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STRUCTURE OF ORNOSE

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ABSTRACT

A new trisaccharide, ornose (**1**), has been isolated from the dried twigs of *Orthenthera viminea* (Family: Asclepiadaceae). On the basis of chemical and spectroscopic evidence, the structure of **1** has been established as *O*-α-L-cymaropyranosyl-(1→4)-*O*-α-L-cymaropyranosyl-(1→4)-β-L-cymaropyranose.

INTRODUCTION

In a chemical investigation of the dried twigs of *Orthenthera viminea*, four novel oligosaccharides, isolated as amorphous products, were provisionally designated A, B, C, and F, in the order of decreasing mobility in p.c. In a preliminary communication[1], one of these reducing oligosaccharides, namely, C, $[\alpha]_D$ +31°, which gave color reactions[2,3] characteristic of 2-deoxy sugars, was reported to afford, on mild hydrolysis with acid[4], only one sugar, having the same mobility in p.c. as cymarose (2,6-dideoxy-3-*O*-methyl-*ribo*-hexose)[5]. This oligosaccharide of cymarose has now been named ornose (**1**). Its mass spectrum (c.i.) shows the highest-mass peak at *m/z* 406 (42%), and, by assuming this fragment to be (M − CH_3CHO), the molecular formula of **1** was derived as $C_{21}H_{38}O_{10}$ (*m/z* 450), suggesting that **1** is a cymarotriose. Although, from plants, a few oligoglycosides containing 2-deoxy sugars exclusively have been reported[6,7], the isolation of their oligosaccharides has not been described in the literature. This situation therefore prompted us to elucidate the structure of **1**, reported in the present communication.

RESULTS AND DISCUSSION

Mild hydrolysis[4] of **1** with acid afforded monosaccharide **6**, which showed the same mobility in p.c. and t.l.c. as cymarose[5], and was characterized as L-cymarose[5] on the basis of its optical rotation, $[\alpha]_D$ −46°.

Oxidation of **1** with bromine–water furnished **7**, $[\alpha]_D$ +26°. Product **7** showed in its i.r. spectrum a strong absorption band at 1780 cm^{-1}, indicative of a

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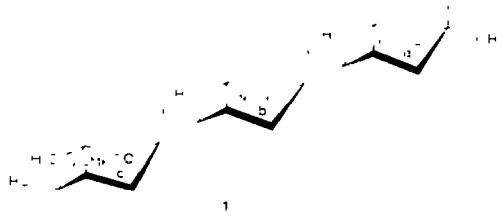
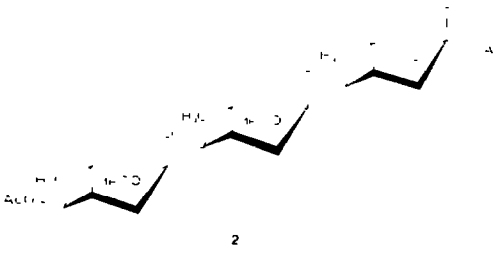


1



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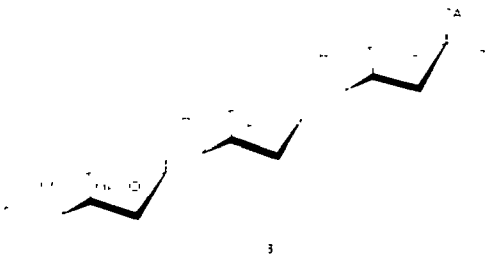
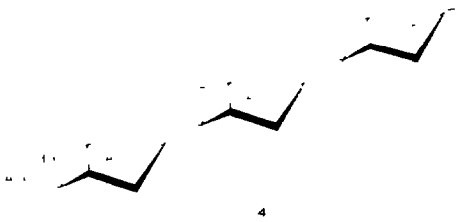


3

2 or 3 →



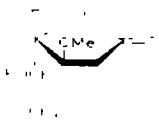
4

1 → 

5

→ 

6



7

→ 

8

γ-lactone grouping[8]. The formation of a γ-lactone by oxidation of **1** containing L-cymarose units in the pyranoid form is not possible, as the 4-hydroxyl group of its reducing residue is engaged in a glycosidic linkage (see later discussion). The γ-lactone **7** is, therefore, presumably a monosaccharide lactone. The ^{1}H-n.m.r. spectrum of **7** indicated that **7** was a dideoxyhexono-1,4-lactone, as the spectrum contained a 3-proton singlet at δ 3.28 and a 3-proton doublet at δ 1.13 (*J* 6.5 Hz), respectively indicating a methoxyl group and a secondary methyl group. A two-proton multiplet in the region δ 2.40–3.0 was assigned to the methylene-group protons at C-2, and a narrow multiplet at δ 4.17, to H-4.

