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A SECOIRIDOID GLUCOSIDE FROM *FRAXINUS ANGUSTIFOLIA*

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Key Word Index—*Fraxinus angustifolia*; Oleaceae; secoiridoid; nuezhenide; angustifoliside C.

Abstract—The known glucoside nuezhenide, and a new secoiridoid glucoside, angustifolioside C [6′-*O*-β- D-glucopyranosyl)-neooleuropein], were isolated from a methanolic extract of the leaves of *Fraxinus angustifolia*. Their structures were elucidated on the basis of chemical and spectral data.

INTRODUCTION

The Oleaceae family is a rich source of secoiridoid, phenylpropanoid and lignan glucosides. A number of secoiridoid glycosides including oleoside, 10-hydroxyoleoside, secologanoside, morroniside as well as secoiridoid lactone types [1–3] have been identified in Oleaceous plants. Members of the genus *Fraxinus* have been used in folk medicine [4]. Among various *Fraxinus* secondary metabolites examined, some secoiridoids exhibited a wide spectrum of biological activities [5]. Of particular interest are oleuropein and its derivatives, which showed hypotensive and related cardiovascular activities [6, 7]. Several plants of the genus *Fraxinus* have been reported to contain secoiridoid compounds, namely, oleuropein, from the leaves of *F. japonica* [8], ligstroside, from the bark of *F. griffithii* [9], and 10-hydroxyligstroside, from the bark of *F. excelsior* [10]. More complex dimeric secoiridoids consisting of two oleoside moieties linked to glucose and/or tyrosol, designated Gl-3 and Gl-5, accompanied by nuezhenide, have been identified as secondary metabolites in embryos of *F. americana* [11, 12]. Excelsioside was isolated from the leaves of *F. excelsior* [13]. Two secoiridoid glucosides, frachinoside [14] and fraxiformoside [15], have also been reported from *F. chinensis* and *F. formosana*, respectively.

Previously, we have studied the secoiridoid constituents in a methanolic extract of the leaves of *F. angustifolia*. This led to the isolation of several new secoiridoids including: ligstral (**3**), angustifolioside A (**4**), angustifolioside B (**5**), ligstrobutyl (**6**), oleobutyl (**7**), penta-*O*-methyl-ligstroside (**8**), and tetra-*O*-methyl-oleoside-dimethylester (**9**), along with the two known secoiridoid glucosides, ligstroside (**10**) and oleuropein (**11**) [16, 17]. We now wish to report on the isolation of a new secoiridoid glucoside, angustifolioside C (**1**), together with a known glucoside neuzhenide (**2**).

RESULTS AND DISCUSSION

The water soluble portion of the methanolic extract of the leaves of *F. angustifolia* was fractionated by solvent partitioning. A combination of polyamide column chromatography (CC) and silica gel CC followed by further purification through reversed phase chromatography and Sephadex LH-20 CC led to the isolation of nuezhenide (**2**) and a new glucoside, angustifolioside C (**1**).

Compound **1** was obtained as a white amorphous powder. Its FAB-mass spectrum showed a quasimolecular ion peak, $[M + Na]^+$, at *m/z* 847, indicating its M_r to be 824. It also showed fragments at *m/z* 685 and 501 attributed to $[M + Na - \text{glucose}]^+$ and $[M + H - (\text{glucose} \times 2)]^+$, respectively. These results were compatible with the molecular formula $C_{38}H_{48}O_{20}$. The UV and IR spectra suggested the presence of an enol–ether system conjugated with a carbonyl group (230 nm; 1715, 1700 and 1630 cm^{-1}), which are characteristics of many iridoid and secoiridoid skeletons [1]. In addition, absorptions due to the catechol chromophore (281 nm; 1520 and 1450 cm^{-1}) were observed. The proton signals due to the secoiridoid moiety of **1** present in its 1D and 2D ^1H–^1H-homonuclear NMR spectra were found to be closely related to those of **4** [17], multiroside and multifloroside [18].

