Structure and Configuration of Tecoside, a New Iridoid Glucoside from *Tecomella undulata*

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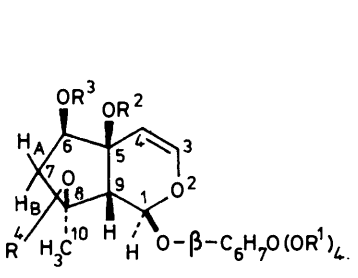
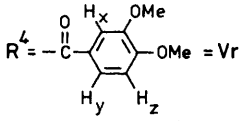
Isolation of a new iridoid glucoside, tecoside from *Tecomella undulata* (Bignoniaceae) is described. It has been proved to be 8-*O*-veratroylharpagide.

EARLIER investigation of the bark of *Tecomella undulata* led to the isolation of two chromone glycosides, undulatoside-A [1] and -B,[2] and an iridoid glucoside, tecomelloside.[2,3] We now report the isolation of a new iridoid glucose, tecoside (I), from the ethyl acetate insoluble portion of the alcoholic extract of the bark.



(I) $R^1 = R^2 = R^3 = H$,

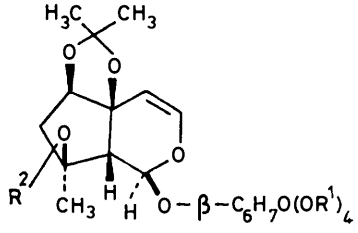


(II) $R^1 = R^2 = R^3 = Ac$, $R^4 = Vr$

(III) $R^1 = R^2 = R^3 = R^4 = H$

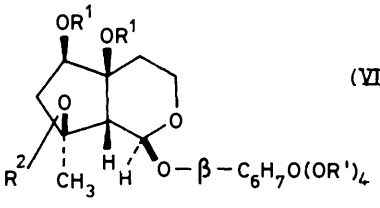
(IV) $R^1 = R^3 = R^4 = Ac$, $R^2 = H$

(V) $R^1 = R^2 = R^3 = R^4 = Ac$



(VI) $R^1 = H$, $R^2 = Vr$

(VII) $R^1 = Ac$, $R^2 = Vr$



(VIII) $R^1 = H$, $R^2 = Vr$

Tecoside is a hygroscopic amorphous powder, $[\alpha]_D^{27}$ −159.26°, with molecular formula $C_{24}H_{32}O_{13}\cdot H_2O$. It responded to Godin's [4] test for polyols, Shear's [5] and Wieffering's [6] test for iridoid glucosides, and other colour reactions [7] for the harpagide moiety. Like other naturally occurring iridoids [8,9] (I) is converted by acid hydrolysis into glucose (1 mol. equiv.) and insoluble black products, due to decomposition of the aglucone.

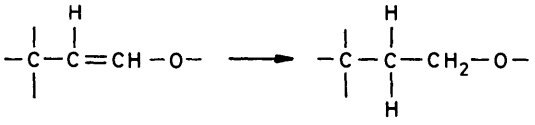
Compound (I) showed $\lambda_{max.}(H_2O)$ 206 nm (log ε 3.39), due to the double bond of a non-conjugated enol ether. The other u.v. absorptions at 225, 262, and 293 nm indicated that the main chromophores of the glucoside were due to the veratroyl group.[10] Compound (I) showed prominent i.r. peaks for hydroxy groups (3 509—3 226 cm^{-1}), ester carbonyl group (1 712 cm^{-1}), and an enolic double bond (1 653 cm^{-1}). (I) formed a hexa-acetyl derivative (II) (Ac_2O–py) on heating at 60° for 40 h, indicating the presence of chelated or tertiary hydroxy group(s).

The 100 MHz ^1H n.m.r. spectrum of (I) (see Table 1) showed double doublets at δ 5.07 and 2.98 due to 4- and 9-H which changed to doublets at δ 5.45 and 3.09 respectively in the n.m.r. spectrum of its acetate (II) confirming thereby the presence of a tertiary hydroxy group at C-5.

The n.m.r. assignments were further confirmed by spin–spin decoupling experiments of (I). The irradiation of the complex multiplet centred at δ 3.46 due to 6-H simplifies the eight line multiplet between δ 2.18 and 2.22 into a quartet due to a simple AB system with a value of the coupling constant (J_{AB} 14.5 Hz) typical of geminal protons. This result confirmed the assignments of the eight line multiplet to the methylene protons at C-7 as the AB part of an ABX system. The reverse irradiation changed the multiplet at δ 3.46 into a doublet.

