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STRUCTURE OF A NOVEL TYPE STEROID GLYCOSIDE, 18-NORSPIROSTANOL OLIGOGLYCOSIDE

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Previously it was reported1) that eight kinds of glycosides of pennogenin, kryptogenin and their related sapogenins were isolated from the fresh rhizomes of Trillium kamtschaticum Pall. (Liliaceae). Now, in addition, a new water-soluble steroid glycoside, named trillenoside (I), was obtained as a white powder (mp 209-220°(decomp.)), $[\alpha]_D$-116°, $C_{47}H_{70}O_{24}$.2) I was hydrolyzed with 1N H_2SO_4 in 50% EtOH to yield apiose,3) arabinose, rhamnose, xylose and the aglycone, named trillenogenin (II), colorless needles, mp 250-251°, $[\alpha]_D$-198°, $C_{26}H_{36}O_8$. II was acetylated to provide the pentaacetate (III), mp 243-245°, $[\alpha]_D$-142°. The spectral data of I, II and III are as follows. I-- IR cm^{-1}: 3700-3200 (OH), 1690 and 1625 (enone). UV nm(ε)2): 249(8600). CD [θ](nm)2):+2990 (325) (positive max.). PMR ($CDCl_3$-CD_3OD-D_2O + CF_3COOH)4): 0.94 (3H, d, J=6Hz, Me-CH<), 1.10 (3H, s, Me-•), 1.30 (3H, d, J=6Hz, 6-Me of rhamnose). II-- IR cm^{-1}: 3600-3100 (OH), 1695 and 1625 (enone). UV nm(ε): 248.5 (12800). CD [θ](nm): +4690 (326) (positive max.). Mass Spectrum m/e: 476 ($C_{26}H_{36}O_8^+$, M^+). PMR (pyridine): 1.01 (3H, d, J=6Hz, Me-CH<), 1.08 (3H, s, Me-•). III-- IR cm^{-1}: 1780-1740 (AcO), 1720 and 1645 (enone). PMR2): 1.94, 1.98, 2.00, 2.06 and 2.09 (AcO×5), 4.09 (1H, q, J=9, 11Hz), 4.43 (1H, q, J=5.5, 11Hz), 4.60, 4.71 (1H, q, J=4, 11.5Hz), 4.98 (1H, d, J=9.5Hz) and 5.13 (1H, t, J=9.5Hz) (AcO-CH< × 6).

II was treated with p-bromobenzenesulfonyl chloride and pyridine and the product was acetylated to give the tetraacetyl monobrosylate (IV), colorless plates, mp 242-244°(decomp.), $[\alpha]_D$ -112°, $C_{40}H_{47}SO_{14}Br$. PMR: 1.96, 2.00, 2.02 and 2.10 (AcO×4), 7.70-7.92 (arom.proton×4).
A single cystal of IV suitable for a X-ray diffraction study was obtained by recrystallization from MeOH. It is orthorhombic, space group $P2_12_12_1$ with unit cell dimensions a=25.10, b=18.61, c=8.98 Å, d(calcd.)=1.377 g/cm³ (for Z=4, mol.wt. 864), d(obsd.)=1.39 g/cm³ (in CCl_4-benzene). The intensities of all reflections with θ<40° were taken with Mo-Kα radiation on a Syntex P1̄ diffractometer (2θ-θ scans). The structure was solved by the heavy atom method using 1748

independent structure factors ($I_o \geq 2\sigma(I_o)$). The bromine and sulfur atoms were located by the three-dimensional Patterson syntheses and the subsequent Fourier syntheses, respectively, and further successive three-dimensional Fourier syntheses revealed all the non-hydrogen atoms. The parameters were refined by the block-diagonal least squares method to an R-factor of 0.092. A view of the molecule of IV is as given in Fig.1 or its mirror image.

Since the CD spectrum of II shows a positive Cotton effect due to enone grouping ($n \rightarrow \pi^*$), according to the Snatzke rule[5] on transoid cyclopentenones, the absolute configuration of II is considered to be as shown in the figure.

Therefore II is defined as 15-oxo-18-nor-25R-spirost-5,13-diene-1β,3β,21,23α,24β-pentaol.

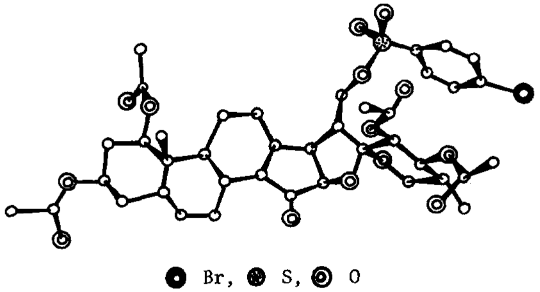
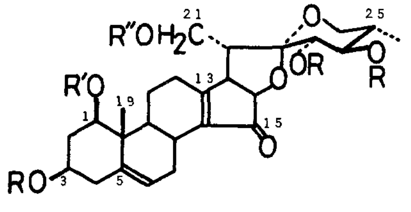


