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NEW CONSTITUENTS FROM *PYRACANTHA COCCINEA* LEAVES

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ABSTRACT.—From the leaves of *Pyracantha coccinea* (Rosaceae), we have isolated and identified four new compounds, 2′(3-methylbut-2-enyloxy)-6-methoxyangelicin (pyracanthin A) [**1**], 2′(3-methylbut-2-enyloxy)-6-hydroxyangelicin (pyracanthin B) [**2**], 5,7,3′,4′-tetrahydroxy-7-*O*-[6″-*O*-(acetyl)-β-D-glucopyranosyloxy]-flavanone (coccinoside A) [**4**], and 5,7,2′,5′-tetrahydroxy-7-*O*-β-D-glucopyranosyloxyflavanone (coccinoside B) [**5**], besides scopoletin, 5,7,2′,5′-tetrahydroxyflavanone, borneolapiosylglucopyranoside, and β-ionol glucopyranoside, never previously isolated from this genus.

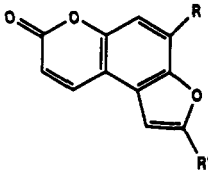
The new structures were established by detailed spectral studies including COSY, 2D ^{1}H-^{13}C direct chemical shift correlation (HETCOR), and 2D ^{1}H-^{13}C correlation via long range coupling (COLOC) nmr techniques. The known compounds were identified by ir and nmr spectral analyses.

In a previous paper (1) we reported the isolation and characterization of a new acylated flavanone glycoside from *Pyracantha coccinea* M.J. Roemer (Rosaceae). In continuing our research on this species, four new constituents were isolated from the leaves. We describe their separation and structural elucidation by ir, uv, ms, ^{1}H- and ^{13}C-nmr spectra, and 2D nmr techniques. The dried leaves were extracted with petroleum ether followed by $CHCl_3$, $CHCl_3$-MeOH (9:1), and MeOH.

The $CHCl_3$ extract, subjected to LH-20 gel filtration and purified by gravity or flash column chromatography, yielded **1**, **2**, and scopoletin. Compounds **1** and **2**, obtained as pale yellow oils, produced violet colorations on treatment with alkaline hydroxylamine followed by $FeCl_3$, indicating their coumarinic nature (2); this was confirmed by ir spectra with bands at 1720 cm^{-1} (coumarin lactone C=O), 1640 cm^{-1} (3,4-double bond in conjugation with C=O), and 1265 cm^{-1} (ether); in addition, **2** had a band at 3580 cm^{-1} (OH). Their uv spectra exhibited absorptions (see Experimental) typical of angular furanocoumarins (3).

The positive ion fabms of **1** showed an $[M-H]^+$ peak at *m/z* 301 corresponding to the molecular formula $C_{17}H_{16}O_5$, which was confirmed by elemental analysis and by the ^{13}C- and DEPT ^{13}C-nmr spectra.

The ^{13}C-nmr spectrum revealed seventeen carbon signals which were sorted by DEPT ^{13}C-nmr into Me × 3, CH_2 × 1, CH × 5, C × 7, C=O × 1 (Table 1). The signal at 163.9 ppm confirmed the coumarin lactone C=O. The complete structural elucidation of the furocoumarin was derived from the chemical shifts and *J* values of the ^{1}H-nmr spectrum and from detailed spectral analyses of HETCOR and COLOC nmr techniques.



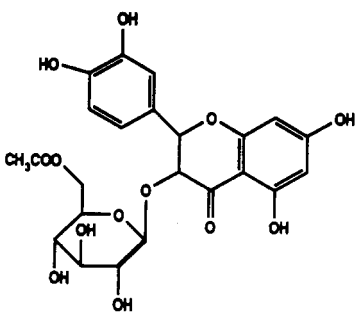
1 R=OMe, R′=$OCH_2CH=C(CH_3)_2$
2 R=OH, R′=$OCH_2CH=C(CH_3)_2$
3 R=OH, R′=H

In the ^{1}H-nmr spectrum, the coupling of doublets at δ 6.15 and 7.77, with $J = 9.5$ Hz, corresponded to H-3 and H-4 of the pyrone ring, and the last chemical shift indicated that C-5 was unsubstituted (4); moreover, the absence of an H-4/H-8 coupling showed that C-8 was substituted and confirmed the angular position of the furan ring (5). The presence of a 2′-substituted furan system was recognizable by a singlet at 7.05 due to H-3′. The oxygen substitution at C-6 arose by the absence of the long-range couplings, $J = 1$ Hz, over five bonds (4) and by the chemical shift of the signal at δ 6.85 due to H-5 (5).