Compound **1** gave rise to signals for two 3,4-dihydroxyphenethyl groups, unlike **4**, which contains a signal for a carbomethoxyl group [17], and these were in good agreement with the reported data for multifloroside [18]

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Table 1. ^{13}C NMR spectral data for angustifolioside C (1) (CD_3OD, 90.50 MHz), multirosid and multifloroside*

	Secoiridoid moiety				Phenolic moiety				Glucosyl moiety		
C	1	Multiroside	Multifloroside		1	Multiroside	Multifloroside		1	Multiroside	Multifloroside
1	94.70 *d*	94.65 *d*	94.57 *d*	1′	66.68 *t*	66.57 *t*	66.88 *t*	1‴	100.80 *d*	100.86 *d*	100.82 *d*
3	155.03 *d*	155.06 *d*	155.02 *d*	2′	35.96 *t*	35.32 *t*	35.29 *t*	2‴	74.73 *d*	74.81 *d*	74.78 *d*
4	109.83 *s*	109.06 *s*	109.26 *s*	3′	131.85 *s*	135.15 *s*	130.90 *s*	3‴	78.36 *d*	78.26 *d*	78.30 *d*
5	31.78 *d*	32.23 *d*	32.17 *d*	4′	117.88 *d*	117.12 *d*	116.46 *d*	4‴	71.35 *d*	71.30 *d*	71.37 *d*
6	41.14 *t*	41.14 *t*	41.07 *t*	5′	145.20 *s*	145.38 *s*	146.10 *s*	5‴	78.08 *d*	78.11 *d*	78.14 *d*
7	173.15 *s*	172.99 *s*	173.07 *s*	6″	144.84 *s*	148.33 *s*	144.78 *s*	6‴	62.67 *t*	62.70 *t*	62.40 *t*
8	126.65 *d*	129.40 *d*	129.33 *d*	7′	119.79 *d*	119.28 *d*	117.04 *d*	1⁗	104.50 *d*	104.52 *d*	
9	130.54 *s*	130.92 *s*	130.64 *s*	8′	122.92 *d*	121.45 *d*	121.32 *d*	2⁗	74.88 *d*	74.64 *d*	
10	13.55 *q*	59.13 *t*	59.19 *t*	1″	67.34 *t*		66.39 *t*	3⁗	78.22 *d*	77.78 *d*	
11	168.20 *s*	168.41 *s*	167.96 *s*	2″	36.42 *t*		35.39 *t*	4⁗	71.30 *d*	71.27 *d*	
$COCH_3$	—	52.02 *q*	—	3″	133.08 *s*		130.92 *s*	5⁗	77.92 *d*	77.49 *d*	
				4″	116.62 *d*		116.35 *d*	6⁗	62.46 *t*	62.33 *t*	
				5″	146.54 *s*		146.14 *s*				
				6″	148.15 *s*		144.78 *s*				
				7″	117.32 *d*		116.97 *d*				
				8″	121.04 *d*		121.32 *d*				

*Date taken from ref. [18].

and neooleuropein [19] with similar 3-4-dihydroxyphenethyl moieties in the secoiridoid skeleton. These were evidenced by the presence of two aromatic ABX spin systems centred between δ6.56–7.04 and two aliphatic ABX_2 spin systems at δ4.26 *dt*, 4.15 *dt* and 2.78 *t* and at δ4.41 *t*, 4.29 *t* and 2.87 *t*. In the ^{13}C NMR spectrum of **1**, two singlets at δ168.20 and 173.15 indicated the presence of two carbonyl groups at C-11 and C-7, respectively. The ^{1}H–^{13}C HMBC spectrum of **1** revealed a significant correlation of the carbonyl resonance at δ168.20(*s*) with the proton of the vinylic singlet (δ7.48, *s*, H-3). The other carbonyl signal at δ173.15(*s*), assigned to C-7, was secured by its correlation with one of the methylene protons (H-6a δ 2.44, *dd*, $J = 14.2$ and 9.5 Hz). One of the glucosyl carbon signals at δ100.80 C-1‴) showed a weak correlation with the proton singlet of H-1 (δ5.89 *brs*), suggesting the locaion of one sugar residue at C-1. Moreover, H-C(7) exhibited correlation to the '*d*' at δ4.74 (H-C-1⁗), suggesting that the second glucosyl unit was attached to the aromatic hydroxyl group at C-6′. Cross comparison of the ^{13}C-NMR spectral data for **1** (Table 1) with those for **4** [17] and multiroside [18] revealed close correspondene in every aspect to those signals arising from the secoiridoid diglucoside moiety. Moreover, an additional set of carbon signals corresponding to a 3,4-dihydroxyphenethoxyl group was observed, as in multifloroside [18] (Table 1). This finding was also supported by the positive FAB-mass spectrum, which showed a quasimolecular ion peak $[M + Na]^+$ at *m/z* 847, indicating an increase of 122 mass units in comparison with that of **4**. The base fragment ion at *m/z* 154 in the EI mass spectrum of **1** also attested to the presence of this side chain.