Fig.1. Molecular Structure of IV



II: R=R′=R″=H III: R=R′=R″=Ac

IV: R=R′=Ac, R″= Br-C_6H_4-SO_2-

VI: R=R″=Me. R′=H XIX: R=R″=Me, R′=Ac

I permethylate (V) prepared by the Kuhn method,[6] $[\alpha]_D$-102°, shows on the mass spectrum the molecular ion peak ($C_{60}H_{96}O_{24}^+$) at m/e 1200 indicating that I consists of one mole each of II, apiose, arabinose, rhamnose and xylose. V was methanolyzed to afford the aglycone (VI) and four kinds of methylated sugars, one of which was isolated by means of chromatography and identified with methyl glycoside of 2,3,5-tri-O-Me-D-apio-D-furanose (VII)[7,8] obtained from apiin[3] permethylate. The mixture of methylated sugars was hydrolyzed with acid and the resulting free sugars were separated over silica gel to give VII, 2,3,4-tri-O-Me-D-xylopyranose (VIII), 2,4-di-O-Me-L-rhamnopyranose and 4-O-Me-L-arabinopyranose (IX). When I was hydrolyzed with 0.2N HCl in MeOH a prosapogenin (X), $[\alpha]_D$-112°, was provided. The permethylate (XI) of X, $[\alpha]_D$-100°, was methanolyzed to give the methyl glycosides of 2,3,4-tri-O-Me-L-rhamnopyranose (XII), VIII and IX. Further partial hydrolysis of XI yielded a compound (XIII), $[\alpha]_D$-98°, of which permethylate (XIV) gave on methanolysis methyl glycosides of XII and 3,4-di-O-Me-L-arabinopyranose. Hydrolysis of X with 0.5N HCl in MeOH gave three prosapogenins, (XV), $[\alpha]_D$-118°, consisting of II, arabinose and rhamnose, (XVI), mp 223-226°, $[\alpha]_D$-133°, II arabinoside, and (XVII), $[\alpha]_D$-110°

consisting of II, arabinose and xylose. The molecular rotation differences (Table I), V - XI, X - XV, XV - XVI and XVI - II, and the signals assigned to the anomeric protons of component monosaccharides on the PMR spectrum (Fig.2) of I in comparison with those of XV, XVI and XVII indicate that the D-apiose, D-xylose, L-rhamnose and L-arabinose units have β, β(C1 form), α(1C form) and α(C1 form) configurations, respectively.

On the basis of all the above data the sugar moiety of I is considered to be the branched-chain tetrasaccharide (XVIII) shown below.

Table I. Molecular Rotation Differences

V	-1224°	-184°	Me 2,3,5-tri-O-Me-D-api•furanoside: α, +239°; β, -163°
XI	-1040°		
X	-992°	-102°	Me D-xyl•pyranoside: α, +253°; β, -108°
XV	-890°	-81°	Me L-rha•pyranoside: α, -111°; β, +170°
XVI	-809°	+133°	Me L-ara•pyranoside: α, +28°; β, +403°
II	-942°		

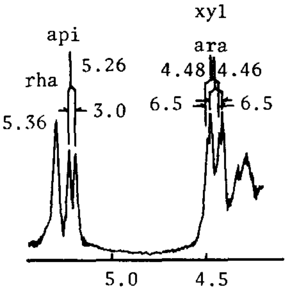
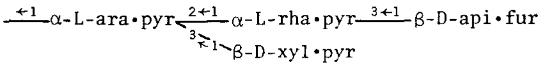


Fig.2. PMR Spectrum of I
($CDCl_3$-CD_3OD-D_2O + CF_3COOH)



$$\overset{\leftarrow 1}{\text{——}}\alpha\text{-L-ara}\cdot\text{pyr}\overset{2\leftarrow 1}{\text{——}}\alpha\text{-L-rha}\cdot\text{pyr}\overset{3\leftarrow 1}{\text{——}}\beta\text{-D-api}\cdot\text{fur}$$
$$\underset{3}{\searrow}\overset{}{\leftarrow}1\text{—}\beta\text{-D-xyl}\cdot\text{pyr}$$

XVIII

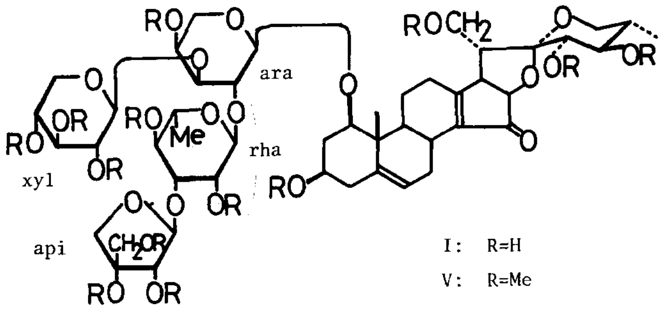
VI was acetylated to give a tetramethyl ether monoacetate (XIX) of II, mp 171-174°, $[\alpha]_D$ -210°. PMR: 2.10 (3H, s, AcO×1), 3.34, 3.37, 3.52 and 3.55 (each 3H, s, MeO×4), 4.65 (1H, q, J=4.5, 11.5Hz). The signal at 4.65 ppm is equivalent to the one-proton quartet (J=4, 11.5Hz) at 4.71 ppm ascribable to C_1-H on the spectrum of III.

Accordingly I is regarded to have the tetrasaccharide XVIII combined with the C_1-OH of II.

Because I has only four monosaccharide units, the possibility1) that I could be the 3,26-O-bisglycoside of the furostanol corresponding to II is ruled out. The IR, UV, CD and PMR spectra of I which show, similarly to those of II, the presence of an enone grouping and only one tertiary and one secondary (besides that of the rhamnose residue) methyl groups indicate II to be the genuine aglycone of I.

Consequently I is trillenogenin (II) 1-O-β-D-apio-D-furanosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside.

II is the first naturally occurring 18-norspirostane derivative, which has the additional structural peculiarities, 1) an enone groupingin the D-ring, 2) a hydroxyl group at C_{21} ,



I: R=H

V: R=Me

3) two hydroxyl groups in the F-ring. Apiose has been reported so far as a component monosaccharide of the flavone,[3] isoflavone,[9] anthraquinone[10] and triterpenoid glycosides.[8,11] I is worth of note as a novel type steroid glycoside in regard to the structures of the aglycone and the sugar moiety as well.

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