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STRUCTURE OF ORTHENTHOSE

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ABSTRACT

A new tetrasaccharide, orthenthose, has been isolated from the dried twigs of *Orthenthera viminea* (Family: Asclepiadaceae). By spectral and chemical procedures, this new tetrasaccharide has been shown to be *O*-α-L-oleandropyranosyl-(1→4)-*O*-α-L-oleandropyranosyl-(1→4)-*O*-α-L-oleandropyranosyl-(1→4)-β-L-oleandropyranose.

INTRODUCTION

In a chemical investigation of the dried twigs of *Orthenthera viminea*, isolation of four oligosaccharides that were provisionally designated A, B, C, and F, in the order of decreasing mobility in p.c., was reported[1]. Oligosaccharide C, named ornose, has already been characterized as L-cymarotriose[2]. Oligosaccharide B has now been named orthenthose (**1**), and its structure elucidation is reported in the present communication.

RESULTS AND DISCUSSION

Orthenthose (**1**), obtained as an amorphous powder, $[\alpha]_D$ +47°, had an elemental analysis agreeing with that calculated for the formula $C_{28}H_{50}O_{13}$. It reduced Fehling solution, and gave color reactions characteristic of 2-deoxy sugars, *viz.*, the xanthydrol[3] and Keller–Kiliani reactions[4]. To identify the sugar units of **1**, it was hydrolyzed with 5mM H_2SO_4 in 1,4-dioxane[5], which afforded only one sugar; this had the same mobility in t.l.c. and p.c. as oleandrose, and was characterized as L-oleandrose (2,6-dideoxy-3-*O*-methyl-L-*arabino*-hexose)[6] (**7**) on the basis of its optical rotation, $[\alpha]_D$ +15°.

Oxidation of **1** with bromine water furnished compound **8**, $[\alpha]_D$ +17°. Product **8** showed in its i.r. spectrum a strong absorption band at 1780 cm^{-1}, suggesting that it was a γ-lactone[7]. The ^{1}H-n.m.r. spectrum of **8** contained a three-proton doublet (*J* 6 Hz) centered at δ 1.20, indicating a secondary methyl group. A two-proton doublet at δ 2.50 (*J* 4 Hz) and a three-proton singlet at δ 3.17 were respec-

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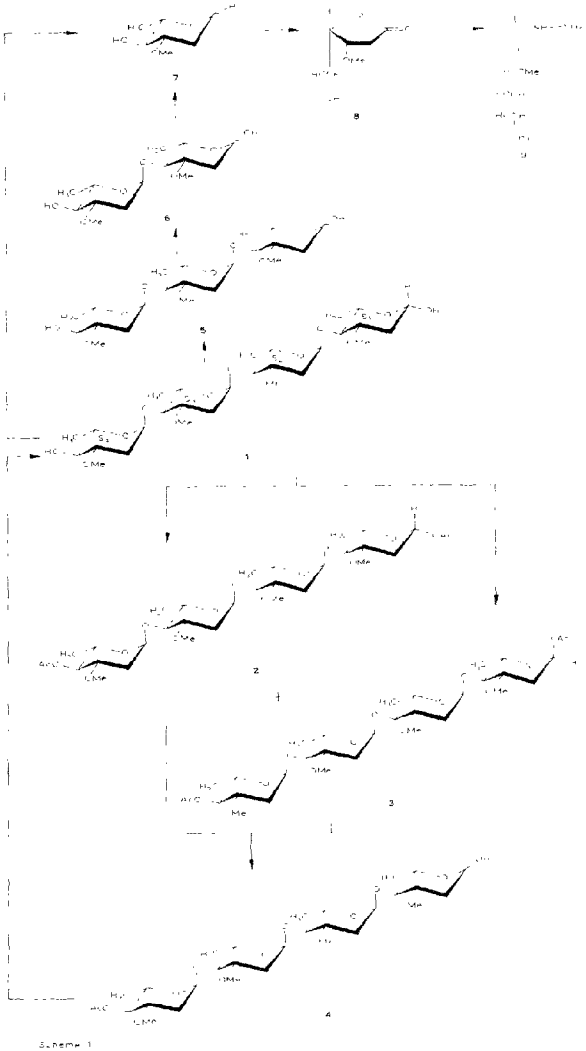