The irradiation of the doublet at δ 6.31 (3-H) reduced the double doublet at δ 5.07 into a doublet, clearly assignable to the olefinic 4-H; the reverse irradiation simplified, as expected, the doublet at δ 6.31 to a singlet.

Irradiation at the doublet at δ 6.08 of the acetal 1-H



SCHEME 1

transformed the double doublet at δ 2.98 (9-H) into a doublet while the reverse irradiation simplified the doublet at δ 6.08 to a singlet.

Tecoside is slowly converted, by reduction over Pd–C at room temperature and pressure, into a dihydro derivative (VIII) as a hygroscopic foam, $C_{24}H_{34}O_{13}$. In the n.m.r. spectrum of (VIII), the peaks at δ 6.31 (3-H) and 5.07 (4-H) disappeared. Furthermore the value of the integral between δ 4.6—3.6 and 2.8—1.4 showed an increase of two protons in (VIII) compared with (I). This indicates the transformation shown in Scheme 1. The absence of absorptions at 1 653 cm^{-1} in the i.r. and 206 nm in u.v. spectrum of (VIII) also supports this transformation.

On basic hydrolysis with $Ba(OH)_2$, (I) yielded veratric

TABLE 1

^{1}H N.m.r. spectral assignments (J in Hz)[a]

Compound (solvent)	Chemical shifts (δ)									
	1-H	3-H	4-H	6-H	7-H_2	9-H	Methyl	Acetyl	Methoxy	Aromatic protons
(I) [(D_2O) + (CD_3)$_2$CO][b]	6.08(d, $J_{1,9}$ 0.5)	6.31(d, $J_{3,4}$ 6.5)	5.07(dd, $J_{3,4}$ 6.5, J 1.2)	3.46(cm)	2.18—2.22 (dq, J 14.5, J 5)	2.98(dd) J 0.5, J 3.0	1.45(s)		3.92(s, 6 H)	7.77(dd, H_z J_{yz} 9, J_{xz} 2) 7.38(d, H_x, J_{xz} 2), 7.07 (d, H_y, J_{yz} 9)
(II) ($CDCl_3$)	6.01(d, J 0.5)	6.47(d, J 6.5)	5.45(d, J 6.5)	3.5(m)	2.5(m)	3.09(d, J 0.5)	1.65	2.1(s, 3 H) 2.98(s, 3 H) 2.09(s, 12 H)	4.1(s, 6 H)	7.83(dd, H_z, J_{yz} 9, J_{xz} 2) 7.27(d, H_x, J_{xz} 2 H), 7.14(d, H_y J_{yz} 9)
(III)[11] (D_2O)[b]	5.75 (J 1.2)	6.41(d, J 6.5)	5.09(dd, J 6.5, J 1.2)	*ca.* 3.9	1.99(dd, J 14.5, 5) 1.88(dd,) J 14.5, 4.5)	2.58(s)	1.25(s)			
(IV)[11] ($CDCl_3$)	6.13(d, J 1.2)	6.38(d, J 6.5)	5.07(dd, J 6.5, J 1.2)	5.4—4.9	2.5—2.0	3.06(s)	1.47(s)	2.03—2.16 (18 H)		
(V)[11] ($CDCl_3$)	6.03(s)	6.38 (J 6.5)	5.52 (J 6.5)	5.5—4.8	2.7—1.8	3.19(s)	1.52(s)	1.93—2.06 (21 H)		
(VII) ($CDCl_3$)	6.2(d, J 0.5)	6.52(d, J 6.5)	5.55(d, J 6.5)	4.0	2.8(d, J 5)	3.4(d, J 0.5)	1.4(s)	2.25 (12 H)	4.2(s, 6 H)	7.85, 7.3, 7.2 1.31(s, 6 H, isopropylidene
(VIII) (D_2O)[b]	5.9(d, J 0.5)	4.6—3.6 (2 H)	2.8—1.4 (2 H)	3.6(cm)	2.6(cm)	2.95(d, J 0.5)	1.37(s)		4.08(s, 6 H)	7.8, 7.2, 7.1

[a] d = Doublet, dd = double doublet, cm = complex multiplet, m = multiplet, s = singlet. [b] Internal reference HDO (δ 4.70).

