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# STRUCTURE OF DIGOXOSE

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ABSTRACT

A new trisaccharide, named digoxose, has been isolated from the dried twigs of *Orthenthera viminea* (Family: Asclepiadaceae). On the basis of chemical and spectroscopic evidence, the structure of this new trisaccharide has been established as *O*-β-D-digitoxopyranosyl-(1→4)-*O*-β-D-digitoxopyranosyl-(1→4)-α-D-digitoxopyranose.

## INTRODUCTION

The natural occurrence of deoxy sugars provides one of the most active areas of research in carbohydrate chemistry. The primary reason for this activity is the widespread occurrence of these compounds in biologically important molecules. In the chemical investigation of *Orthenthera viminea*, the isolation of four novel oligosaccharides[1] of 2-deoxy sugars, and the structure of three of them, *viz.*, vimose[2], orthenthose[3], and ornose[4], have been reported earlier.

In the present paper, structural elucidation of the fourth novel trisaccharide, isolated from the same plant and named "digoxose", is reported. Several cardioactive glycosides involving such digoxose type of sugars, *viz.*, digoxin[5], digitoxin[6], and gitoxin[7], are known to occur in Nature and have been widely used as drugs since long ago. Although chemical and biological studies of these glycosides and their genins have been made in detail, very little has been reported on their sugar component, possibly because their trisaccharide moiety could never be isolated earlier. Lichti and co-workers[8] attempted to isolate the trisaccharide component of digitoxin, but obtained only the monosaccharide digitoxose from it. Obviously, therefore, they could not ascertain the linkages in the digitoxotriose present in the glycoside.

## RESULTS AND DISCUSSION

Digoxose (**1**), obtained as colorless granules, m.p. 171–174°, $[\alpha]_D$ +36°, had an elemental analysis corresponding to $C_{18}H_{32}O_{10}$. It reduced Fehling solution,

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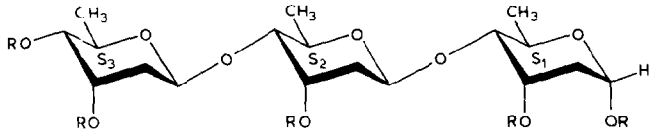
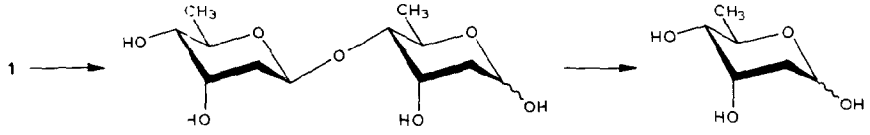
and exhibited positive color reactions characteristic of 2-deoxy sugars, *viz.*, the xanthydrol[9] and Keller–Kiliani[10] reactions.

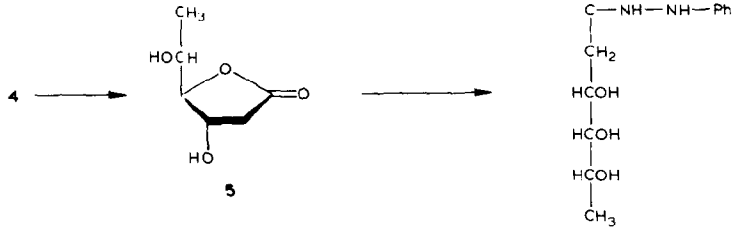
To identify the sugar units of **1**, it was hydrolyzed with 5mM $H_2SO_4$ in 1,4-dioxane[11]. In t.l.c. and p.c., the hydrolyzate showed the presence of only one product, **4**, $[\alpha]_D$ +41°, which was oxidized with bromine water to an amorphous lactone, **5**, $[\alpha]_D$ −27°. A comparison of their optical rotations and their mobility in p.c. with those of authentic samples indicated that **4** was D-digitoxose[12] (2,6-dideoxy-D-*ribo*-hexose) and that **5** was D-digitoxono-1,4-lactone[12]. The hydrazide, **6**, m.p. 123° prepared from **5** possessed the same properties as an authentic specimen of D-digitoxonic phenylhydrazide[12]. On the basis of the formula of digoxose, and the characterization of only digitoxose in its hydrolyzate, it was inferred to be a trisaccharide of D-digitoxose.