This monosaccharide lactone **7** was eventually identified as L-cymarono-1,4-lactone[5], $[\alpha]_D$ +26° ($[\alpha]_D$ hitherto unreported), by p.c. comparison with an authentic sample. Its identification was confirmed by conversion of **7** into crystalline L-cymaronic phenylhydrazide[5] **8**, m.p. 153–156°, $[\alpha]_D$ +5°. The formation of **7** during bromine–water oxidation of **1** could be explained by acid hydrolysis of trisaccharide **1** to the monosaccharide **6**, was due to HBr present in the reaction mixture, and subsequent oxidation of **6** to **7**.

Close analysis of the 400-MHz, ^{1}H-n.m.r. spectrum of **1** in $CDCl_3$ not only confirmed that it was a trisaccharide of L-cymarose, but also permitted ascertaining the configurations of the two glycosidic linkages. For convenience, the three L-cymarose units of **1** are designated *a*, *b*, and *c*. A two-proton, double doublet (*J* 4 and 1 Hz) at δ 5.37 could be assigned to two anomeric protons, those of *b* and *c*, the configuration of C-1 in both being identical. Its small coupling constant (4 Hz) was typical of an equatorial proton of a 2-deoxyhexopyranose in the $^1C_4$(L) conformation[9], also suggesting that units *b* and *c* were both linked through an α-L-(1→4) glycosidic linkage. The assignment of these two equatorial, anomeric-proton signals is also in agreement with the splitting pattern of their adjacent methylene groups. Their equatorial, two-proton multiplet appeared in the region δ 2.34–2.29, and the axial, two-proton multiplet in the region δ 1.54–1.47. A one-proton, double doublet centered at δ 4.83 (*J* 10 and 2 Hz) was attributed to the anomeric proton of cymarose residue *a*. The larger coupling-constant (10 Hz) of this anomeric proton is typical of an axial proton of pyranoid 2-deoxyhexoses, suggesting that unit *a* is a β-L-cymaropyranose residue in the $^1C_4$(L) conformation[9]. In view of the chemical shift and splitting pattern of the one-proton multiplet in the region δ 2.45–2.41, it was attributed to the equatorial proton of the methylene group of unit *a*, whereas the splitting pattern of its axial proton in the region δ 1.38–1.33 was obscured by the secondary methyl-group signals of the molecule. In the higher field, a three-proton doublet centered at δ 1.37 (*J* 6 Hz) was attributed to the secondary methyl group of cymarose unit *a*, whereas another six-proton doublet for two secondary methyl groups at δ 1.30 (*J* 6 Hz) was assigned to the two methyl groups of cymarose units *b* and *c*. A nine-proton singlet at δ 3.43 was assigned to the three methoxyl groups present in the molecule.

A more-direct, chemical support for the hypothesis that **1** is an L-cymarotriose was provided by the results of its very mild hydrolysis with acid[10], which afforded partially and completely hydrolyzed products. Under these conditions, **1** af-

forded three spots in p.c. within 7 days. The fastest spot had the same mobility as the monosaccharide L-cymarose (**6**), which was taken as the reference. The slowest spot (R_{Cym} 0.21) was identical in mobility to the starting material **1**, whereas the third spot (R_{Cym} 0.74) was, presumably, cymarobiose (**5**), formed by partial hydrolysis of **1**. The hydrolysis was complete in 15 days, when the hydrolyzate contained only one sugar, $[\alpha]_D$ −48°, identical in mobility in p.c. to **6**, confirming that **1** is composed of L-cymarose units only.

This conclusion is also in good agreement with the insensitivity of **1** to[11] $NaIO_4$, and with formation of two *O*-acetyl derivatives (t.l.c.). On reaction of **1** with acetic anhydride–pyridine, the major product was **2**, $[\alpha]_D$ +448 (R_f 0.76), and the minor product was **3**, $[\alpha]_D$ +9° (R_F 0.66). When hydrolyzed by the Zemplén method[12], both of these acetates afforded the original sugar **1**, indicating that **2** and **3** are the two anomeric acetates of **1**. As the anomeric acetates originated from the L-cymaropyranose unit *a*, the major acetylation product **2**, exhibiting the higher optical rotation, is taken 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**) had prominent signals for three methoxyl groups as a nine-proton singlet at δ 3.27, for two acetyl groups as two three-proton singlets at δ 2.02 and δ 2.03, and for three secondary methyl groups as a nine-proton doublet centered at δ 1.16 (J 6 Hz). However, other signals (for anomeric protons, methine and methylene groups, *etc.*) did not allow any conclusive interpretation.