Scheme 1

tively assigned to the methylene-group protons at C-2 and the methoxyl group, and a one-proton doublet at δ 4.03 (J 8 Hz), to H-4. Product **8** was thus characterized as a 2,6-dideoxyhexono-1,4-lactone. This monosaccharide lactone **8**, $[\alpha]_D$ +17°, was identified as L-oleandrono-1,4-lactone ($[\alpha]_D$ hitherto unreported) on the basis of its p.c. comparison with an authentic sample. The phenylhydrazide **9**, m.p. 133–135°, $[\alpha]_D$ +19°, prepared from the lactone **8**, possessed properties the same as those reported for L-oleandronic phenylhydrazide[6]. Obviously, the γ-lactone was generated during Br_2 oxidation of **1** by concomitant hydrolysis by the traces of HBr present in the oxidation mixture.

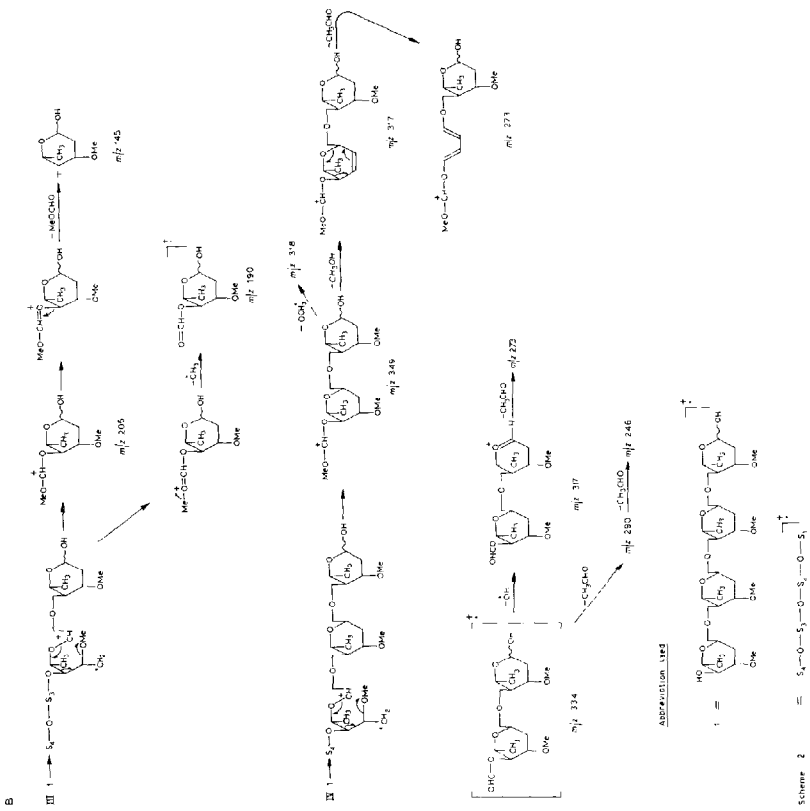
The 400-MHz, ^{1}H-n.m.r. spectrum of **1** in $CDCl_3$ not only confirmed that it was a tetrasaccharide of L-oleandrose but also helped in ascertaining the configuration of each of the three glycosidic linkages. For convenience, the four L-oleandrose units of **1** are designated S_1, S_2, S_3, and S_4. The spectrum contained four three-proton singlets, at δ 3.56, 3.45, 3.38, and 3.32, attributed to four methoxyl groups. Two multiplets, two-proton each, in the regions δ 3.92–4.02 and δ 3.80–3.86, were assigned to H-5 of the four sugar units, and this was confirmed by subsequent, double-resonance experiments. Two other four-proton multiplets, in the regions δ 3.62–3.72 and δ 3.22–3.30, were presumed to be those for H-3 and H-4, respectively. A one-proton, double doublet (J 4 and 1 Hz) centered at δ 5.11, a two-proton, distorted double doublet (J 4 and 1 Hz) centered at δ 5.49, and another one-proton, double doublet (J 10 and 2 Hz) centered at δ 5.03, were assigned to the four anomeric protons of the molecule. The eight C-2 methylene protons of these four 2-deoxy sugar units appeared as two sets of four-proton multiplets, in the regions δ 1.74–2.40 and δ 1.36–1.56, for the equatorial and axial protons, respectively. In the higher field, four three-proton doublets centered at δ 1.33 (J 6 Hz), δ 1.31 (J 6 Hz), δ 1.25 (J 6 Hz), and δ 1.24 (J 6 Hz) were assigned to the four secondary methyl groups of **1**.