acid and an iridoid moiety (III). Compound (III) was obtained as a hygroscopic foam and gave a red-violet colour with Godin's reagent. Its u.v., i.r., and n.m.r spectra showed the presence of the double bond of an enol ether. The existence of one secondary and two tertiary hydroxy groups in the aglycone residue of (III) was demonstrated by acetylation which yielded a mixture of hexa-acetate (IV) and hepta-acetate (V). The n.m.r. spectra of (III)—(V) (Table 1), coupled with the above observations, indicated (III) to be harpagide.[11]

The n.m.r. spectra of tecoside (I) and harpagide (III) and tecoside acetate (II) and harpagide acetate (V) were found to exhibit general similarity (Table 1). Thus compound (I) was confirmed to be a veratroyl ester of harpagide (III). The veratroyl group could be linked either to the glucose moiety or through the three available hydroxy groups at C-5, -6, and -8 in the aglucone part of (III). The absence of a veratroyl moiety in glucose was shown by the formation of 2,3,4,6-tetra-*O*-methyl-D-glucose on permethylation and hydrolysis of (I) and was supported by the isolation of D-glucose by β-glucosidase hydrolysis of (I).

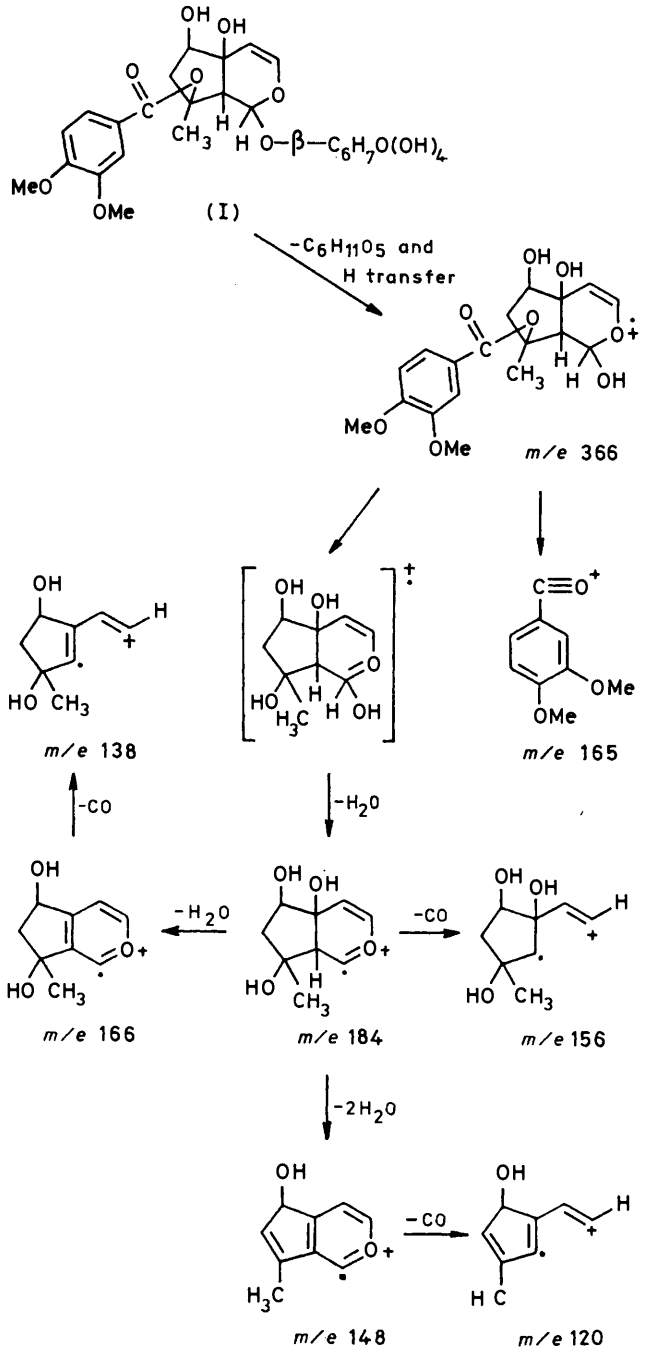
The formation of a mono-*O*-isopropylidene derivative (VI) of tecoside ($SnCl_2$–acetone)[12] proved that the 5- and 6-hydroxy groups of (I) are free. Compound (VI) on acetylation under mild conditions yielded a tetra-acetate (VII) whose n.m.r. spectrum showed an isopropylidene signal at δ 1.31 (6 H) and four acetyl groups shifted to lower field compared with (II). Under normal conditions, glucopyranosides do not give isopropylidene acetals with acetone.[12] Coupled with this fact, the absence of a free hydroxy group in (VII) demonstrated that the isopropylidene function was a part of the aglucone unit and hence further supported the position of the two hydroxy groups at C-5 and -6. Thus, for (III), only the 8-hydroxy group can form an ester, and the veratroyl group is linked to this position. Equal shifts in the signals of the methyl group at C-8 and of the neighbouring protons at C-1 and -9 were observed when (I) was hydrolysed to yield (III)[13] and these signals were shifted back when (III) was acetylated to give (IV) and (V) respectively (Tables 1 and 2). Furthermore, no other proton geminal with a primary or secondary hydroxy group undergoes a shift through this transformation, confirming that the ester arises from a tertiary OH. This confirms the position of veratric acid and the methyl group at C-8 and hence the structure 8-*O*-veratroylharpagide is assigned to tecoside.