A close analysis of the 400-MHz, $^1$H-n.m.r. spectrum of **1** in pyridine-$d_5$ not only confirmed that it was a trisaccharide of D-digitoxose, but also permitted ascertaining the configuration of the two glycosidic linkages. For convenience, the three D-digitoxose units of **1** are designated as S1, S2, and S3 (see Scheme 1). The two, one-proton double doublets ($J$ 7 and 1 Hz) at $\delta$ 5.09 and $\delta$ 5.35 in the spectrum




1 R = H
2 R = Ac



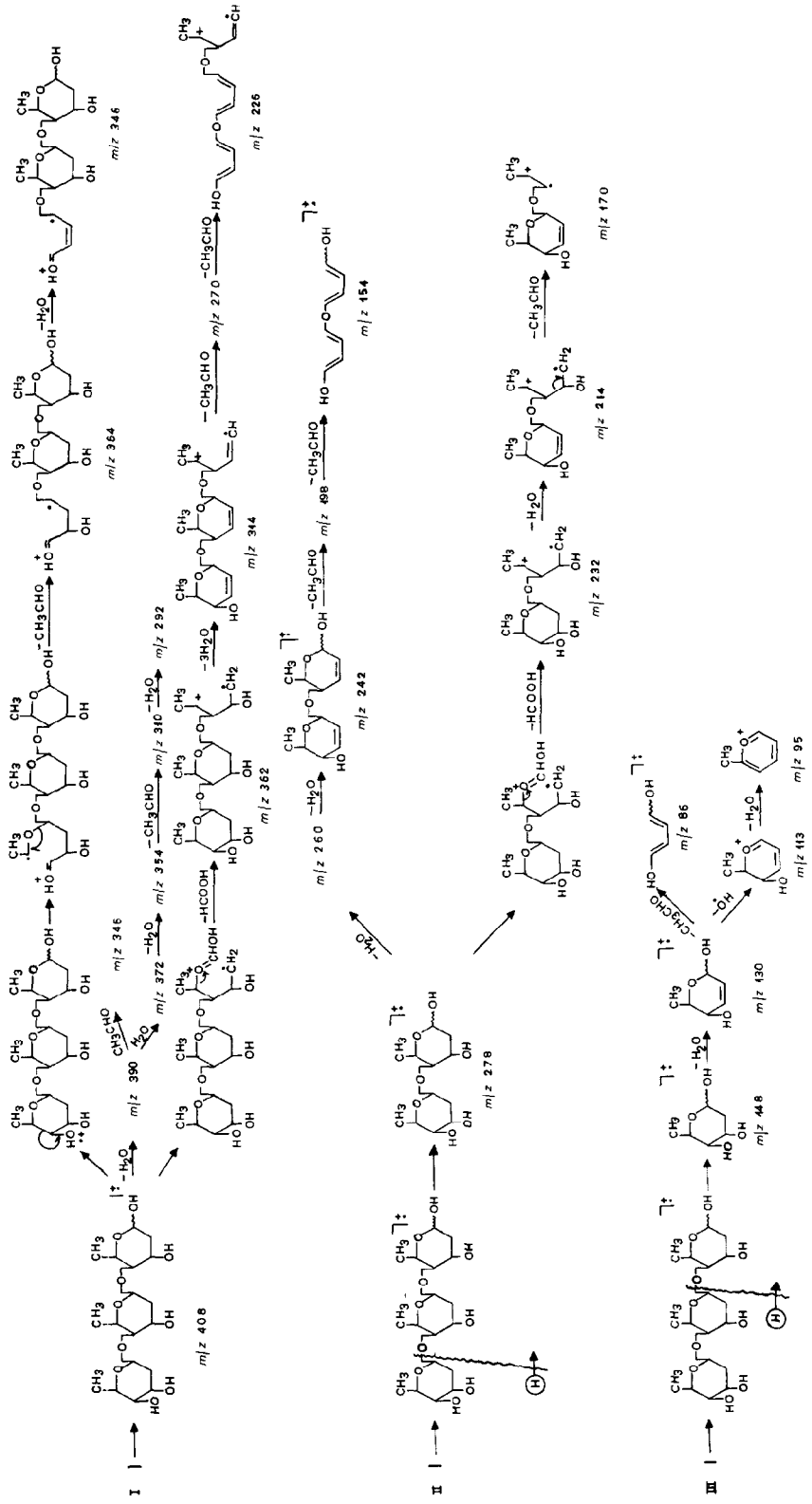




Scheme 1

could be assigned to two identical anomeric protons of S2 and S3. Their large coupling constant (7 Hz) was typical of an axial anomeric proton of a 2-deoxyhexopyranose in the $^4C_1$(D) conformation[13], indicating a $\beta$-glycosidic linkage. The ease of reaction of digoxose with $NaIO_4$ further suggested that S2 and S3 were both linked through a (1→4) linkage. The assignment of these two axial 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 $\delta$ 2.54–2.70, and the axial, two-proton multiplet in the region $\delta$ 2.0–2.14. A one-proton, double doublet centered at $\delta$ 5.77 ($J$ 3 and 1 Hz) was attributed to the anomeric proton of the digitoxose residue S1. The small coupling constant (3 Hz), which is typical of an equatorial orientation, was attributed to the anomeric proton of a 2-deoxyhexopyranose in the $^4C_1$(D) conformation[13]. This suggested that S1 is a $\alpha$-D-digitoxopyranose unit. The splitting pattern of a one-proton multiplet in the region $\delta$ 2.16–2.28, and another one-proton multiplet in the region $\delta$ 1.30–1.48, respectively corresponded to the equatorial and axial protons of the methylene group of S1. A two-proton multiplet in the region $\delta$ 4.68–4.76 and a one-proton multiplet in the region $\delta$ 4.48–4.54 were assigned to H-5 of the S2, S3, and S1 units, respectively. Another two-proton multiplet, in the region $\delta$ 4.04–4.20, was assigned to H-3 of S2 and S3, and a one-proton multiplet in the region $\delta$ 3.84–3.90 was assigned to H-3 of S1. A three-proton multiplet in the region $\delta$ 3.54–3.68 could thus be assigned to the H-4 atoms of the three sugar units. In the higher field, the six-proton doublet ($J$ 6 Hz) at $\delta$ 1.59 was attributed to the two secondary methyl groups of S2 and S3, whereas another three-proton doublet ($J$ 6 Hz), at $\delta$ 1.53, was assigned to the secondary methyl group of S1. A five-proton, broad signal in the region $\delta$ 4.84–5.04, which disappeared after addition of $D_2O$, indicated the five hydroxyl groups present in the molecule. These facts agree with the chemical derivation that **1** is a trisaccharide composed of 2,6-dideoxyhexoses.