Double-resonance experiments were helpful in confirmation of the assignments of the anomeric protons. Irradiation of the signals at 2.195, 2.045, and 2.008 kHz resulted in the collapse of C-2 methylene multiplets, confirming that all four of the anomeric protons belong to 2-deoxy sugar units. The three anomeric protons of lower coupling constant were attributable to their equatorial orientation presumably involved in the glycosidic linkage. They, therefore, were assigned to the S_2, S_3, and S_4 units, suggesting that these L-oleandropyranose units are in the $^1C_4$(L) conformation[8] and linked through an α-L-(1→4)-glycosidic linkage. The large coupling constant (J 10 Hz) of the anomeric proton (at δ 5.03) was attributed to the axial anomeric proton of L-oleandropyranose unit S_1, suggesting that the reducing hexopyranose unit also exists in the $^1C_4$(L) conformation[8], and that its anomeric proton is in the axial orientation, as in β-L-oleandropyranose. Irradiation of the two secondary methyl-group signals, at δ 1.25 and 1.24, caused collapse of the two-proton multiplet in the region δ 3.92–4.02 assigned to be H-5 of two hexose units, and irradiation of the other two secondary methyl-group signals, at δ 1.33 and 1.31, again caused collapse of the two-proton multiplet in the region δ 3.80–

3.86 assigned to be H-5 of the two other hexose units. The foregoing fact is also in good agreement with the decision that orthenthose (**1**) is a tetraose composed of 2,6-dideoxyhexose units.

The mass spectrum (c.i.) of orthenthose (**1**) failed to display its molecular ion, but contained mass peaks only of smaller fragments comprised of those of monosaccharide and disaccharide units. The structurally significant ion-peak was recorded at *m/z* 306 (6%), which corresponded to a disaccharide fragment. The relatively intense peak in the higher-mass region, at *m/z* 290 (100%), corresponded to a fragment formed from the disaccharide fragment resulting from rearrangement cleavage of **1**.

Although very little has been reported on the m.s. of underivatized oligosac-



charides, on the basis of the few decomposition pathways studied, fragmentation routes I and II are presented (see Scheme 2); in these, repeated H transfers in the oligosaccharide, accompanied by the elimination of terminal sugars, less water, are visualized[9], giving rise to an ion of the same minimal mass as the molecular ion of the corresponding oligosaccharide with one less monosaccharide residue, until only the monosaccharide remains.

Fragmentation routes III and IV are presented in Scheme 2 for the genesis of ions to be found in the rearrangement involving migration of methoxyl group after the radical-ion cleavage of the C-1–C-2 bond, followed by migration of the 3-

methoxyl group to C-1, resulting in the cleavage of oligosaccharide[10]. Further fragmentation of the smaller and the monosaccharide units are presumed to arise from the characteristic fragmentation-pattern of 2,6-dideoxyhexoses reported by Pettit and co-workers[9]; these account for most of the major peaks in the spectrum (that fully support the structure elucidated for this tetrasaccharide).