In the mass spectrum of (I), the peak at *m/e* 366 corresponds to the aglucone of (I) indicating, thereby, the absence of a veratroyl moiety in the glucose. The base peak at *m/e* 184 was obtained by the removal of the veratroyl function and a water molecule simultaneously, as expected.[14] The peak at *m/e* 165 was due to the veratroyl function which fragmented to give peaks at *m/e* 137 and 106. Other fragments were at *m/e* 166, 156, 148, 138, and 120 (Scheme 2). Retro-Diels–Alder fragmentations (*m/e* 115 and 86) involving the cleavage of three bonds (Scheme 3) explained the absence of a double bond in the cyclopentane ring of (I) and proved that the veratroyl group in tecoside is at C-8.

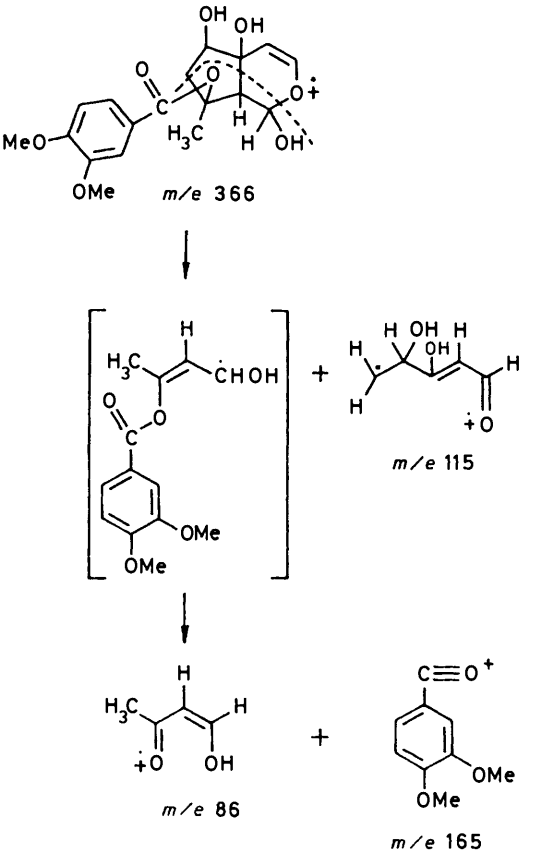
A comparison of the M_D values of (I) (−870°), (III) (−547°), dihydroharpagide[11] (−438°), and methyl α- and β-dihydroharpagenines (+187 and −169°, re-

spectively) [11] indicated that harpagide and therefore tecoside have the β-configuration at C-1 as in all other known iridoid glucosides.[15-19]

The β-orientation was assigned to the 8-hydroxy group of (III) and therefore to the veratroyl group of (I) on the basis of the paramagnetic shifts observed for 1-H, 9-H, and 10-H_3 in (I) compared with those in (III) (Table 2). Similar paramagnetic shifts were observed for 1-H, 9-H, and 10-H_3 in (IV) and (III). These shifts were very similar to those in many other iridoid compounds having the same relative configuration at C-1, -8, and -9 [12,13,19-21] as shown in Scheme 4.