A multiplet at δ 5.39 (one proton), a doublet at δ 4.55 (two protons, $J = 6.8$ Hz), and two singlets at δ 1.81 and δ 1.69 (three protons each) indicated a prenyl ether, while a singlet at δ 3.77 (three protons) indicated an MeO moiety. These substituents were confirmed by the ^{13}C- and DEPT ^{13}C-nmr spectra with signals for an aromatic MeO group at 56.8 ppm and for a methylene moiety at 67.1 ppm attributable to an O-CH_2-CH=system. The C-5 and α-pyrone ring ^{13}C shifts were assigned by comparison with those of sphondin [**3**] (5,6). The 2D ^{1}H-^{13}C nmr direct chemical shift correlation experiments gave the unambiguous assignment of the H-5 proton by correlation of the known C-5 carbon resonance with the signal at δ 6.85 and allowed correlation of the singlet at δ 7.02 (H-3′) with the resonance at δ 109.9 (C-3′). COLOC experiments led to the complete assignment of the C-6, C-7, C-8, and C-2′ quaternary carbons and to elucidation of the relative substituents.

Thus, from the three-bond couplings, the chemical shifts of C-6 and C-2′ were assigned by correlation between the proton resonance at δ 6.85 (H-5) with carbon resonance at δ 148.3 and the signal at δ 7.02 (H-3′) with that at δ 153.9, respectively. Finally, the chemical shifts of C-7 and C-8 were obtained by comparison with those of sphondin [**3**], taking into account the β effects of hydroxylation at C-2′. Since C-6 also correlated with the proton resonance at δ 3.77 (MeO), we concluded that the MeO moiety was linked to C-6. Therefore, compound **1** is 2′(3-methylbut-2-enyloxy)-6-methoxyangelicin, which we have named pyracanthin A.

Compound **2**, in the positive ion fabms, showed an $[M - H]^+$ peak at *m/z* 287. The ^{13}C-nmr spectrum revealed sixteen carbon signals which were sorted by DEPT ^{13}C-nmr into Me × 2, CH_2 × 1, CH × 5, C × 7, C=O × 1 (Table 2). The complete structural elucidation of this furocoumarin was derived from the chemical shifts and J values of the signals in the ^{1}H- and ^{13}C-nmr spectra in comparison with those of compound **1**. The ^{1}H-nmr spectrum showed the same signals except for the lack of the MeO group and the appearance of an OH signal at δ 5.58. The linkage of the OH group at C-6 was confirmed by ^{13}C-nmr analysis which showed the absence of the MeO resonance at δ 56.8 together with the shielding upfield of the C-6 resonance at δ 141.7 (−7.1 ppm shift for the lack of the ethereal linkage) (Table 2). The 2D experiments compared with those of



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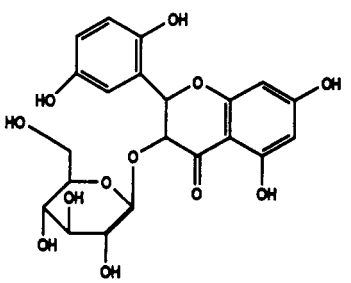

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TABLE 1. ^{1}H- and ^{13}C-nmr Data of Pyracanthin A [**1**] (200 MHz, $CDCl_3$).

