Cardiac Glycosides from *Corchorus olitorius*

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Besides identification of gluco-(1 ⟶ 6)-olitoriside and olitoriside, the structures of olitoriusin and erysimoside isolated from the seeds of *Corchorus olitorius* were proposed to be strophanthidin 3-*O*-β-D-glucopyranosyl-(1 ⟶ 6)-β-D-glucopyranosyl-(1 ⟶ 4)-β-D-digitoxoside (**2**) and strophanthidin 3-*O*-β-D-glucopyranosyl-(1 ⟶ 4)-β-D-digitoxoside (**4**). The structures were elucidated by a combination of fast-atom bombardment mass spectrometry, ^{13}C n.m.r. spectroscopy, and some chemical transformations.

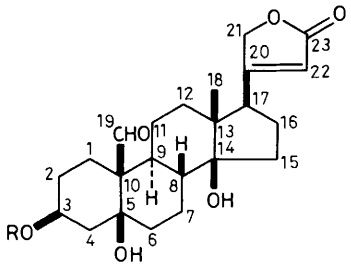
Corchorus olitorius and *C. capsularis* are two sister species, widely cultivated in India for their fibre.[1] The occurrence of corchorin, a cardiac active principle in the seeds of *C. olitorius*, was reported decades ago.[2] However, later work revealed the identity of corchorin with strophanthidin.[3] Several cardiac glycosides have been isolated from *C. olitorius*[4–7] and *C. casularis*[8,9] by various groups of workers. This paper deals with the isolation and structure elucidation of olitoriusin, a new cardiac glycoside, complete structure elucidation of erysimoside, a strophanthidin glucodigitoxoside, along with the isolation of olitoriside and gluco-(1 ⟶ 6)-olitoriside from *C. olitorius*. A new salt-addition technique[10] in fast atom bombardment mass spectrometry (FAB m.s.)[11–13] has been applied for enhancement of the cationized and other fragment ions of the glycosides. The BuOH-soluble fraction of the MeOH extract of the seeds of *C. olitorius* on repeated silica gel column chromatography and preparative t.l.c. (p.l.c.) led to the isolation of four cardiac glycosides, A, B, C, and D, marked according to their decreasing order of polarity. All the four glycosides gave positive Legal and Raymond tests for cardiac glycosides.[14]

Glycoside A (**1**) on acid hydrolysis yielded strophanthidin and carbohydrate constituents identified by paper chromatography and g.l.c. as D-boivinose and D-glucose by comparison with authentic samples. The stereochemistry of strophanthidin was established by *X*-ray crystallography.[15] The physical and spectroscopic data (m.p., $[\alpha]_D$ and ^{13}C n.m.r.) of glycoside A (**1**) indicated its identity with gluco-(1 ⟶ 6)-olitoriside, recently isolated from *C. capsularis*.[9]

