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THE STRUCTURE OF GYPSOSIDE - TRITERPENIC SAPONIN FROM GYPSOPHILA PACIFICA KOM.

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WE have recently isolated[1] a triterpenic saponin named gypsoside from Gypsophila pacifica Kom. roots. The saponin was found to be a gypsogenin(I) glycoside of a molecular weight corresponding approximately to nine monosaccharide units in the sugar moiety. The present communication contains data which can serve a basis for formulating gypsoside as II.

Acid hydrolysis of gypsoside(II) gave rise to gypsogenin lactone, the latter being interconvertible with gypsogenin and 12,13-dihydro-13α-hydroxygypsogenin; one mole of each galactose(Gal), glucose(Gl), arabinose(Ar), fucose(Fu), rhamnose(Rha), glucuronic acid(Glur) and ca. 3 moles of xylose (Xy) were liberated simultaneously.

Methylation by Kuhn's[2] procedure followed by 3-4 times methylation according to Purdie[3] afforded totally methylated II(III), $[\alpha]_D^{20}$ +47.5°(c 3.4 in $CHCl_3$). (Found: C, 58.21;

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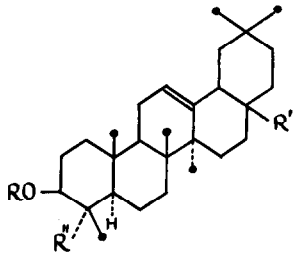
1 A.J.Khorlin, Ju.S.Ovodov and N.K.Kochetkov, Zhur.Obsch. Khim. (U.S.S.R.) 32,782 (1962).

2 R.Kuhn et al, Angew.Chem. 67,32 (1955); 72,805 (1960).

3 T.Purdie and J.C.Irvine, J.Chem.Soc. 83,1021 (1903).



I R'=COOH; R"=CHO; R=H

II R= D-Gal-1→4-D-Gl-1↘4 D-Glur-1-
L-Ar-1↗3

R'= D-Xy-1→3-D-Fu-1↘4 L-Rha-1-OCO-; R"=CHO
D-Xy-1→3-D-Xy-1↗2

IV R=D-Gal-1→4-D-Gl-1→4-D-Glur-1-
R'=COOH; R"=CHO

V R=D-Glur-1-; R'=D-Fu-1→4-L-Rha-1-OCO-; R"=CH_2OH

VI R=D-Glur-1-; R'= D-Fu-1↘4 L-Rha-1-OCO-; R"=CH_2OH
D-Xy-1↗2

VII R= D-Gal-1→4-D-Gl-1↘4 D-Gl-1-; R'=R"=CH_2OH
L-Ar-1↗3

All the sugar residues are in pyranose form.

H, 8.36; CH_3O, 33.75; Calc.for $C_{103}H_{172}O_{44}$: C, 58.28; H, 8.14; CH_3O, 33.75%). Methanolysis of III with 3% methanolic HCl or 72% $HClO_4$-CH_3OH (1:10) gave rise to two moles of 2,3,4-tri-O-methyl-D-xylose and one mole of each 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose, 2,3,6-tri-O-methyl-D-glucose, 2,4-di-O-methyl-D-xylose, 2,4-di-O-methyl-D-fucose, 3-O-methyl-L-rhamnose and methyl 2-O-methyl-D-glucuronate. These methylated monosaccharides were isolated from

the mixture by partition chromatography on a column with SiO_2 (benzene-acetone-water 10:10:1 or $CHCl_3 \rightarrow C_2H_5OH$-$CHCl_3$ 15:85 gradient elution) and after demethylation or total methylation identified with authentic samples of corresponding derivatives.

Partial hydrolysis of II with 10% aq.oxalic acid yielded lactose, $[\alpha]_D^{20}$+80 $\xrightarrow{24h}$ +52°(c 2.5 in H_2O), m.p.200°, and gypsogenin trioside(IV) purified by partition chromatography on a SiO_2 column (n-C_4H_9OH-C_2H_5OH-H_2O 4:1:1). (Found: C, 59.11; H, 8.08; Calc.for $C_{48}H_{74}O_{20}$: C, 59.38; H, 7.65%). Acid hydrolysis of IV afforded galactose,glucose,glucuronic acid; methylation of IV and subsequent methanolysis gave rise to 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-glucose, methyl 2,3-di-O-methyl-D-glucuronate and methyl ester of I. These data suggest structure IV for the trioside produced by partial hydrolysis of II.

Periodate oxydation of II in an aqueous solution,reduction of the product obtained with methanolic $NaBH_4$ and subsequent partial hydrolysis with 0.2N H_2SO_4 lead to a hederagenin trioside(V). (Found: C, 61.45; H, 8.22; Calc.for $C_{48}H_{84}O_{22}$: C, 61.28; H, 8.08%) and a hederagenin tetraoside(VI).Acid hydrolysis of V afforded rhamnose,fucose and glucuronic acid while methylation and subsequent methanolysis gave methyl 2,3,4-tri-O-methyl-D-glucuronate, 2,3,4-tri-O-methyl-D-fucose, 2,3-di-O-methyl-L-rhamnose. Hence,the residues D-Glur-pyr-1→ and D-Fu-pyr-1→4-L-Rha-pyr-1→ in trioside V are bound to different genin I positions.