Further chemical support of the decision that both **2** and **3** are diacetates of **1** came from their very mild hydrolysis at room temperature with 0.5% KOH in methanol. Within 3 h, the hydrolyzate of **2** exhibited three spots in t.l.c., the fastest spot being identical in mobility with the starting material (R_f 0.76). The slowest (R_F 0.11) was identical in mobility to **1**, whereas the third spot, of intermediate mobility (R_F 0.37), is presumed to be that of the monoacetate **4**. In 7 h, **2** was completely converted into **1**. Similarly, the acetate **3** (R_F 0.66) also exhibited three spots within 3 h under identical conditions of hydrolysis. The fastest spot (R_f 0.66) was identical in mobility with the starting material **3**, whereas the two other spots, of lower mobility (R_f 0.37 and 0.11), were identical with the products **4** and **1** formed during the hydrolysis of **2**. This acetate was also completely hydrolyzed to **1** within 7 h. These results indicated that the monoacetate from **2** is identical with that from **3**. Obviously, in the first stage of hydrolysis, the reactive anomeric acetyl groups of **2** and **3** underwent hydrolysis, both diacetates affording the same 4-*O*-acetyl derivative (**4**).

In light of the foregoing evidence, the structure of ornose was established as *O*-α-L-cymaropyranosyl-(1→4)-*O*-α-L-cymaropyranosyl-(1→4)-β-L-cymaropyranose (**1**).

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. 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[13]. Lactones were detected in t.l.c. and p.c. with the NH_2OH–$FeCl_3$ reagent[14]. The adsorbent for t.l.c. was silica gel G (B.D.H.) and, for column chromatography, Silica gel for column (B.D.H.; 60–120 mesh) developed by Duncan's method[15]. Paper chromatography was performed on Whatman No. 1 filter paper, using 4:1 toluene–butanol saturated with water. I.r. spectra were recorded with a Perkin–Elmer I.R.-177 spectrophotometer. ^{1}H-N.m.r. spectra were recorded with a 400-MHz and a 90-MHz Perkin–Elmer R-32 spectrometer for solutions in $CDCl_3$, with Me_4Si as the internal standard. Mass spectra were recorded with a Jeol high-resolution J.M.S.-300 mass spectrometer.

Plant extraction. — Shade-dried, powdered twigs (10 kg) of *Orthenthera viminea* were extracted by the method[16] employed for pregnane glycosides, using 50–95% aqueous ethanol. The ethanolic extracts were combined, and concentrated under diminished pressure, and, without treatment with $Pb(OH)_2$, the concentrate was exhaustively extracted successively with light petroleum (5 × 500 mL), ether (5 ×500 mL), chloroform (5 × 500 mL), 4:1 chloroform–ethanol (5 × 500 mL), and 3:2 chloroform–ethanol (5 × 500 mL). The residues from the last two extracts (9 and 3 g, respectively) were rich in glycosides. They were combined and hydrolyzed with 25mM H_2SO_4 in 50% methanol[4], in order to obtain the genins and sugars. The sugars (2.2 g) exhibited in p.c. 8 spots, designated A, B, C, D, E, F, G, and H, in the order of their decreasing mobilities. This sugar mixture was chromatographed on silica gel (220 g), using 19:1 chloroform–methanol as the eluant, and collecting 250-mL fractions. Fractions 41–48 contained mainly A, fractions 54–63 contained B, fractions 72–84 contained C, and fractions 117–126 were rich in F. Repeated chromatography of these fractions on columns of silica gel afforded pure A (14 mg), $[\alpha]_D^{25}$ +66.3° (*c* 0.63, methanol); B (41 mg), $[\alpha]_D^{25}$ +47.5° (*c* 0.63, methanol); C (40 mg), $[\alpha]_D^{25}$ +31.0° (*c* 0.63, methanol); and F (12 mg), $[\alpha]_D^{25}$ +36.25° (*c* 0.70, methanol). Each of them failed to crystallize, and they were characterized only by their mobility in p.c., and optical rotation.

Amorphous C, $[\alpha]_D^{25}$ +31°, named ornose (**1**), reduced Fehling solution, gave positive tests in the xanthydrol[2] and Keller–Kiliani reactions[3], and a blue coloration with vanillin–perchloric acid[13]; 400-MHz, ^{1}H-n.m.r. data ($CDCl_3$): δ 5.37 (dd, 2 H, *J* 4 and 1 Hz, H-1 in *b* and *c*), 4.83 (dd, 1 H, *J* 10 and 2 Hz, H-1 in *a*), 3.91–3.96 (m, 3 H, H-5 in *a*, *b*, and *c*), 3.54–3.64 (m, 3 H, H-3 in *a*, *b*, and *c*), 3.43 (s, 9 H, 3 OCH_3), 3.22–3.14 (m, 3 H, H-4 in *a*, *b*, and *c*), 2.45–2.41 (m, 1 H, H-2*e* in *a*), 2.34–2.29 (m, 2 H, H-2*e* in *b* and *c*), 1.54–1.47 (m, 2 H, H-2*a* in *b* and *c*), 1.38–1.33 (m, 1 H, H-2*a* in *a*), 1.37 (d, 3 H, *J* 6 Hz, sec. CH_3 in *a*), and 1.30 (d, 6 H, *J* 6 Hz, 2 sec. CH_3 in *b* and *c*); *m/z* 450 (M^+, not observed), 406 (42%, M − CH_3CHO), 366 (71), 320 (10), 308 (20), 290 (80), 273 (27), 210 (12), 178 (30), 162 (44), 148 (9), 145 (15), 130 (45), 128 (17), 113 (100), 101 (19), 97 (50), 95 (39), 86 (94), and 78 (13).

