Lonitoside: a Novel Monoterpenoid Macrolide Diglycoside from *Lonicera nitida*

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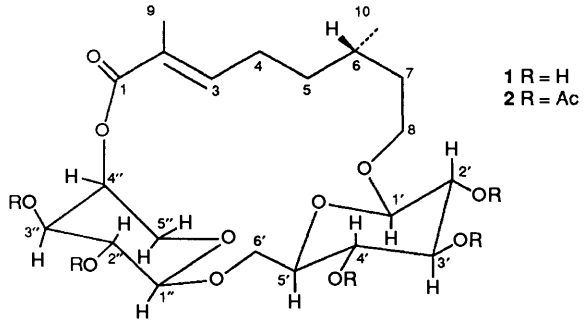
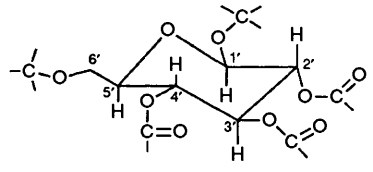
A new macrolide monoterpene diglycoside, lonitoside **1**, has been isolated from *Lonicera nitida* E. H. Wilson (Caprifoliaceae), and its structure elucidated as (*E*)-(6*S*)-8-[β-L-arabinopyranosyl-(1 ″→ 6′)-β-D-glucopyranosyloxy]-2,6-dimethyloct-2-eno-1,4″-lactone by spectroscopic and degradation methods.

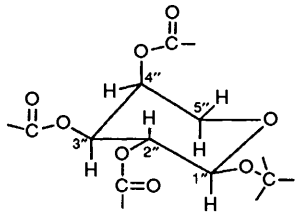
From a methanolic extract of fresh leaves of the honeysuckle species *Lonicera nitida* E. H. Wilson, a new macrolide monoterpene glycoside, lonitoside, has been isolated as its pentaacetate, m.p. 200–202 °C $[\alpha]_D^{20}$ +10° (MeOH) for which structures **1** and **2**, respectively have been deduced on the basis of spectroscopic data and chemical degradations discussed below. The mass spectrum of the acetate showed a strong molecular ion at *m/z* 672, which analysed for $C_{31}H_{44}O_{16}$; it had a UV maximum at 218 nm corresponding to an α,β-unsaturated carbonyl for which the IR spectrum showed corresponding peaks at 1649 and 1716 cm^{-1}, in addition to strong ester absorption at 1747 cm^{-1}.

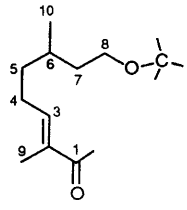


A wealth of structural information was afforded by the ^1H NMR spectrum (500 MHz; $CDCl_3$) together with appropriate decoupling experiments (see Table 1). Five acetate signals between δ 2.00 and 2.08 were consistent with the presence of the sugar indicated by mass spectral fragments. A doublet at δ 4.51 with *J* 7.5 Hz corresponded to an axial β-linked acetal 1′-H coupled to an axial 2′-H (δ 4.98, *J* 9, 7.5 Hz), and further *trans*-diaxial couplings were evident for 3′-, 4′- and 5′-H; the chemical shifts of the corresponding protons showed that the C-3′ and -4′ oxygens were acetylated whereas C-5′ and C-6′ had ether linkages in a β-glucopyranoside **3**. Another sugar unit was established by a set of couplings linking the acetal 1″-H doublet at δ 4.52 to 2″-, 3″-, 4″- and 5″-H_2; the small couplings to 4″-H showing that it alone was equatorial; since the 2′, 3′ and 4′ positions were acylated, the second sugar must be a β-arabinopyranoside **4**.

Furthermore, the nature of the C_{10} remainder of the molecule could also be determined from the NMR spectrum. The chemical shift of an olefinic proton at δ 6.90 was indicative of the β hydrogen of an α,β-unsaturated carbonyl with an *E* configuration [1] and a deshielded methyl singlet at δ 1.82 must be on the α position. Since the olefinic proton was a triplet, it must be linked to a methylene group, duly located by decoupling as an AB pair at δ 2.10 and 2.33; in turn these were linked to another AB methylene pair at δ 1.18 and 1.54, indicating a part structure O=CCMe=CH[CH_2]$_2$–. Again, from their chemical shift at δ 3.58 and 3.98 and couplings, yet another AB pair corresponded to a methylene group linked to ethereal oxygen and another







methylene group at δ 1.37 and 1.64, *i.e.* an –O–[CH_2]$_2$– fragment. Finally a methyl doublet at δ 0.92 was coupled to an obscured proton signal at δ 1.64, which in turn was linked to both terminal methylene groups of the other two fragments, and hence the C_{10} part structure was the monoterpenoid unit **5**.