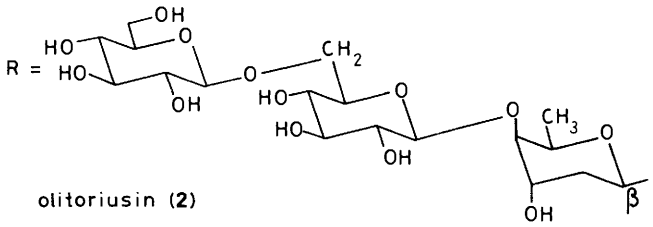
Glycoside B, designated olitoriusin (**2**), on acid hydrolysis yielded strophanthidin (**5**) as the aglycone and monosaccharide constituents identified by paper chromatography and g.l.c. as D-digitoxose and D-glucose. Olitoriusin (**2**) showed a cationized ion at *m/z* 881 attributable to $[M + Na]^+$ as the base peak in the positive-ion FAB spectrum in a glycerol–thioglycerol matrix containing NaCl. Thus the molecular weight of the glycoside (**2**) could be determined to be 858. The fragment ions at *m/z* 405 $[\text{strophanthidin} + H]^+$ and 437 $[\text{glucose–glucose–digitoxose} - 2\,H_2O]^+$ indicated the presence of a strophanthidin moiety and three sugar units in the molecule. The major fragment ions (Scheme 1) are also in full accord with the sequence of the sugar moieties in the assigned structure (**2**) for olitoriusin. ^{13}C N.m.r. spectra of glycoside (**2**) and strophanthidin were determined (C_5D_5N) and signal assignments were made by single-frequency off-resonance decoupling (SFORD) taking into consideration the ^{13}C n.m.r. chemical shifts of strophanthidin,[9,16] methyl β-D-glucoside,[17] β-D-digitoxoside,[18] and glycosylation-shift values.[19–21] The attachment of the trisaccharide moiety in structure (**2**) to the C-3 hydroxy group of strophanthidin was evident from the shift of the carbon atoms C-2, C-3, and C-4 by −1.9, 8.3, and −3.2 p.p.m., respectively, in its ^{13}C n.m.r. spectrum, compared with the corresponding signals of the aglycone. The peak at δ_C 105.0 was ascribed to the anomeric carbon of the terminal glucose (g′-1) and the anomeric carbon (g-1) of the inner glucose, while the resonance due to the anomeric carbon (d-1) of the digitoxose unit could be identified at δ_C 95.3. The 1 ⟶ 6 and 1 ⟶ 4 linkages between the two glucose moieties and between the inner glucose and the digitoxose respectively were deduced from the g-6 and d-4 resonances which experienced downfield glycosylation shifts of 7.4 and 9.0 p.p.m. respectively. Moreover, the linkages of the sugar units were ascertained as follows. The glycoside (**2**) on treatment with NaH–MeI in hexamethylphosphoramide (HMPA) afforded a permethylate which, on hydrolysis, furnished 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and D-cymarose by preparation of their alditol acetates and comparison (g.l.c.) with authentic samples. Thus the structure of olitoriusin is proposed to be strophanthidin 3-*O*-β-D-glucopyranosyl-(1 ⟶ 6)-β-D-glucopyranosyl-(1 ⟶ 4)-β-D-digitoxoside (**2**).

The glucoside C (**3**) appeared to be a strophanthidin bioside from its FAB m.s. data. It was eventually characterized as olitoriside (**3**) by comparison of its m.p., $[\alpha]_D$, and ^{13}C n.m.r. data with those of an authentic sample.[9] The FAB m.s. spectra of olitoriside (**3**) were recorded using a glycerol–thioglycerol matrix in the presence or absence of KCl, NaCl, or NH_4Cl. The details of fragmentation are given in the Experimental section.

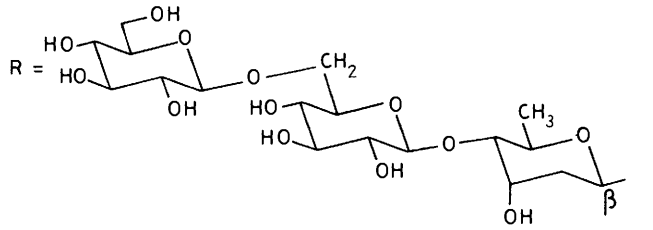
Glycoside D (**4**) on hydrolysis liberated strophanthidin (**5**) as the aglycone and D-glucose and D-digitoxose as the carbohydrate constituents. The molecular weight of (**4**) was determined by FAB m.s. using a glycerol–thioglycerol matrix with $(CD_3)_2SO$ ($[^2H_6]$DMSO) as solvent. The spectrum showed a weak ion at *m/z* 697 assignable to $[M + H]^+$. The other discernible peaks are depicted in Scheme 2. When the FAB m.s. of compound (**4**) was determined in a glycerol–thioglycerol matrix with added KCl, the cationized ion $[M + K]^+$ appeared at *m/z* 735 with high intensity (72%). The other significant peaks appeared at *m/z* 773, 707, and 405 ascribable to $[M + 2K - H]^+$, $[M + K - CO]^+$, and $[\text{strophanthidin} + H]^+$ respectively. The attachment of the carbohydrate moiety at the C-3 position of strophanthidin and the linkage between the sugar units were evident from the ^{13}C n.m.r. data of compound (**4**) (Table). The carbon atoms of the steroid moiety were identified by correlation with the characteristic chemical-shift values of strophanthidin (**5**). The carbon atoms C-2, C-3, and C-4 appeared shifted by −2.8, 6.9, and −3.0 p.p.m. respectively



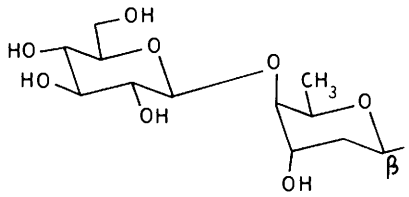
gluco-(1→6)-olitoriside (1)