These features suggested that **1** was a diglucoside of an oleoside-type secoiridoid in which the carbomethoxyl group of **4** was replaced by a 3,4-dihydroxyphenethyl group. It is well known that glycosidation produces an upfield shift of the signal of oxygenated phenolic C-atoms and downfield shifts of the signals for *ortho-* and *para*-related C-atoms [20]. The resonances for H-4′, H-7′ and H-8′ (δ7.09, H-4′; 7.13, H-7′; and 6.77, H-8′), for the glycosylated dihydroxyphenylethanol moiety, were shifted upfield by δ0.23, 0.35 and 0.21, respectively. A similar spectral difference was observed for periclymenoside and periclymenosidic acid, which have the same substituents [21, 22]. Similar glycosidation effects were observed for the carbon signals of C-3′ and C-7′ (downfield shifts of $\Delta = 1.05$ and $\Delta = 2.69$, respectively) (Table 1). This result also suggested that the second glucosyl unit was attached to the aromatic hydroxyl group at C-6′. This is in full agreement with the data reported for **4** [17] and multiroside [18], which have the same substitution pattern for the phenethyl moiety.

Acetylation of **1** gave the fully acetylated undecaacetate **1a**. As expected, the FAB-mass spectrum contained a $[M + H]^+$ ion at *m/z* 1287 corresponding to the molecular formula $C_{60}H_{70}O_{31}$. The main fragment peaks recorded were at *m/z* 978 $[(M + Na) - 331]^+$ for the loss of a [2,3,4,6-*O*-acetyl glucose oxonium ion]$^+$. The ^{1}H NMR spectrum of **1a** revealed, besides those signals for the secoiridoid diglucoside residue, the presence of 11 acetyl signals belonging to three aromatic (δ2.31, 2.29 and 2.28) and eight aliphatic (δ2.13, 2.10, 2.08, 2.06, 2.02, 1.99, 1.90 and 1.75) acetyl groups, thus confirming the presence of two glucosyl and two 3,4-dihydroxyphenethyl moieties, the downfield shifts for the signals of two glucose units indicating that both are terminal. This spectral evidence pointed to structure **1** for angustifolioside C, a new example of a secoiridoid diglucoside which has not been isolated formerly.

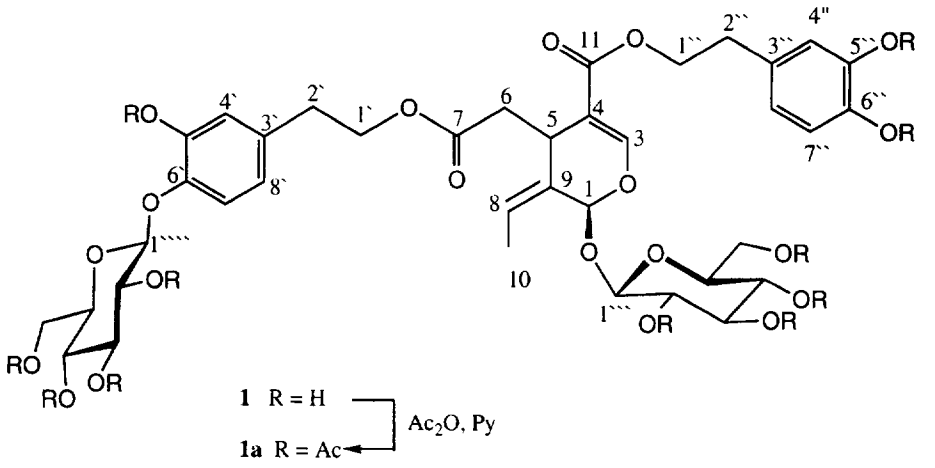
Compound **2** was obtained as an amorphous white powder. Its molecular formula $C_{34}H_{42}O_{17}$ was estimated from a weak $[M + 1]^+$ peak at *m/z* 687 and an intensive $[M + Na]^+$ peak at *m/z* 709 in the FAB-mass spectrum. The presence of an aromatic moiety and a conjugated ester group was indicated by the absorptions in the UV (238 and 273 nm) and IR (1730, 1705, 1630 and 1520 cm^{-1}) spectra of **2**. Its ^{1}H and ^{13}C NMR spectra contained signals characteristic of those for oleoside and one tyrsoyl glucoside. A comparison of the spectral data for **2** with those reported in the literature confirmed that **2** was nuezhenide [23, 24].