Very little has been reported on the mass spectrum of nonderivatized oligosaccharides, presumably owing to their low volatility, which limits the formation of ions of higher mass. However, when nonderivatized digoxose was subjected to mass spectrometry, the mass spectrum exhibited its molecular ion of appreciable abundance, along with several other prominent mass ion peaks. On the basis of the decomposition pathways of a few simple, nonderivatized disaccharides and oligosaccharides studied so far[3,14,15], almost all of the prominent fragment-ions observed in the mass spectrum of **1** could be interpreted, and this fully substantiated the digitoxotriose structure derived for digoxose (**1**).

In the higher-mass region, the prominent mass ion peaks in the mass spectrum of **1** were structurally significant, as they could be interpreted as resulting from the loss of water and acetaldehyde molecules in different fragmentation sequences common to 2,6-dideoxyhexoses[16]. The course of this fragmentation is presented in route I (see Scheme 2). Fragmentation routes II and III are based on the cleavage of the trisaccharide digoxose by repeated H-transfers, where elimination of terminal sugar less water is visualized[16] to give an ion of the corresponding disac-



Scheme 2 Mass-spectral fragmentation of digoxose.

charide and finally, a monosaccharide. The fragment-ion peaks in the lower-mass region could be interpreted as arising from the characteristic fragmentation-pattern of 2,6-dideoxyhexoses reported by Pettit and co-workers[16].

The fragmentation routes IV and V (see Scheme 2) present the genesis of ions to be formed in the rearrangement involving migration of the 3-hydroxyl group to C-1, after the radical-ion cleavage of the C-1–C-2 bond resulting in cleavage of the oligosaccharide[17].

Substantive chemical support in favor of the D-digitoxotriose structure for digoxose was obtained from the results of its very mild hydrolysis[6] with acid at room temperature, which, in 5 days, exhibited three spots in t.l.c. and p.c. for the products of partial and complete hydrolysis. The fastest-moving spot had the same mobility as the completely hydrolyzed product digitoxose (**4**), which was taken as the reference; the slowest spot ($R_{Dig}$ 0.36) was identical in mobility with the starting material **1**, whereas the third spot ($R_{Dig}$ 0.72) was presumably that of digitoxobiose (**3**), formed by the partial hydrolysis of **1**. This hydrolysis was complete within 9 days, when the hydrolyzate afforded only one product, $[\alpha]_D$ +39°, which, in p.c., showed identical mobility to **4**, confirming that **1** is composed of three D-digitoxose units.