Proton	δ (ppm)	H-H COSY correlations	Carbon	δ (ppm)	HETCOR correlations	COLOC correlations
			C-2	163.9 (C)		
H-3	6.15, d, J = 9.5	H-4	C-3	113.4 (CH)	H-3	
H-4	7.77, d, J = 9.5	H-3	C-4	146.0 (CH)	H-4	
			C-4a	113.0 (C)		
H-5	6.85, s		C-5	102.1 (CH)	H-5	
			C-6	148.3 (C)		6.85 (H-5)
						3.77 (MeO)
			C-7	151.1 (C)		
			C-8	110.8 (C)		
			C-8a	140.8 (C)		
			C-2′	153.9 (C)		7.02 (H-3′)
H-3′	7.02, s		C-3′	109.9 (CH)	H-3′	
H-1′	4.55, d, J = 7.0	H-2″	C-1″	67.1 (CH_2)	H-1″	
H-2′	5.39, m	H-1″, Me	C-2″	120.1 (CH)	H-2″	1.69 (Me)
			C-3″	140.0 (C)		1.69 (Me)
Me	1.81, d, J < 1	H-2″	Me	25.9 (Me)	Me	1.69 (Me)
Me	1.69, s		Me	18.3 (Me)	Me	
MeO	3.77, s		MeO	56.8 (Me)	MeO	

1 confirmed the structure of **2** as 2′(3-methylbut-2-enyloxy)-6-hydroxyangelicin, which we have named pyracanthin B.

The $CHCl_3$/MeOH extract subjected to LH-20 gel filtration and purified on a Lobar RP8 column by eluting with MeOH/H_2O yielded 5,7,2′,5′-tetrahydroxyflavanone and compound **4**. Compound **4**, a pale yellow powder, in the positive ion fabms showed an $[M-H]^+$ peak at m/z 493 corresponding to the molecular formula $C_{23}H_{24}O_{12}$, derived by elemental analysis and by the ^{13}C- and DEPT ^{13}C-nmr analyses. The flavanoid nature was deduced by the positive test with magnesium/HCl acid reagent and by the uv spectra with the usual shift reagents which were similar to eriodictyol 7-glucoside

TABLE 2. ^{1}H- and ^{13}C-nmr Data of Pyracanthin B [**2**] (200 MHz, $CDCl_3$).

Proton	δ (ppm)	H-H COSY correlations	Carbon	δ (ppm)	HETCOR correlations	COLOC correlations
			C-2	162.7 (C)		
H-3	6.20, d, J = 9.5	H-4	C-3	114.4 (CH)	H-3	
H-4	7.53, d, J = 9.5	H-3	C-4	147.1 (CH)	H-4	
			C-4a	112.7 (C)		
H-5	6.75, s		C-5	100.9 (CH)	H-5	
			C-6	141.7 (C)		6.75 (H-5)
			C-7	149.8 (C)		
			C-8	111.3 (C)		
			C-8a	140.8 (C)		
			C-2′	154.5 (C)		6.89 (H-3′)
H-3′	6.89, s		C-3′	111.5 (CH)	H-3′	
H-1′	4.59, d, J = 7.0	H-2″	C-1″	67.0 (CH_2)	H-1″	
H-2′	5.40, m	H-1″, Me	C-2″	118.7 (CH)	H-2″	1.71 (Me)
			C-3″	140.6 (C)		1.71 (Me)
Me	1.75, d, J < 1	H-2″	Me	26.5 (Me)	Me	1.71 (Me)
Me	1.71, s		Me	19.0 (Me)	Me	
OH	5.58					

and its hexacetyl derivative (1); ir spectrum showed bands at 1655 cm^{-1} (C=O function of a γ-pyrone nucleus) and at 1715 cm^{-1} (ester moiety), besides OH and aromatic functions and the glycosidic nature (1). The ^{1}H-nmr spectrum confirmed the above suggestion, since it showed the characteristic ABX system signals of a flavanone nucleus at δ 5.25 (H-2) and 2.88 (H-3) with $J_{2ax,3ax}$ = 11.8 Hz (axial-axial coupling), indicative of a 2-aryl group equatorial to the heterocyclic ring.

The aromatic region of **4** was defined by two doublets for ring A, while ring B displayed an ABX system due to protons at C-5′, C-6′, and C-2′. The 5,7,3′,4′-tetrahydroxy substitution pattern was confirmed by the ^{13}C-nmr spectrum and suggested the structure of eriodictyol for the aglycone (7). The linkage of the sugar moiety at the 7-OH was confirmed by superposition of the ^{13}C-nmr chemical shifts for C-2 to C-8 with those of the aglycone (8) and the chemical shifts of H-6 and H-8 protons with those of eriodictyol 7-glucoside (9).