Substantial chemical support for the L-oleandrotetraose structure for orthenthose was provided by the results of its very mild hydrolysis[11] with acid at room temperature; within 7 days the hydrolyzate exhibited four spots in p.c. for the products of partial and complete hydrolysis. The fastest-moving spot had the same mobility as the product of complete hydrolysis, L-oleandrose (**7**), which was taken as the reference; the slowest spot (R_{Ole} 0.29) was identical in mobility to the starting material **1**, whereas the third and fourth spots (R_{Ole} 0.77 and 0.38) were presumably those of oleandrobiose (**6**) and oleandrotriose (**5**), respectively, formed by partial hydrolysis of **1**. This hydrolysis was complete within 15 days, when the hydrolyzate afforded only one product, $[\alpha]_D$ +13°, which, in p.c., showed a mobility identical to that of **7**, confirming that **1** is composed of L-oleandrose units only.

Acetylation of **1** with acetic anhydride in pyridine furnished two *O*-acetyl derivatives (1.l.c., 99:1 chloroform–methanol), the major product **2**, $[\alpha]_D$ +149° (R_f 0.60), and the minor product **3**, $[\alpha]_D$ +79° (R_f 0.68). When deacetylated by the Zemplén method[12], both of these acetates afforded the same product **1**, indicating that the two *O*-acetyl derivatives are acetates of the anomers of **1**. As the anomeric acetates originated from the L-oleandropyranose unit S_1, the major acetylation product **2**, exhibiting the higher optical rotation, was presumed to be the β anomer, and the other (minor) acetylation product **3** (of lower optical rotation) to be the α anomer.

The 90-MHz, ^{1}H-n.m.r. spectrum of the major acetylation product **2** was not properly resolved to give complete information. However, it had prominent signals for four methoxyl groups as a twelve-proton singlet at δ 3.45, for two acetyl groups as two three-proton singlets at δ 2.03 and δ 2.04, and for four secondary methyl groups as four three-proton doublets (J 6 Hz) centered at δ 1.30, 1.26, 1.18, and 1.13.

Further chemical support for the decision that **2** and **3** are diacetates of **1** came from very mild, alkaline hydrolysis (0.5% KOH in methanol) of **2** at room temperature. Within 3 h, the hydrolyzate exhibited three spots in t.l.c., the fastest spot (R_f 0.60) was identical in mobility to the starting material, the slowest spot (R_f 0.13) identical in mobility to the completely deacetylated product **1**, and the third spot, of intermediate mobility (R_f 0.24), was presumed to be the monoacetate **4**. In 7 h, compound **2** was completely converted into **1**. Similarly, the acetate **3** also exhibited three spots in 3 h under identical hydrolytic conditions. The fastest spot (R_f 0.68) was identical in mobility to the starting material **3**, whereas the two spots of lower mobility (R_f 0.24 and 0.13) were the same as the products formed during the hydrolysis of **2**, the slowest spot possessing the same mobility as **1** (R_f 0.13). This reaction was complete within 7 h, when the hydrolyzate showed only one product, namely **1**.

These results indicated that the monoacetate **4** from **2** is identical with that from **3**. Obviously, in the first stage of hydrolysis, the reactive anomeric acetyl group of **2** and **3** underwent hydrolysis, both affording the same 4-*O*-acetyl derivative **4**. A negative $NaIO_4$ reaction[13] of **1** precluded the presence of a vicinal diol grouping in the molecule, and the formation of a di-*O*-acetyl derivative from **1** is in agreement with its tetraose structure.

The structure of orthenthose (**1**) was thus established as *O*-α-L-oleandropyranosyl-(1→4)-*O*-α-L-oleandropyranosyl-(1→4)-*O*-α-L-oleandropyranosyl-(1→4)-β-L-oleandropyranose. This is the first time that a tetrasaccharide composed exclusively of 2-deoxy sugars is reported as being obtained from plants, although oligoglycosides of these rare sugars had been described[14,15] earlier.