Acid hydrolysis of the tetraoside VI afforded rhamnose, fucose,glucuronic acid and xylose; methanolysis of the

methylated VI, $[\alpha]_D^{20}$ 0±2°(c 2.0 in CH_3OH), (Found: C, 62.89; H, 8.79; Calc.for $C_{65}H_{108}O_{22}$: C, 62.90; H, 8.79%),lead to 2,3,4-tri-O-methyl-D-xylose, 2,3,4-tri-O-methyl-D-fucose, 3-O-methyl-L-rhamnose and methyl 2,3,4-tri-O-methyl-D-glucuronate. This leads to a conclusion that VI contains residues D-Glur-pyr-1→ and $\begin{matrix}\text{D-Xy-pyr-1}\searrow 2\\ \text{D-Fu-pyr-1}\nearrow 4\end{matrix}$ L-Rha-pyr-1→ bound to the genin I.

Reduction of III with $LiAlH_4$ in tetrahydrofuran under reflux (12h) afforded methylated tetraoside VII, $[\alpha]_D^{20}$ +28.2° (c 1.7 in $CHCl_3$). (Found: C, 62.40; H, 9.12; Calc.for $C_{64}H_{110}O_{22}$: C, 62.44; H, 9.00%). Methanolysis of VII produced 2,3,4,6-tetra-O-methyl-D-galactose, 2-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-arabinose thus suggesting structure VII for the tetraoside. Besides VII, a methylated oligosaccharide VIII, $[\alpha]_D^{20}$ -5°(c 2.2 in CH_3OH), is formed in the reduction of III with $LiAlH_4$. Methanolysis of VIII produced two moles of 2,3,4-tri-O-methyl-D-xylose and one mole of each 2,4-di-O-methyl-D-xylose, 2,4-di-O-methyl-D-fucose and 3-O-methyl-L-rhamnitol. These data together with the structures of sugar chains in V and VI allow to formulate VIII as

$$\begin{matrix}\text{D-Xy-pyr-1}\rightarrow 3\text{-D-Fu-pyr-1}\searrow 4\\ \text{D-Xy-pyr-1}\rightarrow 3\text{-D-Xy-pyr-1}\nearrow 2\end{matrix}\ \text{L-Rhamnitol.}$$

Hence,II contains two branched sugar chains. The C_3-position of the genin I is substituted by a grouping:

$$\begin{matrix}\text{D-Gal-pyr-1}\rightarrow 4\text{-D-Gl-pyr-1}\searrow 4\\ \text{L-Ar-pyr-1}\nearrow 3\end{matrix}\ \text{D-Glur-pyr-1}\rightarrow \quad \text{(IX)}$$

The position of the remaining sugar chain :

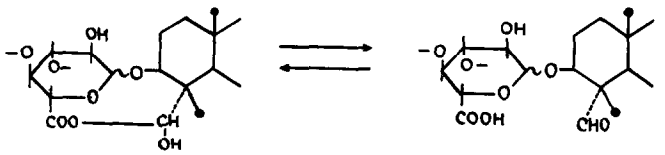
D-Xy-pyr-1→3-D-Fu-pyr-1 ↘4
D-Xy-pyr-1→3-D-Xy-pyr-1 ↗2 L-Rha-pyr-1→ (X)

was elucidated by following experiments.

On treatment with CH_2N_2 II gave a monomethyl ester. The ester readily produced II on hydrolysis with aq.$Ba(OH)_2$. Acid hydrolysis of the ester gave rise to gypsogenin lactone but not a methyl ester of I.

Hence, the unsubstituted carboxyl belongs to the glucuronic acid residue and the sugar chain X is bound to the genin (I) carboxyl (cf.[4]) and gypsoside may be formulated as II.

It has been shown earlier that gypsoside can be isolated in two readily interconvertible by the action of pH forms, differing from each other by the presence or absence of a free CHO-group (as indicated by the IR-spectrum). This ready interconversion may be satisfactory explained by a reversible formation of a hemiacylal grouping by the glucuronic acid carboxyl and the genin CHO-group:



As far as we know, gypsoside is the first example of a plant glycoside of a firmly established structure with a relatively large number of monosaccharide units in its carbohydrate chain. This type of compounds can be really named oligosides; it seems to be widely represented in

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[4] N.K.Kochetkov, A.J.Khorlin and V.E.Vaskovsky, Tetrahedron Letters 713 (1962).

Nature according to evidence obtained in our as well as in some other laboratories[5-7]. It may be noted now that the presence of a branched carbohydrate chain composed of a large number of different monosaccharide units seems to be characteristic of triterpenic oligosides. The other peculiarity of triterpenic acid oligosides is the presence of a stable acyl-glycoside bond connecting the sugar moiety to the sterically hindered genin carboxyl(cf.[4,8]).

It may seem interesting that gypsoside II is present in some other plants of the Gypsophila series.We have now proved II to be identical with G.paniculata triterpenic saponin.

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5 B.Lithgoe and S.Trippett, Nature 163,259 (1949).

6 Y.Lin, T.Lo and W.Tai, J.Chinese Chem.Soc. (Taiwan) Ser.II,157 (1954).

7 R.Ruyssen and J.Rowan, J.Pharm.Belg. 21,869 (1939).

8 J.Polonsky, E.Sach and E.Lederer, Bull.Soc.Chim.Fr. 880 (1959).