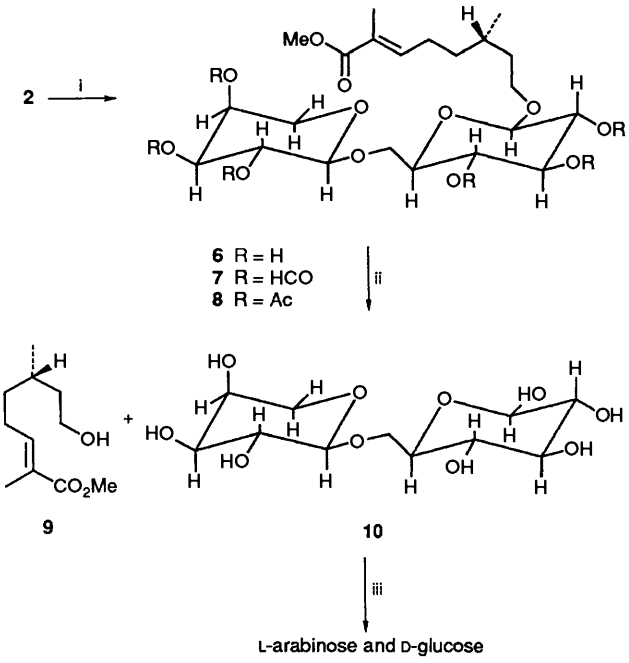
However, at this stage the mode of linkage of these units could not be established: the ether bond between the two sugars could be from C-1″ to C-1′ or C-6′, and conversely that from C-8 of the monoterpene to either C-6′ or C-1′; furthermore, the C-1 carbonyl was presumably in a lactone ring, which could incorporate any of the acylated positions on the sugars. Again, although the relative configuration of both sugars were clear from the NMR studies, the absolute configurations of these and the monoterpene remained to be established.

Confirmation of the presence of a lactone ring was obtained when Zemplen deacylation of the pentaacetate in methanol also led to a cleavage to a methyl ester, which on formylation gave a methyl ester hexaformate $[\alpha]_D^{20}$ −10.5° ($CHCl_3$). Its NMR spectrum now showed a methyl ester singlet at δ 3.70 and one-proton singlets between δ 7.98 and 8.11 corresponding to six formyl groups.

Hydrolysis of the methyl ester glycoside with β-glucosidase in pH 5.0 buffer and extraction with chloroform afforded the monoterpene aglucone (Scheme 1), $C_{11}H_{20}O_3$, $[\alpha]_D^{20}$ −7.6° ($CHCl_3$), which was shown to be methyl (*E*)-(*S*)-2,6-dimethyl-8-hydroxyoct-2-enoate **9** from spectroscopic and literature data.[2] Evaporation of the aqueous portion and acetylation gave a disaccharide heptaacetate, as evidenced by a (CI) molecular ion analysing for $C_{25}H_{34}O_{17}$, and its NMR spectrum. In particular the NMR signal for 1-H of glucose had shifted downfield to δ 6.30 (*J* 4 Hz) compared to δ 4.51 in lonitoside pentaacetate, showing it had been deshielded by acetylation, whereas the C-6 methylene protons had not. Hence the