EXPERIMENTAL

General. — Melting points were determined on a Boetius micro melting-point apparatus, and are uncorrected. Optical rotations were measured in a 1-dm tube with a Jasco dip 180 automatic polarimeter. The sugars were made visible in t.l.c. with 50% aqueous H_2SO_4. In p.c., the sugars were detected with the vanillin–perchloric acid reagent[16]. The lactones were detected in t.l.c. and p.c. with the NH_2OH–$FeCl_3$ reagent[17]. The adsorbent for t.l.c. was silica gel G (BDH), and, for column chromatography, silica gel for column (BDH; 60–120 mesh) developed by Duncan's method[18]. Paper chromatography was performed on Whatman No. 1 filter paper, using 4:1 toluene–1-butanol saturated with water. I.r. spectra were recorded with a Perkin–Elmer IR-177 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 90 MHz with a Perkin–Elmer R-32 spectrometer, for solutions in $CDCl_3$, with Me_4Si as the internal standard. The ^{1}H-n.m.r. spectrum of orthenthose was recorded with a Bruker 400-MHz instrument. Mass spectra were recorded with a Jeol high-resolution, J.M.S.-300 mass spectrometer.

Orthenthose (**1**). — Shade-dried twigs of *Orthenthera viminea* were extracted by an earlier method[19]. Mild hydrolysis of the isolated glycosides with acid afforded a sugar mixture (2.2 g) which was chromatographed on silica gel (220 g), using 19:1 chloroform–methanol as the eluant, and collecting 250-mL fractions. Evaporation of fractions 54–63 gave a residue (50 mg). For further purification, the viscous material (50 mg) obtained from the column was rechromatographed on silica gel (30 g). Fractions 12–18, eluted with 19:1 chloroform–methanol (collection of 30-mL fractions), afforded pure, amorphous orthenthose (41 mg); $[\alpha]_D^{25}$ +47.5° (*c* 0.63, methanol). It gave a blue coloration (for 2-deoxy sugar) with vanillin–perchloric acid spray-reagent[16], gave positive tests in the xanthydrol[3] and Keller–Kiliani reactions[4], and reduced Fehling solution. It also gave a negative $NaIO_4$ test, and the following 400-MHz, ^{1}H-n.m.r. data ($CDCl_3$): δ 5.49 (dd, 2 H, *J* 4 and 1 Hz, H-1 in S_2 and S_3), 5.11 (dd, 1 H, *J* 4 and 1 Hz, H-1 in S_4), 5.03 (dd, 1 H, *J* 10 and 2 Hz, H-1 in S_1), 3.92–4.02 (m, 2 H, 2 H-5), 3.80–3.86 (m, 2 H, 2 H-5), 3.62–3.72 (m, 4 H, 4 H-3), 3.56 (s, 3 H, OCH_3), 3.45 (s, 3 H, OCH_3), 3.38 (s, 3 H, OCH_3),

3.32 (s, 3 H, OCH_3), 3.22–3.30 (m, 4 H, 4 H-4), 2.20–2.40 (m, 3 H, 3 H-2*e*), 1.74–1.82 (m, 1 H, H-2*e*), 1.48–1.56 (m, 3 H, 3 H-2*a*), 1.36–1.40 (m, 1 H, H-2*a*), 1.33 (d, 3 H, *J* 6 Hz, sec. CH_3), 1.31 (d, 3 H, *J* 6 Hz, sec. CH_3), 1.25 (d, 3 H, *J* 6 Hz, sec. CH_3), and 1.24 (d, 3 H, *J* 6 Hz, sec. CH_3); *m/z* 594 (M^+ not observed), 420 (6%), 366 (2), 349 (9), 324 (2), 318 (2), 317 (2), 308 (28), 306 (6), 290 (100), 283 (2), 276 (38), 273 (17), 260 (3), 254 (6), 246 (4), 242 (3), 222 (3), 211 (2), 205 (12), 196 (3), 190 (2), 178 (16), 162 (51), 148 (12), 145 (9), 130 (21), 113 (31), 101 (29) 97 (42), 95 (54), 86 (13), and 78 (5).