Scheme 2



Scheme 3

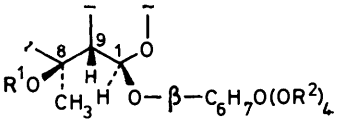
Table 2

N.m.r. chemical shift (δ) differences

Δδ	1-H	9-H	10-H_3
(I) – (III)	0.33	0.40	0.20
(III) – (IV)	0.38	0.48	0.22

EXPERIMENTAL

Visualisation of spots on t.l.c. was achieved by spraying either with 2N-H_2SO_4 and heating for 2—3 min at 100° (silica gel plates) or with a 0.7% solution of vanillin in 2% methanolic HCl and heating for 2—3 min at 100° (cellulose plates and paper chromatograms). M.p.s were determined on a Kofler block and are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 137 and u.v. spectra on a Beckman DU 32 spectrophotometer. ^1H N.m.r. spectra



(I) R^1 = Vr, R^2 = H
(III) R^1 = R^2 = H
(IV) R^1 = R^2 = Ac

Scheme 4

were registered with Varian A-60 and HA-100 instruments, with Me_4Si as internal reference for spectra in $CDCl_3$ while for those in D_2O the HDO signal (δ 4.70) was taken as internal reference with Me_4Si as external reference. Spin decoupling experiments were performed with the spin decoupler accessory of the Varian HA-100 instrument using the frequency sweep mode. Mass spectra were determined at 70 eV and 180° on a Varian MAT CH4-B instrument using a direct inlet system.

Isolation.—Defatted fresh bark (2 kg) on *T. undulata* was extracted with EtOH (Soxhlet) and the extract on concentration gave a brown viscous solid which was macerated with EtOAc (3 × 500 ml). The EtOAc insoluble portion was dissolved in MeOH (100 ml) and precipitated with dry ether. The solid thus obtained was chromatographed over silica gel impregnated with 10% $AgNO_3$ solution using $CHCl_3$–MeOH (24 : 1) as eluant.

Tecoside (I) was obtained as a hygroscopic powder (400 mg), m.p. 139—142°; $[\alpha]_D^{27}$ −159.26° (*c* 0.40, MeOH) (Found: C, 52.4; H, 6.3. $C_{24}H_{32}O_{13}\cdot H_2O$ requires C, 52.7; H, 6.2%); R_F 0.58 (EtOAc–pyridine–H_2O 40 : 11 : 6; cellulose), 0.72 ($CHCl_3$–MeOH 9 : 1), and 0.55 (EtOAc–MeOH–H_2O 100 : 16.5 : 13.5). It gave positive Godin's, Shear's, and Wieffering's tests, $\lambda_{max.}(H_2O)$ 206 nm (log ε 3.39); $\lambda_{max.}$(MeOH) 225 (log ε 4.59), 262 (4.43), 293 (4.14), and 330 nm (3.26); $\nu_{max.}$(KBr) 3 509—3 226, 1 712, 1 653, 1 603, 1 220, and 770 cm^{-1}; *m/e* 366(7.3%), 184(100), 166(60.4), 165(75.2), 156(2.2), 148(30.4), 138(29.7), and 137(30.2).

Hexa-acetyltecoside (II).—Tecoside (50 mg) was treated with pyridine (1.5 ml) and Ac_2O (2 ml) at 60° for 40 h. The mixture was worked up as usual. The crude acetate thus obtained crystallised from $CHCl_3$–MeOH as *needles*, m.p. 167—168°; $[\alpha]_D^{26}$ −271.4° (*c* 0.14, $CHCl_3$) (Found: C, 54.9; H, 5.8. $C_{36}H_{44}O_{19}$ requires C, 55.4; H, 5.6%); $\lambda_{max.}$(MeOH) 225, 260, and 293 nm; $\nu_{max.}$(KBr) 1 653, 1 710, and 1 600 cm^{-1}.

Acid Hydrolysis of Tecoside (I).—Tecoside (10 mg) underwent complete hydrolysis with 10% H_2SO_4 in 2 min. The aglucone was obtained as a black insoluble product. The aqueous hydrolysate after neutralisation ($BaCO_3$) and subsequent concentration on paper chromatography (Bu^nOH–pyridine–H_2O 6 : 4 : 3) showed the presence of glucose by direct comparison with an authentic sample.

Dihydrotecoside (VIII).—To a solution of tecoside (100 mg) in 95% EtOH (5 ml) was added 100 mg of 10% Pd–C. About one mole of hydrogen per mole of tecoside was absorbed during 2 h. The solution was filtered, washed with EtOH, and evaporated to give an amorphous residue. This residue on chromatography on silica gel (8 g) using Bu^nOH saturated with H_2O as eluant, afforded the *dihydro derivative* (VIII) as a hygroscopic foam (Found: C, 54.1; H, 6.5. $C_{24}H_{34}O_{13}$ requires C, 54.3; H, 6.4%), $\lambda_{max.}$ (MeOH) 225, 262, 295, and 332 nm; $\nu_{max.}$(KBr) 3 509—3 226, 1 712, 1 603, 1 515, 1 430, 1 075, and 770 cm^{-1}.