Periodate oxidation of **1**. — To a solution of **1** (2 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** (5 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 (4 mg) that exhibited one spot in t.l.c. (19:1 chloroform–methanol), and that, in p.c., had the same mobility as cymarose. For purification, it was distilled under high vacuum, yielding colorless, syrupy **6** (3 mg); $[\alpha]_D^{25}$ −46.8° (*c* 0.63, methanol). It reduced Fehling solution, gave positive tests for a 2-deoxy sugar in the xanthydrol[2] and Keller–Kiliani reactions[3], and did not undergo periodate oxidation. Sugar **6**, obtained from the hydrolyzate of **1**, was thus identified as L-cymarose[5].

Very mild hydrolysis of **1** *with acid*. — To a solution of **1** (2 mg) in methanol (0.5 mL) was added 0.01M HCl (0.5 mL) in 99.5% aqueous methanol, and the solution was kept at room temperature. After 7 days, it showed three spots in p.c., two of them having mobilities identical to those of **6** ($R_{c,ym}$ 1.0) and **1** ($R_{c,ym}$ 0.21), respectively; the third spot ($R_{c,ym}$ 0.74) was, presumably, the partially hydrolyzed product **5**. After 15 days, the hydrolyzate showed only one spot, which had the same mobility as **6**. Evaporation of the solution afforded a colorless syrup (2 mg) having $[\alpha]_D^{25}$ −48.4° (*c* 0.63, methanol), which is comparable to that of L-cymarose.

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 **7** (7 mg), $[\alpha]_D^{25}$ +26.5° (*c* 0.63, methanol), showing only one spot with the NH_4OH–$FeCl_3$ spray-reagent[14], and having the same mobility as L-cymarono-1,4-lactone in t.l.c. (19:1 chloroform–methanol) and p.c.; $\nu_{max}^{CHCl_3}$ 3580 (ass. OH), 2980, 1780 (C=O, γ-lactone), 1582–1568, 1190, 1165, 1100, 1015, 955, and 850 cm^{-1}; ^1H-n.m.r. data: δ 4.17 (m, 1 H, H-4), 3.5 (s, 1 H), 3.28 (s, 3 H, OCH_3), 3.0–2.40 (m, 2 H, CH_2) and 1.13 (d, 3 H, *J* 6.5 Hz, sec. CH_3).

Phenylhydrazide (**8**). — A solution of **7** (6 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. This residue (4 mg) crystallized from methanol–ether as colorless needles (2 mg); m.p. 153–156°, $[\alpha]_D^{25}$ +5.0° (*c* 0.9, methanol).

Di-O-acetylornose (**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_2SO_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 residue (15 mg); these acetates were separated on a column of silica gel (15 g), using 99:1 chloroform–methanol as the eluant. The major product **2** (R_F 0.7, 10 mg), $[\alpha]_D^{25}$ +448.5° (*c* 0.63, methanol), and **3** (R_F 0.66, 3 mg), $[\alpha]_D^{25}$ +9.0° (*c* 0.63, methanol), were obtained as amorphous products; ^{1}H-n.m.r. data for **2** (90 MHz; $CDCl_3$): δ 5.65 (m, 3 H), 5.5 (m, 2 H), 3.27 (s, 9 H, 3 OCH_3), 2.03 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), and 1.16 (d, 9 H, *J* 6 Hz, 3 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 solution was kept at room temperature. After 10 min, it showed only 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 only 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 temperature. After 3 h, it showed three spots (t.l.c., 99:1 chloroform–methanol). The R_F (0.76) of the fastest spot was identical with that of **2**, and the two other spots had mobilities identical to those of **4** (R_F 0.37) and **1** (R_F 0.11), 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 (0.66) of the fastest spot was identical with that of **3**, and the two other spots had mobilities identical with those of **4** (R_F 0.37) and **1** (R_F 0.11), respectively. After 7 h, **3** was completely hydrolyzed, and showed only one spot, which had the same mobility as **1**.

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