EXPERIMENTAL

General experimental procedures. MPLC: Sepralyte C18, 40 μm (Analytichem); TLC: Silica gel 60 F_{254} (Merck) plates; CC: Silica gel 60 (0.063–0.2 mm, Merck), Polyamide (ICN Biomedicals) or Sephadex LH-20 (Pharmacia). Secoiridoids were detected by spraying with 1% vanillin/H_2SO_4, followed by heating at 100° for 5 min. ^{1}H and ^{13}C NMR: Bruker NMR 360 MHz and Varian NMR 600 MHz high field spectrometers equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1b, respectively, using TMS as int. standard in appropriate solvents.

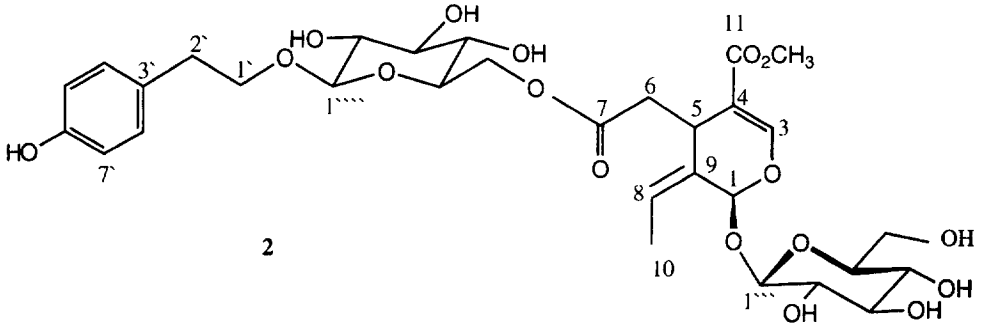
Plant material. The leaves of *F. angustifolia* Vohl were collected in September 1989 from the Royal Plant Garden, Al-Ribat, Morocco. The plant material was kindly identified by Prof. Dr Lotfi Bolous and Dr Mohammed El-Gibaly (Plant Taxonomy Unit, National Research Centre, Cairo, Egypt). A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and Isolation. Air-dried powdered leaves of *F. angustifolia* (600 g) were subjected to exhaustive extraction with MeOH (4 × 2.5 l). The combined MeOH extracts were concd *in vacuo* at 40° to dryness (103 g). The conc. MeOH extract was dissolved in H_2O (500 ml) and filtered through Celite. The filtrate and washings were combined and defatted with petrol. The defatted crude extract (89 g) was extracted successively with *n*-BuOH satd with H_2O. Then *n*-BuOH-soluble frs were pooled and concd *in vacuo* at 40° to afford a residue which was chromatographed on a polyamide column (130 × 2.5 cm) with H_2O, followed by increasing concns of MeOH to yield five main frs (A: H_2O; B: 25% MeOH; C: 50% MeOH; D: 75% MeOH; and E: MeOH).

