.6, H–C(1″)); 6.68 (*s*, H–C(3)); 6.96 (*s*, H–C(6)). ^{13}C-NMR: *Table 2*. FD-MS: 474 (*M*$^+$). FAB-MS (pos. mode): 475 ([*M* + H]$^+$), 329 ([(*M* + H] – Rha]$^+$), 167 ([(*M* + H – Rha – Glc]$^+$). HR-MS: 474.4982 ($C_{22}H_{33}O_{11}^+$; calc. 474.4990).

The AcOEt-soluble part was submitted to CC (silica gel). The fraction eluted with 20% $CHCl_3$/hexane was further purified by repeated CC and then by prep. TLC (AcOEt/hexane 20:80): *nonyl 4-hydroxybenzoate* (**4**; 10.4 mg). Gum. IR ($CHCl_3$): 3550, 1720. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.05 (*d*, *J* = 6.8, H–C(2), H–C(6)); 6.68 (*d*, *J* = 6.8, H–C(3), H–C(5)); 4.19 (*d*, *J* = 7.3, CH_2(1′)); 1.16–1.25 (br. *s*, CH_2(2′) to CH_2(8′)); 0.88 (*t*, *J* = 7.0, Me(9′)). ^{13}C-NMR: *Table 2*. CI-MS: 264 (*M*$^+$), 121 (100). HR-MS: 264.3618 ($C_{16}H_{24}O_3^+$; calc. 264.3604).

From the CC of the AcOEt-soluble part, the fraction eluted with 30% $CHCl_3$/hexane was further purified by repeated CC and finally by prep. TLC (10% acetone/hexane): *4-hydroxybenzoic acid* (8.2 mg). Amorphous powder.

Acid Hydrolysis of Compounds **1**–**3**. At 100°, 5 mg of **1**, **2**, or **3** was refluxed with 10% aq. HCl soln. for 3 h. On cooling, the aglycone of **1** and **2** was separated, recrystallized from $CHCl_3$, and identified as genkwanin (= *5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4*H*-1-benzopyran-4-one*) by comparison of its spectral data [15][22]. The aglycone of compound **3** was identified as *2-isopropyl-5-methylbenzene-1,4-diol* by comparison with the reported data [26].

The aq. hydrolyzate was neutralized with $AgCO_3$ and concentrated; by co-TLC with authentic sugars in different solvent systems, glucose, galactose, arabinose, and rhamnose were identified. The concentrated residue was further treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.125 ml) at 60° for 1 h. The soln. was then treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.05 ml) at 60° for 1 h. The supernatant was applied to GLC (*Supelco SPB™-1* column (30 m × 0.25 mm), column temp. 230°, N_2 flow rate 0.8 ml min^{-1}): t_R of derivatives of D-glucose 10.32 (L-glucose 10.78), of D-galactose 11.09 (L-galactose 11.89), of L-rhamnose 9.41 (D-rhamnose 9.72), and of L-arabinose 7.79 (D-arabinose 8.40).

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