The acetyl structure of the ester moiety of **4** was deduced by the fabms data that gave a prominent fragment at *m/z* 289 due to the loss of an acetyl-hexose unit with the glycosidic oxygen. The acetyl group was confirmed by a singlet at δ 1.92 in the ^{1}H nmr, integrating for three protons, and by a carbonyl signal at δ 172.8 and an Me resonance at δ 20.7 in the ^{13}C-nmr spectrum. The sugar region of the ^{1}H-nmr spectrum, with the anomeric proton signal shifted to lower magnetic field (δ 5.11) and a large coupling constant (J = 7.3 Hz), indicated a β linkage. The ^{13}C-nmr spectrum exhibited the presence of six methynes as in eriodictyol 7-glucoside (9); but with respect to this, a

TABLE 3. ^{13}C-nmr Spectral Data [values in δ (ppm) Downfield from TMS, in CD_3OD, 200 MHz] of Flavanones **4** and **5**.

Carbon	Compound			
	4		**5**	
	^{13}C (δ)	DEPT	^{13}C (δ)	DEPT
C-2	80.8	CH	80.7	CH
C-3	44.2	CH_2	44.1	CH_2
C-4	198.6	C	197.8	C
C-5	164.8	C	164.5[a]	C
C-6	97.0	CH	97.9	CH
C-7	166.7	C	166.9	C
C-8	96.2	CH	96.9	CH
C-9	164.5	C	104.9	C
C-10	101.2	C	164.1[a]	C
C-1′	131.3	C	131.4	C
C-2′	114.8	CH	146.9[b]	C
C-3′	147.0	C	119.3	CH
C-4′	146.5	C	116.2	CH
C-5′	116.2	CH	146.4[b]	C
C-6′	119.4	CH	114.7	CH
C-1″	98.1	CH	101.2	CH
C-2″	74.6	CH	74.6	CH
C-3″	77.7	CH	77.7	CH
C-4′	71.6	CH	71.1	CH
C-5″	75.5	CH	77.9	CH
C-6″	66.7	CH_2	62.4	CH_2
CH_3	20.8	CH_3		
C=O	172.8	C		

[a,b]Assignments with the same superscript may be interchanged.

careful examination of the sugar portion showed a downfield shift of C-6″ (from 60.5 to 62.9 ppm) and an upfield shift of C-5″ (from 76.3 to 73.7 ppm) indicating a C-6″ acetyl in **4** (10).

Moreover, the ^{1}H-nmr spectrum of **4** showed a broad signal at δ 4.40 (H-6″) that underwent the 0.3 ppm downfield shift due to the acetylation at C-6″; also H-5″ evidenced a minor downfield shift (0.20 ppm) (11), while the remaining signals were practically identical with those of eriodictyol 7-glucoside. Therefore, the compound is 5,7,3′,4′-tetrahydroxy-7-*O*-[6″-*O*-(acetyl)-β-D-glucopyranosyloxy]-flavanone which we named coccinoside A.

The MeOH extract, subjected to LH-20 gel filtration and purified with a gravity Si gel chromatography ($CHCl_3$/MeOH) and with a semipreparative reversed-phase hplc (MeOH/H_2O), yielded borneol apiosylglucopyranoside, β-ionol glucopyranoside, and a yellow-orange amorphous solid **5**. Compound **5**, in the positive ion fabms, showed an $[M-H]^+$ peak at *m/z* 451 corresponding to $C_{21}H_{22}O_{11}$ (derived by the elemental and by ^{13}C- and DEPT ^{13}C-nmr analyses) and a prominent fragment at *m/z* 272 due to the loss of an hexose unit with the glycosidic oxygen. Uv and ir spectra were similar to those of 5,7,2′,5′-tetrahydroxyflavanone and **4** which suggested they were flavanoids. This was confirmed by the ^{1}H-nmr spectral data (see Experimental). The 5,7,2′,5′-tetrahydroxy substitution pattern and the location of the hexose at C-7 were derived by comparison of the ^{13}C-nmr spectrum with that of 5,7,2′,5′-tetrahydroxyflavanone 7-*O*-β-D-rutinoside (12). The unusual 2′,5′-dihydroxy substitution pattern was confirmed by an overlapped signal, integrating for two protons, at δ 6.69 (H-3′ and H-4′) and by a signal, integrating for one proton, at δ 6.83 attributable to H-6′.