On acetylation with acetic anhydride in pyridine, **1** furnished *O*-acetyl derivative **2**, $[\alpha]_D$ +75°. The 80-MHz, $^1$H-n.m.r. spectrum of **2** was not properly resolved to give complete information. However, it had prominent signals for three anomeric protons, as a two-proton double doublet at $\delta$ 5.35 ($J$ 7 and 1 Hz) and a one-proton double doublet at $\delta$ 4.56 ($J$ 3 and 1 Hz). Five acetyl groups appeared as two singlets, a three-proton singlet at $\delta$ 2.10 and a twelve-proton singlet at $\delta$ 2.0. Three secondary methyl group signals appeared as a nine-proton doublet ($J$ 6 Hz) at $\delta$ 1.25.

Chemical support for **2** being a pentaacetate of **1** came from its very mild hydrolysis with 0.5% KOH in methanol at room temperature. Within 2 h, the hydrolyzate exhibited six spots in t.l.c. The fastest-moving spot ($R_F$ 0.90) showed the same mobility as the starting material **2**. The slowest spot ($R_F$ 0.1) was identical in mobility with **1**, and the spots of intermediate mobility ($R_F$ 0.81, 0.72, 0.60, and 0.36) were presumed to be the partially hydrolyzed products of **2**, *viz.*, the tetra-, tri-, di-, and mono-*O*-acetyl derivative respectively of **1**. In 9 h, **2** was completely deacetylated to **1**.

The digitoxotriose structure of **1** is fully supported by the formation of its penta-*O*-acetyl derivative, and the presence of a vicinal-diol grouping in the molecule (through the positive $NaIO_4$ reaction[18]).

In light of the foregoing evidence, the structure of digoxose was thus established as *O*-β-D-digitoxopyranosyl-(1→4)-*O*-β-D-digitoxopyranosyl-(1→4)-α-D-digitoxopyranose (**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. 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[19] reagent. The lactones were detected in t.l.c. and in p.c. with the $NH_2OH$–$FeCl_3$ reagent[20]. 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[21]. Paper chromatography was performed on Whatman No. 1 filter paper, using 4:1 toluene–butanol saturated with water. $^1$H-N.m.r. spectra were recorded at 80 MHz with a CFT-20 Varian spectrometer, for solutions in $CDCl_3$ with $Me_4Si$ as the internal standard. The $^1$H-n.m.r. spectrum of digoxose was recorded with a Bruker 400-MHz spectrometer for a solution in pyridine-$d_5$. The mass spectrum was recorded with a Jeol High Resolution JMS-300 mass spectrometer.

*Digoxose* (**1**). — Shade-dried twigs of *Orthenthera viminea* were extracted by an earlier method[22]. Mild hydrolysis, with acid, of the glycosides isolated 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 117–126 gave a single substance as a viscous residue (18 mg). For further purification, this material was rechromatographed on silica gel (15 g). Fractions 15–21, eluted with 93:7 chloroform–methanol (collection of 10-mL fractions), afforded pure digoxose as colorless granules (12 mg) from ethyl acetate–pentane; m.p. 171–174°, $[\alpha]_D^{25}$ +36.25° (*c* 0.70, methanol). It gave a blue coloration with the vanillin–perchloric acid spray-reagent, gave positive tests in the xanthydrol and Keller–Kiliani reactions, and reduced Fehling solution; it also gave a positive $NaIO_4$ test. For **1**, 400-MHz, $^1$H-n.m.r. data ($C_5D_5N$): $\delta$ 5.77 (dd, *J* 3 and 1 Hz, H-1 in S1), 5.35 (dd, 1 H, *J* 7 and 1 Hz, H-1), 5.09 (dd, 1 H, *J* 7 and 1 Hz, H-1), 4.84–5.04 (b, 5 H, 5 OH), 4.68–4.76 (m, 2 H, H-5 in S2 and S3), 4.48–4.54 (m, 1 H, H-5 in S1), 4.04–4.20 (m, 2 H, H-3 in S2 and S3), 3.83–3.90 (m, 1 H, H-3 in S1), 3.54–3.68 (m, 3 H, H-4 in S1, S2, and S3), 2.54–2.70 (m, 2 H, H-2*e* in S2 and S3), 2.16–2.28 (m, 1 H, H-2*e* in S1), 2.0–2.14 (m, 2 H, H-2*a* in S2 and S3), 1.59 (d, 6 H, *J* 6 Hz, sec. $CH_3$ in S2 and S3), 1.53 (d, 3 H, *J* 6 Hz, sec. $CH_3$ in S1), and 1.30–1.48 (m, 1 H, H-2*a* in S1); *m/z* 408 ($M^+$, 1%), 390 (2), 387 (4), 372 (1), 364 (1), 362 (1), 356 (3), 354 (1), 346 (2), 339 (5), 314 (1), 310 (2), 308 (4), 307 (2), 306 (4), 292 (5), 290 (8), 289 (4), 288 (10), 278 (7), 270 (7), 262 (14), 261 (2), 260 (7), 254 (4), 253 (7), 244 (1), 243 (3), 242 (4), 237 (10), 232 (2), 226 (7), 217 (8), 214 (2), 210 (15), 209 (100), 199 (2), 198 (2), 177 (2), 176 (1), 170 (3), 166 (5), 160 (2), 154 (1), 153 (5), 148 (38), 140 (2), 131 (3), 130 (15), 113 (17), 95 (23), 86 (51), and 35 (51).