Anal. Calc. for $C_{28}H_{50}O_{13}$: C, 56.56; H, 8.42. Found: C, 56.98; H, 8.12.

Periodate oxidation of **1**. — To a solution of **1** (1 mg) in methanol (0.2 mL) was added a solution of sodium metaperiodate (6 mg) in water (0.1 mL), and the mixture was kept for 2 h at room temperature, diluted with water (0.4 mL), and evaporated under diminished pressure. The residue was unreacted **1** (t.l.c., 9:1 chloroform–methanol).

Mild hydrolysis of **1** *with acid*. — To a solution of **1** (4 mg) in 1:1 water–1,4-dioxane (0.5 mL) was added 5mM H_2SO_4 (0.5 mL), and the solution was warmed for 30 min at 50°, cooled, and made neutral with freshly precipitated barium carbonate; the suspension was filtered, and the filtrate was evaporated to dryness under diminished pressure. The residue was extracted with hot acetone; evaporation of the extract yielded a syrup (3 mg) that exhibited one spot in t.l.c. (23:2 chloroform–methanol), and that, in p.c., had the same mobility as an authentic sample of oleandrose. For purification, it was distilled under high vacuum, yielding colorless, syrupy **7** (2 mg); $[\alpha]_D^{25}$ +15.0° (*c* 0.63, methanol). It reduced Fehling solution, gave positive tests for a 2-deoxy sugar in the xanthydrol[3] and Keller–Kiliani reactions[4], and did not undergo periodate oxidation[13]. Sugar **7**, obtained from the hydrolyzate of **1**, was thus identified as L-oleandrose[6].

Very mild hydrolysis of **1** *with acid*. — To a solution of **1** (2 mg) in methanol (0.5 mL) was added 0.01MHCl (0.5 mL) in 99.5% aqueous methanol, and the solution was kept at room temperature. After 7 days, it showed four spots in p.c., two of them having mobilities identical to those of **7** (R_{Oli} 1.0) and **1** (R_{Oli} 0.29), respectively; the third spot (R_{Hle} 0.77) and the fourth spot (R_{Oli} 0.38) were, presumably, the partially hydrolyzed products **6** and **5**, respectively. After 15 days, the hydrolyzate showed only one spot, which had the same mobility as **7**. Evaporation of the solution afforded a colorless syrup (2 mg) having $[\alpha]_D^{25}$ +15.4° (*c* 0.70, methanol), a specific rotation comparable to that of L-oleandrose.

Oxidation of **1** *with bromine-water*. — A solution of **1** (10 mg) in water (0.8 mL) was mixed with bromine (13 μL), and shaken in a stoppered flask in the dark for 24 h at room temperature. The excess of bromine was then removed under diminished pressure, the acidic mixture was made neutral with freshly precipitated silver carbonate, and the suspension was filtered. H_2S was passed through the filtrate to remove Ag^+ ions, and the suspension was filtered. The filtrate was evaporated to dryness under diminished pressure, yielding syrupy lactone **8** (8 mg), $[\alpha]_D^{25}$ +17.3° (*c* 0.63, methanol), showing only one spot with the NH_2OH–$FeCl_3$ rea-

gent[17], and having the same mobility as L-oleandrono-1,4-lactone in t.l.c. (19:1 chloroform–methanol) and p.c.; $\nu_{max}^{CH_2Cl_2}$ 3580 (as. OH), 2980, 1780 (C=O, γ-lactone), 1582–1568, 1190, 1165, 1100, 1015, 955, and 850 cm^{-1}; 90-MHz ^{1}H-n.m.r. data ($CDCl_3$): δ 4.03 (d, 1 H, *J* 8 Hz, H-4), 3.5 (d, not assigned), 3.17 (s, 3 H, OCH_3), 2.50 (d, 2 H, *J* 4 Hz, CH_2), and 1.20 (d, 3 H, *J* 6 Hz, sec. CH_3).