Enzymatic Hydrolysis of Compound (I).—Tecoside (5 mg) was hydrolysed using emulsin (5 mg) in phosphate buffer (2 ml; 0.02M; pH 7.0) at 36° for 4 days. D-Glucose was the only sugar present in the aqueous hydrolysate.

Permethylation of Compound (I).—Tecoside (5 mg) was permethylated according to Hakomori's method [22] and the product was hydrolysed with Killiani's reagent (HCl–CH_3CO_2H–H_2O 2 : 7 : 11).[23] The methylated sugar was identified as 2,3,4,6-tetra-*O*-methyl-D-glucose by direct comparison with an authentic sample on paper chromatography (Bu^nOH–EtOH–H_2O 5 : 1 : 4, upper layer).

Basic Hydrolysis of Compound (I).—Tecoside (150 mg) was hydrolysed with $Ba(OH)_2$ (0.1N, 10 ml) at room temperature for 30 h. The mixture was neutralised with HCl (0.1N), extracted with ether, dried, and the solvent evaporated. The residue crystallised from MeOH as needles, m.p. 181—182° (Found: C, 59.2; H, 5.3. $C_9H_{10}O_4$ requires C, 59.3; H, 5.4%); $\lambda_{max.}$(MeOH) 225, 260, and 295 nm; $\nu_{max.}$(Nujol) 1 666, 1 587, 1 505, 1 260, and 870 cm^{-1}. It was identified as veratric acid by comparison with an authentic sample (mixed m.p., co-t.l.c., co-i.r.).

Isolation of Compound (III).—The aqueous hydrolysate after neutralisation with acid was at once treated with $BaCO_3$, filtered, and evaporated. The residual mixture after animal charcoal treatment was chromatographed over silica gel using $CHCl_3$–MeOH 1 : 1 as eluant and compound (III) was obtained as a hygroscopic foam. It gave a red-violet colour with Godin's reagent, $[\alpha]_D^{22}$ −150° (*c* 1.2, H_2O) (Found: C, 49.1; H, 6,7. $C_{15}H_{24}O_{10}$ requires C, 49.5; H, 6.6%), $\lambda_{max.}$ (H_2O) 206 nm (log ε 4.01) $\nu_{max.}$ (KBr) 3 510, 1 655, and 1 515 cm^{-1}. On the basis of spectral data (III) was shown to be harpagide.[11]

Acetylation of Compound (III).—To a solution of (III) (100 mg) in dry pyridine (2 ml) was added Ac_2O (2 ml) and the mixture heated at 50° for 30 h. The mixture was worked up as usual. The viscous syrup so obtained was chromatographed over silica gel (containing 10% water). On elution with $CHCl_3$–MeOH (1 : 4), the hepta-acetyl derivative (V) (60 mg), m.p. 185—187°, and on elution with $CHCl_3$–MeOH (15 : 1), the hexa-acetyl derivative (IV) (30 mg), m.p. 225—226°, were obtained.

5,6-*Isopropylidenetecoside* (VI).—To a solution of tecoside (40 mg) in dry acetone (1 ml) was added $SnCl_2$ solution (283 mg) in acetone (1.7 ml). The mixture was left at room temperature overnight and then it was treated with saturated $NaHCO_3$ solution and diluted with H_2O. The mixture was filtered through decolorising charcoal (1 g). Soluble salts were removed by elution with water and the isopropylidine derivative was eluted with MeOH which on concentration gave a residue. On chromatography over silica gel using Bu^nOH saturated with H_2O as eluant the isopropylidene derivative (VI) was obtained as a foam (30 mg).

5,6-*Isopropylidenetecoside Tetra-acetate* (VII).—Compound (VI) (30 mg) was treated with pyridine (1 ml) and Ac_2O (1.5 ml) at room temperature for 24 h. The mixture was worked up as usual. The crude acetate thus obtained on attempted crystallisation was obtained as a foam, $\nu_{max.}$(KBr) 1 695, 1 516, 1 449, 1 370—1 351br, and 1 220 cm^{-1}.

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