The nature and the site of linkage of the hexose moiety of **5** were established by the ^{13}C-nmr spectrum, which was compared with those of eriodictyol 7-glucoside (9) and compound **4** and with other literature data (13). These comparisons led to the conclusion that a glucose was β-linked in a pyranose form to the OH at C-7 and identified **5** as 5,7,2′,5′-tetrahydroxy-7-*O*-β-D-glucopyranosyloxyflavanone. This compound constitutes the second example of a 5,7,2′,5′-tetrahydroxyflavanone glycoside found in nature (12).

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; hplc, Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401; lpc, Duramat pump. Ir and uv spectra were determined with Perkin-Elmer spectrophotometer models 684 and 330, respectively. One- and two-dimensional nmr spectra were measured in CD_3OD or $CDCl_3$. Chemical shifts are reported in δ (ppm) downfield from internal standard TMS, and coupling constants (*J*) are given in Hz. The COLOC, DEPT, direct HETCOR, and COSY-90 experiments were carried out using Bruker commercial microprograms. The 2D homonuclear proton chemical shift correlations (COSY) experiments were measured by employing the conventional pulse sequence and were obtained using a data set ($t_1 \times t_2$) of 1024 × 1024 points for a spectral width of 1165 Hz (relaxation delay 1 sec). The DEPT experiments were performed using polarization transfer pulses of 90° to obtain only CH groups and 135° to obtain positive signals for CH and Me and negative ones for the CH_2. Polarization transfer delays were adjusted to an average CH coupling of 130 Hz.

These delays were also applied for 2D direct ^{13}C-^{1}H shift correlations (HETCOR) on a 512 × 2048 data matrix; 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 10 Hz to obtain the maximum polarization transfer.

PLANT MATERIAL.—The leaves of *P. coccinea* were collected in Pisa in May 1990. A dried voucher specimen is deposited in the Department of Bioorganic Chemistry, Pisa, Italy.

EXTRACTION MATERIAL.—Dried ground leaves (1 kg) were extracted in a Soxhlet sequentially with petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), and MeOH at room temperature. Each of these extracts, evaporated to dryness, was subjected to Sephadex LH-20 gel filtration eluting with MeOH or MeOH-

$CHCl_3$ (9:1). The $CHCl_3$ residue (5.8 g) fractionated by gel filtration with MeOH-$CHCl_3$ (9:1) yielded five main fractions. Fraction IV was further separated by gravity and flash cc on Si gel eluting with $CHCl_3$-hexane (99:1) and $CHCl_3$-MeOH (95:5), repectively, to yield the pure coumarinic compounds.

The $CHCl_3$-MeOH residue (6.2 g) with MeOH gave nine fractions. Fraction VII was further fractionated by gravity cc on Si gel eluting with $CHCl_3$-MeOH (7:3) and then by Lobar RP 8 with MeOH-H_2O (3:1) to yield 5,7,2′,5′-tetrahydroxyflavanone and compound **4**.

The MeOH residue (12.8 g) eluting with MeOH gave six fractions. Fraction II was further fractionated by gravity cc on Si gel, and then by hplc on a C_{18} μ-Bondapack (30 cm × 7.8 mm, flow rate 3.5 ml min^{-1}) to yield pure β-ionol glucopyranoside. Fraction III, by flash cc on Si gel, and then Lobar RP 8 gave borneol apiosylglucopyranoside; fraction VII, by flash cc on Si gel with $CHCl_3$-MeOH (85:15) and then by Lobar RP 8 with MeOH-H_2O (7:3) yielded compound **5**.

KNOWN COMPOUNDS.—Scopoletin (13 mg) and 5,7,2′,5′-tetrahydroxyflavanone (6 mg) were identified by comparison of ^{1}H-nmr, uv, and ir data with those in the literature (4,14); borneol apiosylglucopyranoside and β-ionol glucopyranoside were identified by comparison of ^{1}H- and ^{13}C-nmr data with those from the literature (15–17).