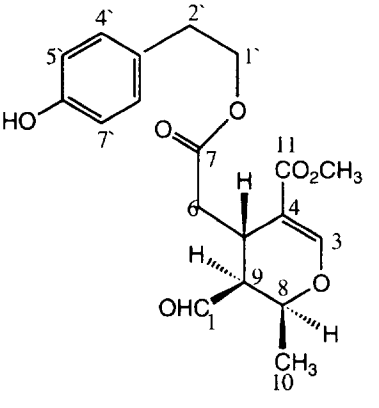


1 R = H
1a R = Ac

Ac_2O, Py



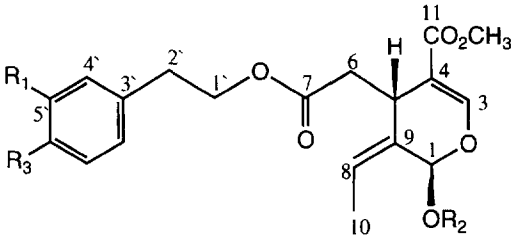
2



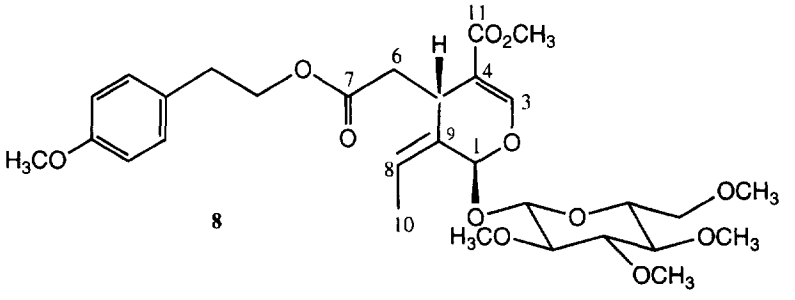
3

Fr. A (10 g) was rechromatographed on silica gel with $CHCl_3$–MeOH–H_2O (80:20:1–15:10:1) to give 7 frs (A1–A7). Fr. A3 was subjected to CC on silica gel eluting with a CH_2Cl_2–MeOH gradient (4:1–7:3), and three frs ($A3_a$–$A3_c$) were collected. The second fr. was purified by MPLC (325 × 18 mm) using a gradient of MeOH in H_2O (5–35%) to yield a mixt. of **1** and **2**, which were then sepd on a silica gel column eluted with $CHCl_3$–MeOH–H_2O (40:10:1–70:30:3) to afford **1** and **2**. Final purification of **1** and **2** on Sephadex LH-20 columns eluted with MeOH yielded **1** (29 mg) and **2** (22 mg), respectively.

Anqustifolioside C (**1**). Amorphous powder; UV λ_{max}^{MeOH} nm: 230, 281; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1715 (ester), 1700 (C=O), 1630 (C=C), 1520 (arom. ring); FAB-MS *m/z*: 847 $[M + Na]^+$, 685 $[M + Na - Glc]^+$, 501 $[M + Na - 2Glc]^+$; ^1H NMR (360, 600 MHz, CD_3OD): secoiridoid moiety: δ5.89 (*br s*, H-1), 7.48 (*s*, H-3), 3.98 (*dd*, J = 9.5, 4.5 Hz, H-5), 2.44 (*dd*, J = 14.2, 9.5 Hz, H-6a), 2.72 (*dd*, J = 14.5, 4.5 Hz, H-6b), 6.19 (*br t*, J = 7.0 Hz, H-8), 1.72 (*dd*, J = 7.0, 1.5 Hz, H-1), phenolic moiety: δ4.26 (*dt*, J = 11.0, 6.7 Hz, H-1′a), 4.15 (*dt*, J = 11.0, 6.7 Hz, H-1′b), 2.78 (*t*, J = 6.7 Hz, H-2′), 7.09 (*d*, J = 1.7 Hz, H-4′), 7.13 (*d*, J = 8.0 Hz, H-7′), 6.77 (*dd*, J = 8.0, 1.7 Hz, H-8′), 4.41 (*t*, J = 7.0 Hz, H-1″a), 4.29 (*t*, J = 7.0 Hz, H-1″b), 2.87 (*t*, J = 7.0 Hz, H-2″), 6.86 (*d*, J = 1.7 Hz, H-4″), 6.78 (*d*, J = 8.0 Hz, H-7″), 6.56



	R_1	R_2	R_3
4	OH	Glc	OGlc
5	H	Glc	OGlc
6	OH	$CH_2CH_2CH_2CH_3$	H
7	OH	$CH_2CH_2CH_2CH_3$	OH
10	H	Glc	H
11	OH	Glc	H



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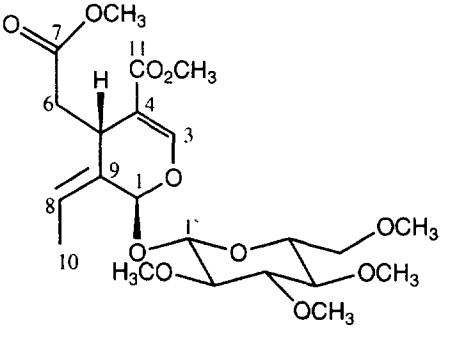