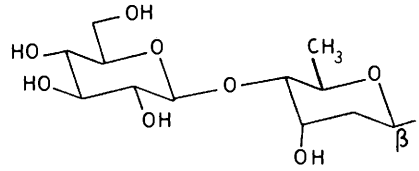
olitoriusin (2)



olitoriside (3) R =



erysimoside (4) R =



strophanthidin (5) R = H

from the corresponding signals of the aglycone.[16] In the spectrum of glycoside (**4**) having a β-D-glucose linked to digitoxose by a (1 ⟶ 4) linkage, the peak at δ_C 104.1 was assigned to the anomeric carbon (g-1). The resonance due to d-4 could be identified at δ_C 82.0, taking into consideration the expected 9.2 p.p.m. downfield shift (glycosylation shift) from the corresponding signal of β-D-digitoxoside. The linkage between the sugar units was further confirmed by permethylation of glycoside (**4**) followed by hydrolysis, which resulted in the liberation of 2,3,4,6-tetra-*O*-methyl-D-glucose and D-cymarose, both identified (g.l.c.) by comparison with authentic samples. Consequently the structure of glycoside (**4**) is suggested to be strophanthidin 3-*O*-β-D-glucopyranosyl-(1 ⟶ 4)-β-D-digitoxoside. It is noteworthy that the physical data (m.p. and $[\alpha]_D$) of glycoside (**4**) seem to be identical with those reported for erysimoside isolated by Kowalewski *et al.*[22] from *Erysimum perotskianum* and by Rao and Rao from *C. capsularis*.[23,24] However, the linkage between the sugar units in erysimoside is yet to be determined. It may be mentioned that the cold methanolic extract of the defatted powdered seeds of *Corchorus olitorius* was subjected to t.l.c. examination and that spots of olitoriside (**3**) and erysimoside (**4**) were clearly visible. This observation demonstrated that olitoriside (**3**) and erysimoside (**4**) are not artefacts.