Table 1 ^{1}H NMR (500 MHz; $CDCl_3$) of lonitoside pentaacetate **2**

H	Chemical shift (δ)	Multiplicities	J/Hz (coupled H)
3	6.90	t	7.5 ($4_{a,b}$)
4_a	2.33	dq	15 (4_b), *ca.* 7 ($5_{a,b}$), 7.5 (3)
4_b	2.10	m	15 (4_a), 7.5 (3)
5_a	1.54	m	15 (5_b), *ca.* 7 (4_a)
5_b	1.18	m	15 (5_a), *ca.* 7 (4_a)
6, 7_a	1.64	m	—
7_b	1.37	m	—
8_a	3.89	dd	16 (8_b), 8 (7_a)
8_b	3.58	m	16 (8_a), 4 ($7_{a,b}$)
9	1.82	s	—
10	0.92	d	6.5 (6)
1′	4.51	d	7 (2′)
2′	4.98	dd	9 (3′), 7 (1′)
3′	5.17	t	9 (2′, 4′)
4′	5.06	t	9 (3′, 5′)
5′	3.70	m	9 (4′), 2 ($6'_a$), 5 ($6'_b$)
$6'_a$	3.83	dd	10 ($6'_b$), 2 (5′)
$6'_b$	3.65	m	10 ($6'_a$), 5 (5′)
1″	4.52	d	7 (2″)
2″	5.29	dd	10 (3″), 7 (1″)
3″	5.03	dd	10 (2″), 3 (4″)
4″	5.27	td	3 (3″, $5''_a$), 1.5 ($5''_b$)
$5''_a$	3.99	dd	13 ($5''_b$), 3 (4″)
$5''_b$	3.61	dd	13 ($5''_a$), 1.5 (4″)
OAc	2.0–2.08	5 × s	—

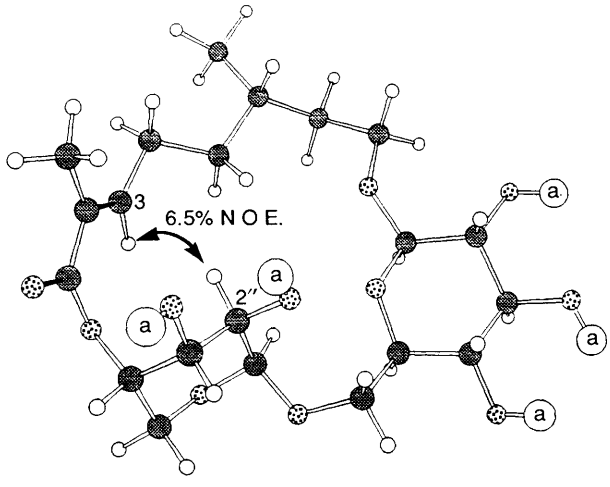


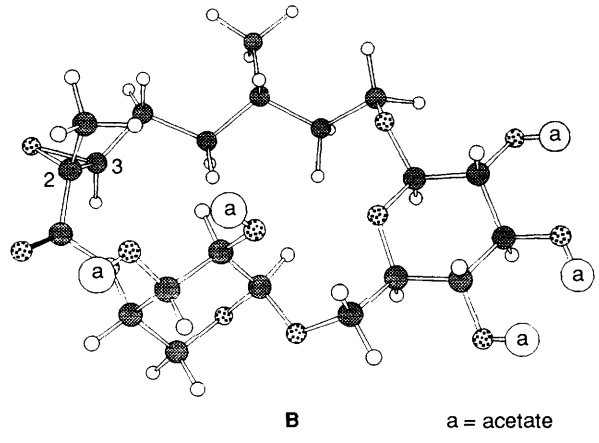
Scheme 1 *Reagents and conditions:* i, NaOMe, 2 h, room temperature; ii, β-glucosidase, pH 5, 3 d, 37 °C; iii, HCl, 2 h, 60 °C

disaccharide must comprise a glucose unit with an ether link from C-6 to C-1′ in arabinose rather than the 1-1′ alternative. Inevitably glucose will be the unique D-enantiomer but both D- and L-arabinose occur in Nature. In order to distinguish between these possibilities, the disaccharide was hydrolysed by heating with 2 mol dm^{-3} HCl and the products were separated by chromatography on Whatman DE32 microcellulose[3] to afford D-(+)-glucose and L-(+)-arabinose. Hence the disaccharide must have structure **10**, corresponding to vicianose.[4]

The crucial feature left to be established was the point of lactonisation in the sugar moiety. A small amount of lonitoside itself was obtained as a gum $[\alpha]_D^{20}$ −30° (MeOH) by careful chromatography of the original plant extract. Examination of the NMR spectrum of the free glycoside in $(CD_3)_2CO$–D_2O revealed the sugar hydrogen at the single acylated position at δ 5.08 as a narrow multiplet with only small ax.–eq. and eq.–eq. couplings which meant that it must be equatorial. Consequently the oxygen function must be axial, which can only correspond to the unique 4″ position in the arabinose moiety. Hence lonitoside has the novel macrolide structure **1**, (*E*)-(6*S*)-8-(β-L-arabinopyranosyl-(1″ → 6′)-β-D-glucopyranosyloxy)-2,6-dimethyloct-2-eno-1,4″-lactone. It is interesting to note that the absolute configuration at C-6 in the monoterpene is the same as that of the iridoid loganin which is also found in *L. nitida*[5] suggesting that both may have a common chiral precursor.