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(*dd*, J = 8.0, 1.7 Hz, H-8″), glucosyl moiety: δ4.81 (*d*, J = 7.9 Hz, H-1‴), 5.18 (*dd*, J = 7.8, 9.2 Hz, H-2‴), 3.25–3.52 (2H, *m*, overlapping, H-3‴ and H-3⁗), 3.68 (2H, *dd*, J = 12.0, 1.5 Hz, H-6‴a, H-6⁗a), 3.86 (*dd*, J = 12.0, 5.0 Hz, H-6‴b), 4.74 (*d*, J = 7.6 Hz, H-1⁗), 3.89 (*dd*, J = 12.0, 5.5 Hz, H-6⁗b); ^{13}C NMR: Table 1.

Undecaacetyl angustifolioside C (**1a**). Treatment of **1** (12 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temp. overnight, followed by CC over silica gel using C_6H_6–Me_2CO (3:1) gave the undecaacetate **1a** as an amorphous powder; IR^{KBr} ν_{max}^{KBr} cm^{-1}: 1760 (ester), 1710 (C=O), 1630 (C=C), FAB-MS *m/z*: 1287 $[M + H]^+$, 1309 $[M + Na]^+$, 978 $[(M + Na) - 331]^+$; ^{1}H NMR (360, 600 MHz, $CDCl_3$): secoiridoid moiety: δ5.67 (*br s*, H-1), 7.41 (*s*, H-3), 3.89 (*dd*, J = 9.0, 4.5 Hz, H-5), 2.38 (*dd*, J = 14.5, 9.0 Hz, H-6a), 2.65 (*dd*, J = 14.5, 4.5 Hz, H-6b), 5.98 (*q*, J = 7.0 Hz, H-8), 1.81 (*dd*, J = 7.0, 1.5 Hz, H-10), phenolic moiety: δ4.18 (*dt*, J = 11.0, 7.0 Hz, H-1′a), 4.24 (*dt*, J = 11.0, 7.0 Hz, H-1′b), 2.90 (*t*, J = 6.7 Hz, H-2′), 7.04 (*d*, J = 2.0 Hz, H-4′), 6.86 (*d*, J = 8.0 Hz, H-7′), 7.04 (*dd*, J = 8.0, 2.0 Hz, H-8′), 4.30 (*m*, H-1″a), 4.36 (*m*, H-1″b), 2.94 (*t*, J = 7.0 Hz, H-2″), 7.17 (*d*, J = 2.0 Hz, H-4″), 7.08 (*d*, J = 8.0 Hz,

H-7″), 7.08 (*dd*, $J = 8.0$, 2.0 Hz, H-8″), glucosyl moiety: δ5.04 (*d*, $J = 7.9$ Hz, H-1‴), 5.34 (*dd*, $J = 9.2$, 9.4 Hz, H-3‴), 5.15 (*dd*, $J = 9.4$, 10.0 Hz, H-4‴), 3.79 (*m*, H-5‴), 4.14 (*dd*, $J = 12.2$, 2.8 Hz, H-6‴a), 4.19 (*dd*, $J = 12.0$, 5.0 Hz, H-6‴b), 5.25 (*d*, $J = 7.6$ Hz, H-1⁗), 5.12 (*dd*, $J = 7.5$, 9.2 Hz, H-2⁗), 5.36 (*dd*, $J = 9.2$, 9.4 Hz, H-3⁗), 5.13 (*dd*, $J = 9.4$, 10.0 Hz, H-4⁗), 3.86 (*ddd*, $J = 10.0$, 5.5, 2.5 Hz, H-5⁗), 4.08 (*dd*, $J = 12.4$, 2.5 Hz, H-6⁗a), 4.30 (*dd*, $J = 12.4$, 5.5 Hz, H-6⁗b), 2.31, 2.29, 2.28 [each 3H, *s*, (3 × arom. acetoxyl)], 2.13, 2.10, 2.08, 2.06, 2.02, 1.99, 1.90, 1.75 (each 3H, *s*, (8 × aliph. acetoxyl)].

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