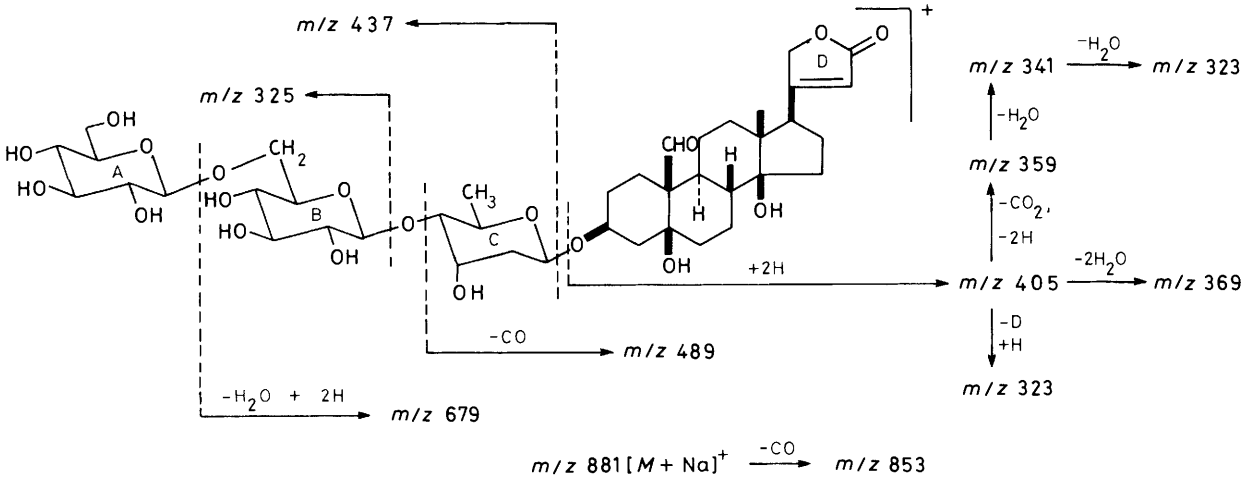
Experimental

M.p.s were measured on a capillary melting-point apparatus and are uncorrected. T.l.c. was carried out on silica gel G with the solvent $CHCl_3$ (60 ml)–water (5 ml); MeOH was then added till the solution became clear. Paper chromatography was done on Whatman paper No. 1 with solvent system BuOH–C_5H_5N–water (6:4:3); a saturated solution of aniline oxalate in water was used as staining agent. G.l.c. was performed on a Hewlett-Packard model 5730A instrument with the following columns: (i) ECNSS-M, 3% on Gas Chrome Q at 190 °C for alditol acetates and (ii) OV-225 on Gas Chrome Q for partially methylated alditol acetates. ^1H and ^{13}C n.m.r. spectra were recorded on a JEOL FX-100 spectrometer operating at 99.6 MHz and 25.05 MHz, for $CDCl_3$ or C_5D_5N or $[^2H_6]$DMSO solutions respectively, with tetramethylsilane as internal standard. Optical rotations were measured on a JASCO automatic polarimeter. Fast-atom bombardment mass spectra (FAB m.s.) were obtained on a VG ZAB-SE mass spectrometer equipped with a FAB source operating at an accelerating voltage of 8 kV. FAB mass spectra were also obtained using a Finningan MAT 312 mass spectrometer operating at an accelerating voltage of 2—3 keV. Samples were dissolved in $[^2H_6]$DMSO (2—10 $\mu g \mu l^{-1}$) and deposited on a FAB probe tip. A thin layer of either glycerol or thioglycerol was applied to the probe tip containing the samples and mixed thoroughly with a Pasteur pipette before insertion into the source. The primary atom (xenon) was produced using a saddle-field ion source operating at a tube current of 1—1.5 mA at an energy of 8 keV. For the salt-addition technique, samples were dissolved in $[^2H_6]$DMSO (2—5 $\mu g ml^{-1}$) to which salt (KCl, NaCl, or NH_4Cl) was added such that the sample to salt ratio was roughly 1:3. A thin layer of a glycerol–thioglycerol mixture (50:50) was applied to the copper probe tip to which the sample solution containing salt was added and the whole was thoroughly mixed. The probe was then introduced into the source of the mass spectrometer for data acquisition. Electron-impact mass spectra were recorded on a Hitachi RMU-6L mass spectrometer by direct inlet at 70 eV.

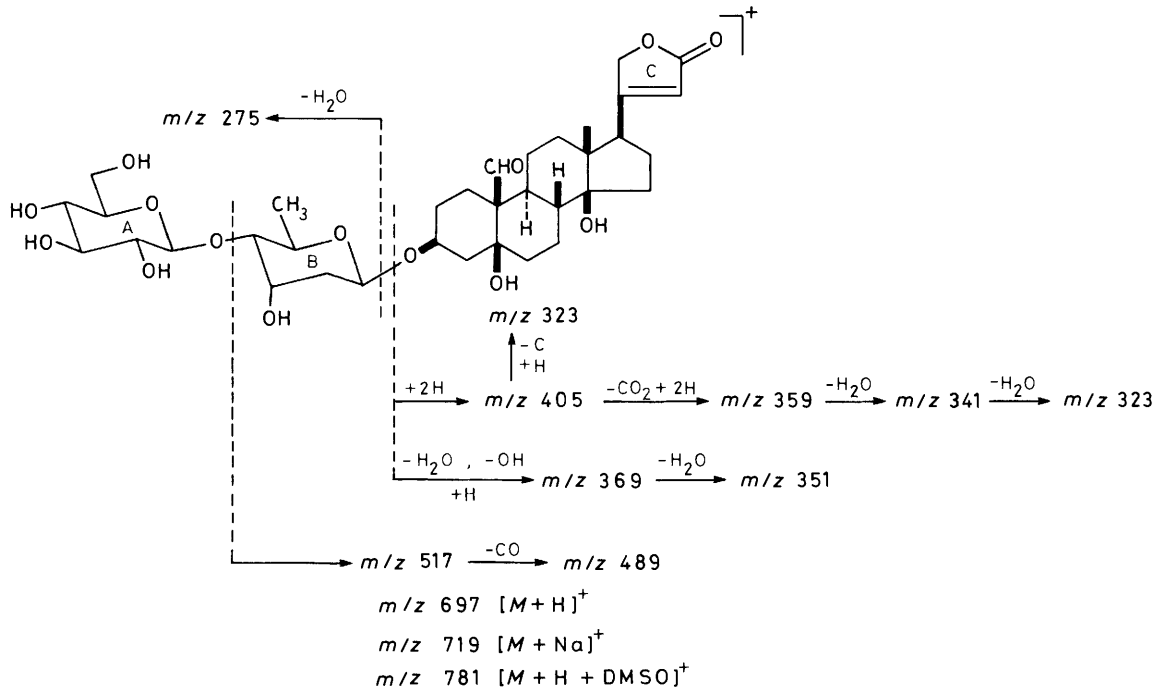
Extraction and Chromatography.—The air-dried powdered seeds of *C. olitorius* (2 kg) was successively extracted with light petroleum (b.p. 60—80 °C), chloroform, and methanol. The residue (30 g) left on removal of MeOH under reduced pressure was partitioned between BuOH and water and the organic layer was evaporated to dryness under reduced pressure to give a dark brown mass (20 g). This extract was chromatographed on silica gel (300 g). Graded elution was carried out with light petroleum, light petroleum–chloroform, (1:1), chloroform, chloroform–methanol (19:1, 9:1, 17:3, and 4:1). A total of 70 fractions (250 ml each) was collected and fractions giving similar spots on t.l.c. were combined.