Phenylhydrazide (**9**). — A solution of lactone **8** (5 mg) in absolute ethanol (0.05 mL) was mixed with freshly distilled phenylhydrazine (0.04 mL), and the mixture was heated for 30 min at 100°. The viscous mass was cooled, and repeatedly triturated with absolute ether (to remove the excess of phenylhydrazine), yielding a brown powder (4 mg) that crystallized from methanol–ether as colorless needles (2 mg); m.p. 133–135°, $[\alpha]_D^{25}$ +19.4° (*c* 0.73, methanol).

Di-O-acetylorthenthose (**2** *and* **3**). — A solution of **1** (15 mg) in pyridine (0.3 mL) and acetic anhydride (0.3 mL) was kept for 48 h at room temperature. The pyridine and the excess of acetic anhydride were then evaporated under diminished pressure. A solution of the viscous residue in chloroform was successively washed with 2M HCl, 2M Na_2CO_3 solution, and water, dried (Na_2SO_4), and evaporated, affording a mixture of two di-*O*-acetyl derivatives of **1** (t.l.c., 99:1 chloroform–methanol) as an amorphous mass (14 mg); these acetates were separated by preparative t.l.c.: major product **2** (R_F 0.60; 9 mg), $[\alpha]_D^{25}$ +149.5° (*c* 0.63, methanol), and minor product **3** (R_F 0.68; 3 mg), $[\alpha]_D^{25}$ +79.0° (*c* 0.63, methanol); 90-MHz, ^{1}H-n.m.r. data for **2** ($CDCl_3$): δ 6.28 (dd, *J* 4 and 1 Hz, 2 H, 2 H-1), 5.86 (m, 2 H, 2 H-1), 4.40–4.10 (m, not assigned), 3.45 (s, 12 H, 4 OCH_3), 2.04 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 1.30 (d, 3 H, *J* 6 Hz, sec. CH_3), 1.26 (d, 3 H, *J* 6 Hz, sec. CH_3), 1.18 (d, 3 H, *J* 6 Hz, sec. CH_3), and 1.13 (d, 3 H, *J* 6 Hz, sec. CH_3).

Deacetylation of **2**. — To a solution of **2** (1 mg) in methanol (0.3 mL) was added sodium methoxide (0.3 mL), and the mixture was kept at room temperature. After 10 min, it showed one spot (t.l.c., 9:1 chloroform–methanol), which had the same mobility as **1**.

Deacetylation of **3**. — Compound **3** (1 mg) in methanol was treated with a solution of sodium methoxide as in the deacetylation of **2**; after 10 min, it showed one spot (t.l.c., 9:1 chloroform–methanol), which had the same mobility as **1**.

Very mild hydrolysis of **2** *with alkali*. — To a solution of **2** (1 mg) in methanol (0.5 mL) was added 0.5% KOH (0.5 mL) in 99.5% aqueous methanol, and the solution was kept at room temperaure. After 3 h, it showed three spots (t.l.c., 99:1 chloroform–methanol). The R_F (0.60) of the fastest spot was identical with that of **2**, and the two other spots had mobilities identical to those of **4** (R_F 0.24) and **1** (R_F 0.13), respectively. After 7 h, the hydrolyzate showed only one spot, which had the same mobility as **1**.

Very mild hydrolysis of **3** *with alkali*. — Similarly, **3** (1 mg) was hydrolyzed with 0.5% KOH in aqueous methanol, as for **2**. After 3 h, it showed three spots (t.l.c., 99:1 chloroform–methanol). The R_F (0.68) of the fastest spot was identical with that of **3**, and the other spots had mobilities identical with those of **4** (R_F 0.24) and **1** (R_F 0.13), respectively. After 7 h, **3** was completely hydrolyzed, and showed only one spot, which had the same mobility as **1**.

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