It might be expected that the macrocyclic lactone would be conformationally very mobile. However, epoxidation of the alkene gave essentially a single product, as did catalytic hydrogenation, and we attribute this interesting stereofacial selectivity to one predominant conformation of the macrolide ring. Computerised molecular modelling and MM2 energy minimisation indicated the preferred conformation **A** for **2**, and hence predicted a 2*R*,3*S* configuration for the epoxide, corresponding to structure **B**. This conclusion has been corroborated by NMR studies on **2**, which *inter alia* reveal a substantial nuclear Overhauser effect of 6.5% between 3-H and 2-H″ in accordance with their close proximity in conformation **A**.





Experimental

General Procedures.—For analytical and preparative thin layer chromatography, Merck silica plates pre-coated with Kieselgel 60 F_{254} were used. Column chromatography was carried out on Merck Kieselgel 60 (230–400 mesh). Melting points were determined on a Kofler block without correction.

Optical rotations were determined on an Optical Activity AA-100 polarimeter. Ultra violet absorption spectra (λ_{max}) were recorded on a Shimadzu UV-260 spectrometer and infra-red spectra (ν_{max}) were recorded on a Perkin-Elmer P-E 1710 FT-IR. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM500 or AC300 spectrometer. Line positions or centres of multiplets are given on the δ scale with reference to tetramethylsilane (TMS) as an internal standard. The multiplicities, types of protons and coupling constants are indicated. All J values are in Hz. Mass spectra (MS) were run on a Kratos Concept spectrometer using electron (EI) and chemical ionisation (CI). Molecular formulae were determined from accurate mass measurement (MM). Solvents and reagents were purified when necessary by standard methods.[6] Organic solutions were dried by being washed with brine and set aside over anhydrous sodium sulphate.

Isolation of Lonitoside **1**.—A methanolic extract (10 dm^3) of macerated fresh twigs and leaves of *Lonicera nitida* (1 kg) was evaporated to a green aqueous suspension (*ca.* 350 cm^3), which was defatted with light petroleum (b.p. 40–60 °C; 5 × 400 cm^3) and ethyl acetate (3 × 300 cm^3). The aqueous layer was then extracted with chloroform–ethanol (2:1; 3 × 400 cm^3), dried and evaporated to a gummy residue (20 g). Careful chromatography of a small portion of this crude material (0.1 g) on silica with acetone–chloroform (2:1) gave a fraction of pure lonitoside as a gum (2 mg), R_f 0.27 ($CHCl_3$–$PhCH_3$–MeOH, 5:3:2); $[\alpha]_D^{20}$ −30° (*c* 0.05 in MeOH); δ_H[300 MHz; $(CD_3)_2CO$–D_2O] 0.98 (3 H, d, *J* 6.5, 10-H), 1.7–1.78 (1 H, m, 6-H), 1.85 (3 H, s, 9-H), 2.06 (1 H, m, 4_b-H), 2.43 (1 H, m, 4_a-H), 3.68 (1 H, dd, *J* 14 and 1.2, $5''_b$-H), 3.88 (1 H, d, *J* 14, $5''_a$-H), 4.10 (1 H, dd, *J* 11.9 and 2.4, $6'_a$-H), 4.36 (1 H, d, *J* 8.5, 1″-H), 4.51 (1 H, d, *J* 6, 1′-H), 5.08 (1 H, dt, *J* 3 and 0.8, 4″-H) and 7.3 (1 H, t, *J* 9.5 and 5, 3-H). Acetylation with acetic anhydride–pyridine gave a product identical with lonitoside pentaacetate **2**.

Isolation of Lonitoside Pentaacetate **2**.—A portion of the extract (4 g) was acetylated with acetic anhydride and pyridine overnight at room temperature. The solvents were removed under reduced pressure, the residue was taken up in chloroform (40 cm^3), washed with dilute hydrochloric acid and sodium carbonate, and dried (sodium sulphate). After evaporation, the crude acetate was treated with Jones' reagent for 90 min to remove oxidisable impurities. Excess oxidant was then destroyed by addition of methanol and after work-up the products were chromatographed on silica to yield lonitoside pentaacetate as colourless fluffy crystals (660 mg) from methanol, m.p. 200–202 °C, $[\alpha]_D^{20}$ +10° ($CHCl_3$); ν_{max}(film)/cm^{-1} 1747, 1716 and 1649; δ_H(500 MHz; $CDCl_3$) see Table 1; *m/z* 672 $(M)^+$, 613, 553 and 485 (Found: 672.2611. $C_{31}H_{44}O_{16}$ requires 672.2654).