*Anal.* Calc. for $C_{18}H_{32}O_{10}$: C, 52.94; H, 7.84. Found: C, 53.31; H, 7.47.

*Periodate oxidation of* **1**. — To a solution of **1** (1 mg) in methanol (0.5 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. By cochromatography, the residue showed complete consumption of digoxose (t.l.c., 9:1 chloroform–methanol).

*Mild hydrolysis of* **1** *with acid.* — To a solution of **1** (3 mg) in 1:4 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 (2.5 mg) that exhibited one spot in t.l.c. (9:1 chloroform–methanol), and, in p.c., mobility identical to that of digitoxose. For purification, it was distilled under high vacuum, yielding colorless, syrupy **4** (2 mg); $[\alpha]_D^{25}$ +41.3° (*c* 0.60, methanol). It reduced Fehling solution, gave positive tests for a 2-deoxy sugar in the xanthydrol and Keller–Kiliani reactions, and underwent periodate oxidation. Sugar **4**, obtained from the hydrolyzate of **1**, was thus identified as D-digitoxose.

*Oxidation of* **4** *with bromine water.* — A solution of **4** (3 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 acid was neutralized 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 **5** (2 mg), $[\alpha]_D^{25}$ −27.3° (*c* 0.41, methanol), showing only one spot with the $NH_2OH$–$FeCl_3$ reagent, and having the same mobility as D-digitoxono-1,4-lactone in t.l.c. (19:1 ethyl acetate–methanol), and in p.c.

*Phenylhydrazide* (**6**). — A solution of lactone **5** (1.5 mg) in absolute ethanol (0.05 mL) was mixed with freshly distilled phenylhydrazine (0.05 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 crystallized from methanol–ether as colorless needles (1 mg), m.p. 123°, identified as D-digitoxonic phenylhydrazide (**6**).

*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% methanol, and the solution was kept at room temperature. After 5 days, it showed three spots in p.c., two of them having mobilities identical to those of **4** ($R_{Dig}$ 1.0) and **1** ($R_{Dig}$ 0.36), respectively; the third spot ($R_{Dig}$ 0.72) was, presumably, the partially hydrolyzed product **3**. After 9 days, the hydrolyzate showed only spot. The hydrolyzate was made neutral by the addition of IRA-400 ($OH^-$) resin, the suspension filtered, and the filtrate evaporated to dryness, yielding a syrup (2 mg) having $[\alpha]_D^{25}$ +39.6° (*c* 0.54, methanol). It showed only one spot in p.c., and this had the same mobility as **4**, comparable to that of D-digitoxose.

*Penta-*O*-acetyldigoxose* (**2**). — A solution of **1** (2 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. Penta-*O*-acetyldigoxose (**2**; t.l.c., 49:1 chloroform–methanol) was obtained as an amorphous residue (2 mg); $[\alpha]_D^{25}$ +75.29° (*c* 0.60, methanol); 80-MHz, $^1$H-n.m.r. data for **2** ($CDCl_3$): $\delta$ 5.35 (dd, *J* 7 and 1 Hz, 2 H, 2 H-1 in S2 and S3), 4.56 (dd, *J* 3 and 1 Hz, 1 H, H-1 in S1), 2.10 (s, 3 H, OAc), 2.0 (s, 12 H, 4 OAc), and 1.25 (d, *J* 6 Hz, 9 H, 3 sec. $CH_3$).

*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% methanol, and the solution was kept at room temperature. After 2 h, it showed 6 spots in t.l.c. in 97:3 chloroform–methanol, two of them having mobilities identical to those of **2** ($R_F$ 0.90) and **1** ($R_F$ 0.1), respectively. The other four spots, $R_F$ 0.81, 0.72, 0.60, and 0.36 were, presumably, the partially deacetylated products, namely, tetra-, tri-, di-, and mono-*O*-acetyldigoxose, respectively. After 9 h, the hydrolyzate showed only one spot, which had the same mobility as **1**.

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