Isolation of Olitoriusin (**2**) *and Gluco-*(1 ⟶ 6)*-olitoriside* (**1**).—Further purification of the chloroform–methanol (4:1) eluate by rechromatography followed by p.l.c. afforded olitoriusin (**2**) and gluco-(1 ⟶ 6)-olitoriside (**1**) as homogeneous compounds.

Olitoriusin (**2**) was obtained as a solid from MeOH–diethyl ether (50 mg), m.p. 185—188 °C; $[\alpha]_D$ + 21.2° (*c* 0.45 in MeOH) (Found: C, 51.4; H, 7.3. $C_{41}H_{62}O_{19}$ requires C, 51.33; H, 7.28%).



Scheme 1. Major fragments in the FAB m.s. of olitoriusin (**2**)



Scheme 2. Major fragment ions in the FAB m.s. of erysimoside (**4**)

Hydrolysis of Olitoriusin (**2**).—Olitoriusin (**2**) (100 mg) was hydrolysed with water (60 ml) and aq. HCl (0.25 ml) at 70 °C for 2.5 h. After work-up in the usual way, the aglycone was obtained as long needles from 90% EtOH, m.p. 234—235 °C; $[\alpha]_D$ + 41.2° (*c* 0.6 in MeOH), which data were in good agreement with those of authentic strophanthidin (m.p., i.r., and t.l.c.). The filtrate from the hydrolysate was neutralized with Ag_2CO_3, then filtered, and a portion of the filtrate was concentrated under reduced pressure and tested for carbohydrates by p.c. D-Glucose and D-digitoxose were identified by comparison with authentic samples. D-Digitoxose was isolated in (p.p.c.) and its optical rotation was measured. The initial $[\alpha]_D$ value was observed to be +44.4° and the equilibrium value after 1 h was +49.5° (*c* 0.02 in H_2O). The corresponding values for an authentic sample were observed to be +46.5° and +50.0°. The other portion of the concentrated filtrate was reduced with $NaBH_4$ and worked up in the usual manner. The residue was acetylated with Ac_2O–pyridine (1 : 1) at water-bath temperature for 1 h, dried *in vacuo*, and purified by chromatography over silica gel. Only two peaks, corresponding to D-glucitol acetate and D-digitoxitol acetate, were obtained.

Permethylation of Olitoriusin (**2**) *and Hydrolysis.*—A stirred solution of compound (**2**) (100 mg) in HMPA (5 ml) was treated with NaH (300 mg) and MeI (8 ml) at room temperature for 3 h under N_2. The reaction mixture was extracted with diethyl ether washed with water, dried over Na_2SO_4, and evaporated to dryness to yield a gummy residue, which was purified by chromatography over silica gel. The product was hydrolysed with 2M-HCl in aq. MeOH (10 ml) for 2 h. The filtrate was worked up in the usual way and tested for methylated sugars by g.l.c. on column (ii). Three peaks were detected and identified as those of the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose, and cymarose by comparison with authentic samples.

Gluco-(1 ⟶ 6)-*olitoriside* (**1**).—This was crystallized from ethanol–ether (70 mg), m.p. 208—211 °C (decomp.); $[\alpha]_D$ −7.2°

Table. Chemical shifts [δ_C ($\pm$0.1)] of olitoriusin (**2**), erysimoside (**4**), and strophanthidin (**5**)