2′(3-Methylbut-2-enyloxy)-6-methoxyangelicin (pyracanthin A) [**1**].—Compound **1** (12 mg): tlc R_f 0.37 [$CHCl_3$-hexane (9:1)]; ir ν max (NaCl) 1720, 1640, 1265 cm^{-1}; uv λ max (MeOH) 225, 250 (sh), 285, 340 nm; ^{1}H and ^{13}C nmr see Table 1. Found C 42.81, H 3.45; $C_{17}H_{16}O_5$ requires C 42.87, H 3.39%.

2′(3-Methylbut-2-enyloxy)-6-hydroxyangelicin (pyracanthin B) [**2**].—Compound **2** (7 mg): tlc R_f 0.17 [$CHCl_3$-hexane (98:2)]; ir ν max (NaCl) 3580, 1720, 1640, 1265 cm^{-1}; uv λ max (MeOH) 228, 253 (sh), 289, 338 nm; ^{1}H and ^{13}C nmr see Table 2.

5,7,3′,4′-Tetrahydroxy-7-O-[6″-O-(acetyl)-β-D-glucopyranosyloxy]-flavanone (coccinoside A) [**4**].—Compound **4** (14 mg): tlc R_f 0.41 [$CHCl_3$-MeOH (8:2)]; $[\alpha]^{25}D$ −41.4 (c = 0.7, MeOH); ir ν max (NaCl) 3480, 3240, 1715, 1655, 1265, 1065 cm^{-1}; uv λ max (MeOH) 282, 335 (sh) nm; MeOH/$AlCl_3$ 305, 366 nm; MeOH/$AlCl_3$/HCl 303, 366 nm; ^{1}H nmr (CD_3OD) (δ) aglycone 7.06 (1H, d, J = 2.1 Hz, H-2′), 7.04 (1H, dd, J = 8.9 and 2.1, H-6′), 6.91 (1H, d, J = 8.9 Hz, H-5′), 6.06 (1H, d, J = 2.3 Hz, H-8), 5.98 (1H, d, J = 2.3 Hz, H-6), 5.25 (1H, dd, J = 11.8 and 3.3 Hz, H-2), 2.68 (1H, dd, J = 3.3 and 17.5 Hz, H_b-3), 3.08 (1H, dd, J = 11.8 and 17.5 Hz, H_a-3), glucosyl moiety 5.11 (1H, d, J = 7.3 Hz, H-1″), 4.47 (1H, br d, J = 12.2 Hz, H_a-6″), 4.21 (1H, dd, J = 9.2 and 12.2 Hz, H-6_b″), 4.03 (1H, m, H-5″), 3.82–3.25 (4H, m), acetyl moiety 1.92 (3H, s); ^{13}C nmr see Table 3. Found C 56.02, H 4.98; $C_{23}H_{24}O_{12}$ requires C 56.10, H 4.91%.

5,7,2′,5′-Tetrahydroxy-7-O-β-D-glucopyranosyloxyflavanone (coccinoside B) [**5**].—Compound **5** (9 mg): tlc R_f 0.83 [$CHCl_3$-MeOH (8:2)]; $[\alpha]^{25}D$ −38.8 (c = 0.7, MeOH); ir ν max (NaCl) 3480, 3240, 1715, 1655, 1265, 1065 cm^{-1}; uv λ max (MeOH) 278, 330 (sh) nm, MeOH/$AlCl_3$ 302, 358 nm, MeOH/$AlCl_3$/HCl 303, 359 nm; ^{1}H nmr (CD_3OD) (δ) aglycone 6.83 (1H, br s, H-6′), 6.69 (2H, br s, H-3′ and H-4′), 6.16 (1H, d, J = 2.1 Hz, H-8), 6.08 (1H, d, J = 2.1 Hz, H-6), 5.24 (1H, dd, J = 12.1 and 3.8 Hz, H-2), 2.66 (1H, dd, J = 3.8 and 17.3 Hz, H_b-3), 3.06 (1H, dd, J = 12.1 and 17.3 Hz, H_a-3), glucosyl moiety 4.95 (1H, d, J = 7.3 Hz, H-1″), 3.75–3.21 (6H, m); ^{13}C nmr see Table 3. Found C 55.92, H 4.99; $C_{21}H_{22}O_{11}$ requires C 56.00, H 4.92%.

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