Lonitoside Methyl Ester Hexaformate **7**.—Zemplen deacetylation of the pentaacetate (10 mg) with sodium methoxide in methanol followed by formylation with formic/acetic anhydride and pyridine gave the methyl ester hexaformate **7**, $[\alpha]_D^{20}$ −10.5° ($CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.88 (3 H, d, *J* 6, 10-Me), 1.25–1.58 (6 H, m, 4′-, $5_{a,b}$-, 6-, $7_{a,b}$-H), 1.8 (3 H, s, 9-Me), 2.16 (1 H, m, 4_a-H), 3.48 (1 H, m, 8_b-H), 3.62 (1 H, dd, *J* 5 and 10, $6'_b$-H), 3.68 (1 H, d, *J* 10, $5''_b$-H), 3.71 (2 H, m, $5'_{a,b}$-H), 3.7 (3 H, s, CO_2Me), 3.9 (2 H, m, $6'_a$-, 8_a-H), 3.95 (1 H, dd, *J* 2 and 10, 6′-H), 4.08 (1 H, dd, *J* 3 and 10.5, $5''_a$-H), 4.51 (1 H, d, *J* 9, 1′-H), 4.52 (1 H, d, *J* 6.5, 1″-H), 4.99 (1 H, t, *J* 10, 2″-H), 5.1 (1 H, t, *J* 10, 4′-H), 5.18 (1 H, dd, *J* 3 and 9, 3″-H), 5.26 (1 H, dd, *J* 6.5 and 9, 2″-H), 5.44 (1 H, t, *J* 10, 3′-H), 5.46 (1 H, br s, 4″-H), 6.7 (1 H, dt, *J* 1 and 7.5, 3-H) and 7.98–8.11 (6 × 1 H, s, HCO_2).

Cleavage of the Methyl Ester Glycoside with β-Glucosidase.—A solution of lonitoside pentaacetate (300 mg) in sodium methoxide (0.1 mol dm^{-3}, 5 cm^3) was set aside at room temperature for 2 h, after which time a lump of carbon dioxide was added and the methanol evaporated under reduced pressure. The residue was dissolved in pH 5.0 buffer (40 cm^3), β-glucosidase (30 mg) added and the solution left at 37 °C for 3 d. The aqueous solution was then extracted with chloroform (3 × 10 cm^3), the combined extract dried (sodium sulphate) and evaporated under reduced pressure. Flash chromatography on silica in chloroform afforded the aglucone, identified as methyl (*E*)-(*S*)-2,6-dimethyl-8-hydroxyoct-2-enoate (63 mg), $[\alpha]_D^{20}$ −7.6° ($CHCl_3$); ν_{max}(film)/cm^{-1} 3410, 1708 and 1649; δ_H(300 MHz; $CDCl_3$) 0.93 (3 H, d, *J* 7, 10-Me), 1.88 (3 H, s, 9-Me), 2.18 (2 H, q, *J* 8), 3.72 (3 H, s, CO_2Me) and 6.76 (1 H, dt, *J* 1, 8, 3-H); *m/z* 200 $(M)^+$.

The aqueous portion (40 cm^3) was then extracted with chloroform–ethanol (2:1, 3 × 15 cm^3) and the combined extract was dried and evaporated. Acetylation with acetic anhydride and pyridine as above afforded the methyl ester hexaacetate derivative **8** (72 mg), δ_H(300 MHz; $CDCl_3$) 0.89 (3 H, d, *J* 7, 10-Me), 1.79 (3 H, s, 9-Me), 1.92–2.15 (6 × 3 H, s, OAc), 3.71 (3 H, s, CO_2Me), 4.48 (2 H, d, *J* 8, 1′-H, 1″-H), 6.73 (1 H, dt, *J* 1, 8, 3-H).