Carbon	(2)[a]	(4)[b]	(5)[a]	Carbon	(2)[a]	(4)[b]
1	24.2	23.4	24.6	d-1	95.3	96.1
2	25.3	24.4	27.2[c]	d-2	38.2	38.0
3	74.9	73.5	66.6	d-3	66.3[c]	65.8[c]
4	35.0	35.2	38.2	d-4	81.9	82.0
5	73.5	73.3	74.3	d-5	67.7[c]	67.8[c]
6	36.8	35.8	37.6	d-6	18.1	18.0
7	22.6	21.8	22.2	g-1	102.8	104.1
8	41.4	41.8	42.0	g-2	74.5	73.1
9	39.7	38.4	39.3	g-3	78.1	79.1
10	55.0	54.3	55.4	g-4	71.6[d]	71.6
11	18.8	17.3	18.2	g-5	76.3	75.6
12	39.7	38.2	39.4	g-6	70.0	60.9
13	49.0	48.9	49.7	g'-1	105.0	
14	84.5	83.4	84.6	g'-2	74.6	
15	32.3	31.1	32.4	g'-3	78.1	
16	27.3	26.2	27.3[c]	g'-4	71.7[d]	
17	50.8	49.7	51.0	g'-5	77.1	
18	16.0	15.4	16.2	g'-6	62.6	
19	208.8	208.5	208.8			
20	176.0[c]	176.1	175.7[d]			
21	73.8	73.1	73.9			
22	116.8	116.2	117.7			
23	174.8[c]	173.7	174.2[d]			

d = Digitoxose; g,g' = glucose; [a] In C_5D_5N. [b] In [$^2H_6$]DMSO. [c,d] Assignments within a column may be interchanged.

(*c* 0.72 in MeOH) {lit.,[9] 175 °C (sintering)—215 °C; $[\alpha]_D$ −8.1°}; its ^{13}C n.m.r. data were comparable with those reported in the literature.

Isolation of Erysimoside (**4**) *and Olitoriside* (**3**).—Fractions eluted with $CHCl_3$—MeOH (17:3) yielded a solid, which on further purification by rechromatography afforded a light yellow solid which was found to be a mixture of two compounds (t.l.c.). This was subjected to p.l.c. and erysimoside (**4**) (0.15 g) and olitoriside (**3**) (0.13 g) were obtained.

Erysimoside (**4**) was crystallized from MeOH–ether, m.p. 172—173 °C; $[\alpha]_D$ + 18.5° (*c* 0.51 in MeOH) (Found: C, 60.4; H, 7.5. $C_{35}H_{52}O_{14}$ requires C, 60.33; H, 7.52%). Erysimoside (100 mg) was hydrolysed with aq. HCl (60 ml) as described for olitoriusin and worked up in the usual way. The residue, on chromatographic purification over silica gel followed by crystallization from aq. MeOH, yielded strophanthidin (40 mg), m.p. 236—238 °C, $[\alpha]_D$ + 42° (*c* 0.5 in MeOH).

The filtrate from the hydrolysate were worked up in the usual way and the sugar constituents identified as D-digitoxose and D-glucose (p.c. and g.l.c.) by comparison with authentic samples.

Permethylation of Erysimoside (**4**) *and Hydrolysis.*—Compound (**4**) (50 mg) was permethylated in HMPA (5 ml), NaH (300 mg), and methyl iodide (6 ml) as described earlier. The residue obtained was hydrolysed with 2M-HCl in aq. MeOH (8 ml) for 3 h, worked up as usual, and subjected to g.l.c. analysis on column (ii). 2,3,4,6-Tetra-*O*-methyl-D-glucitol diacetate and cymaritol diacetate could be identified using authentic specimens.

Olitoriside (**3**). This was crystallized from MeOH–$CHCl_3$ (90 mg), m.p. 203—205 °C; $[\alpha]_D$ − 4.8 (*c* 0.5 in MeOH); FAB m.s. (positive) (glycerol–thioglycerol matrix with [$^2H_6$]DMSO as solvent) *m/z* 781 $[M + H + DMSO]^+$ (24%), 719 $[M + Na]^+$ (17.5), 679 $[M + H - H_2O]^+$ (29), 517 $[M + H - \text{glucose}]^+$ (3.6), 489 $[M + 2H - \text{glucose} - CO]^+$ (67), 405 [strophanthidin + H]$^+$ (100), 369 $[405 - 2H_2O]^+$ (46), 359 $[405 - CO_2 - 2H]^+$ (39.1), 341 $[359 - H_2O]^+$ (31.7), 323 $[359 - 2H_2O]^+$ (36.4), and 275 [glucose–boivinose − $2H_2O$]$^+$ (51.8) (Found: C, 60.25; H, 7.6. Calc. for $C_{35}H_{52}O_{14}$: C, 60.33; H, 7.52%).

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