Part of the aqueous portion (10 cm^3) was evaporated under reduced pressure and the residue acetylated in the usual way. The isolated product was found to be the heptaacetate of the disaccharide vicianose **10** as a 2:1 mixture of β and α epimers (3 mg), δ_H(300 MHz; $CDCl_3$) 1.97–2.12 (21 H, 7 × OAc), 5.68 (d, *J* 8, 1-Hβ) and 6.32 (d, *J* 4, 1-Hα); *m/z* (CI) 596 $(M + 18)^+$, 366, 331, 317, 263 and 259.

Hydrolysis of Vicianose **10**.—The remainder of the aqueous portion (30 cm^3) was acidified with hydrochloric acid 2 mol dm^{-3} (5 cm^3) and warmed for 2 h at 60 °C. The solution was then neutralised with 10% aq. sodium carbonate and evaporated under reduced pressure. Column chromatography of the residue on Whatman DE 32 microcellulose in aq. acetone (1:9) yielded L-arabinose (5 mg) $[\alpha]_D^{20}$ +71° (H_2O), and D-glucose (3 mg) $[\alpha]_D^{20}$ +30° (H_2O); identified by paper chromatography (BuOH–H_2O) and $[\alpha]_D$ comparison with authentic samples. Acetylation with acetic anhydride and pyridine in each case afforded a mixture of β- and α-D-arabinose tetraacetates (2:1 ratio) (3 mg), $[\alpha]_D^{20}$ +47° ($CHCl_3$); δ_H(300 MHz; $CDCl_3$) 2.0–2.19 (12 H, 4 OAc), 3.78 (dd, *J* 2 and 13), 3.82 (dd, *J* 2 and 13.5), 4.05 (m), 5.11 (dd, *J* 3.5 and 8), 5.26–5.41 (m), 5.66 (d, *J* 7, 1-Hβ) and 6.36 (d, *J* 2.5, 1-Hα); *m/z* (CI) 336 $(M + 18)^+$ and 259, and a mixture of β- and α-D-glucose pentaacetates (3:2 ratio) (2 mg), $[\alpha]_D^{20}$ +103° ($CHCl_3$); δ_H(300 MHz; $CDCl_3$) 2.01–2.19 (24 H, 8 OAc), 3.85 (dq, *J* 9 and 2), 4.11 (m), 4.28 (m), 5.08–5.2 (m), 5.25 (d, *J* 9), 5.29 (d, *J* 4), 5.48 (t, *J* 10), 5.71 (d, *J* 8, 1-Hβ), 6.34 (d, *J* 4, 1-Hα); *m/z* (CI) 408 $(M + 18)^+$, 331, 318 and 259, again identical with similar mixtures prepared from authentic samples.

Epoxidation of Lonitoside Pentaacetate.—To a stirred solution of lonitoside pentaacetate (330 mg) in dichloromethane (1 cm^3) and phosphate buffer pH 8.0 (1 cm^3) in an ice-bath was added *m*-CPBA (98 mg) in small portions during 10 min. After stirring for 5 h at room temperature, more *m*-CPBA (98 mg) was again added in small portions to the cooled mixture in an ice-bath during 10 min. The mixture was stirred at room temperature for 5 h, the organic layer separated, washed successively with saturated aq. sodium thiosulphate, 10% aq. sodium carbonate and water and dried (anhydrous sodium sulphate). Evaporation under reduced pressure and flash chromatography of the residue on silica in chloroform yielded, in addition to unchanged starting material, 2,3-epoxylonitoside pentaacetate (198 mg, 59%), ν_{max}(film)/cm^{-1} 1755, 1370, 1220

and 1053; δ_H(300 MHz; $CDCl_3$) 0.92 (3 H, d, *J* 6, 10-Me), 1.2–1.8 (7 H, m, $4_{a,b}$-, $5_{a,b}$-, 6-, $7_{a,b}$-H), 1.55 (3 H, s, 9-Me), 2.01–2.12 (5 × 3 H, s, OAc), 2.99 (1 H, dd, *J* 3 and 9, 3-H), 3.5–3.9 (4 H, m, 5′-, $6'_{a,b}$-, $5''_b$-H), 4.05 (1 H, dd, *J* 4.5 and 12, $5''_a$-H), 4.51 (1 H, d, *J* 7, 1′-H), 4.52 (1 H, d, *J* 7.5, 1″-H) and 4.95–5.25 (6 H, m, 2′-, 3′-, 4′-, 2″-, 3″-, 4″-H); *m/z* 688 (M^+) (Found: 688.2596. $C_{31}H_{44}O_{17}$ requires 688.2578).

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