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SHORT COMMUNICATION

STRUCTURE OF A SAPONIN FROM LUCERNE (*MEDICAGO SATIVA*)*

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Abstract—The carbohydrate moiety of a highly toxic lucerne saponin was found to be a trisaccharide, which has been characterized as *O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl (1 → 3) β-D-glucopyranose by enzymatic hydrolysis, full and partial acid hydrolysis and gas chromatographic analysis of the methanolysis products of the methylated saponin. The results of enzymatic degradation of the saponin indicate a glycosidic bond between the carbohydrate moiety and the aglycone, analogy to other saponins and theoretical considerations point toward an attachment at C_3 of the aglycone.

INTRODUCTION

THE ISOLATION of a saponin from lucerne tops and roots that exerts a variety of physiological properties, such as haemolysis, larval and fungal growth inhibition has been reported.[1,2] It is composed of glucose and medicagenic acid (2β,3β-dihydroxy-Δ^{12}-oleanene-23,28-dioic acid[3]), and it was shown[1] that this saponin is the main factor to which the activities exerted by the mixture of saponins isolated from lucerne can be attributed. In view of the physiological significance of this saponin, the determination of its structure was undertaken and the results of this investigation are hereby reported.

RESULTS AND DISCUSSION

Determination of glucose: medicagenic acid ratios in the acid hydrolysate of the saponin were 1:1 by weight; as the molecular weight of the sapogenin is 502, this implies a 3:1 molar ratio between carbohydrate and aglycone moieties. When the saponin was hydrolysed mildly for 2 hr, TLC of the hydrolysate showed the presence of one main spot, with R_f 0·85, whereas the intact saponin has an R_f of 0·75. Glucose and medicagenic acid determinations in the eluted material gave a 1:1·5 ratio, by weight, indicating a 2:1 molar ratio between carbohydrate and aglycone moieties.

Gas chromatography of the methanolysis products from the methylated intact saponin showed components having the retention times of methyl glucosides of three differently methylated glucose residues (Table 1). Only two of these could be identified by gas chromatography of the methanolysis products of the methylated, partially degraded, saponin showing that as the result of mild hydrolysis the 1 → 6 linkage of the sugar moiety was opened.

Incubation of the intact saponin with β-glucosidase released, after 24 hr, free glucose and after 36 hr also free medicagenic acid, as indicated by chromatography; when yeast α-glucosidase was used, no degradation of the saponin could be observed. This finding,

* Part V in a series "Lucerne Saponins"; for Part IV see *J. Sci. Fd Agric.* (in press).

[1] B. GESTETNER, S. SHANY, Y. TENCER, Y. BIRK and A. BONDI, *J. Sci. Fd Agric* **21**, 502 (1970).
[2] S. SHANY, B. GESTETNER, Y. BIRK and A. BONDI, *J. Sci. Fd Agric.* **21**, 508 (1970).
[3] C. DJERASSI, D. B. THOMAS, A. L. LIVINGSTON and C. R. THOMPSON, *J. Am. Chem. Soc.* **79**, 5292 (1957).

TABLE 1. GAS CHROMATOGRAPHIC IDENTIFICATION OF METHYLATED METHYL GLYCOSIDES OF SUGARS FOUND IN AN INTACT AND PARTIALLY DEGRADED LUCERNE SAPONIN

Compound	Relative retention time* (T) Column a	Column b	Assignment
Authentic compounds			
Methyl 2,3,4,6 tetra-*O*-methylglucopyranoside	1·00, 1·46	1 00, 1 52	
Methyl 2,3,4 tri-*O*-methyl glucopyranoside	2·35, 3·35	2 80, 4·35	—
Methyl 2,4,6 tri-*O*-methyl glucopyranoside	2 95, 4·95	3·81, 5·96	—
Methanolysis products of methylated saponin			
Peaks 1, 2	1·00, 1·46	1·00, 1·52	2,3,4,6 tetra-*O*-methyl glucopyranoside
Peaks 3, 5	2·32, 3 34	2 82, 4·32	2,3,4 tri-*O*-methyl glucopyranoside
Peaks 4, 6	2·92, 5·00	3·78, 5 94	2,4,6 tri-*O*-methyl glucopyranoside
Methanolysis products of methylated partially degraded saponin			
Peaks 1, 2	1 00, 1 46	1·00, 1 52	2,3,4,6 tetra-*O*-methyl glucopyranoside
Peaks 3,4	2·90, 4·95	3·80, 5·97	2,4,6 tri-*O*-methyl glucopyranoside

* Relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (T = 1·00).

supported by the low specific rotation of the saponin $[\alpha]_D^{23}$ +10·2° ($c = 1$) provide proof that the glycosidic linkages of the saponin are of β-configuration. In view of these findings the following structure of the carbohydrate moiety of this lucerne saponin is proposed (Fig. 1).

With regard to the point of attachment of the carbohydrate residue to the aglycone, the ability of β-glucosidase to attack the saponin excludes the possibility of an ester linkage at C_{23} or C_{28}. As for the hydroxyls at C_2 and C_3, the greater reactivity toward substitution of an equatorial position (3β) is well known;[4] furthermore, in the case of medicagenic acid,

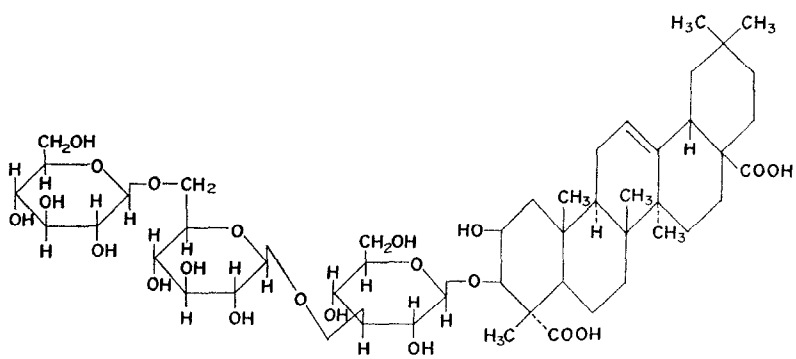

FIG. 1.

[4] E. ELIEL, *Stereochemistry of Carbon Compounds*, p. 222, McGraw-Hill, New York (1962).

the axial CH_3 groups at C_{23} and C_{25} would cause considerable steric interference to substitution by a bulky carbohydrate moiety at the axial 2β-hydroxy group. In view of these considerations and by analogy to a variety of other triterpenoid saponins,[5,6,7] it seems safe to assume that the attachment of the carbohydrate moiety is at C_3 of the aglycone.

EXPERIMENTAL

Isolation of Saponins

Saponin extracts were prepared either from commercially dehydrated lucerne tops meal, Hairy Peruvian variety, or from its roots by the method of Shany *et al.*[8] The saponin extracts were subjected to TLC on Kieselgel HR plates[2] and the area having R_f 0·75 was eluted with ethanol. Optical rotation was observed in 80% ethanol at 23°.

Hydrolysis and Determination of Composition of Lucerne Saponin

Full acid hydrolysis of the saponin was carried out in 1 N H_2SO_4 in dioxane–water (1:3) for 5 hr.[8] Mild acid hydrolysis was performed in 0·01 N H_2SO_4 in the above medium for different time intervals. Two-hr mild hydrolysis was found to be the most suitable for the purposes of this work. Glucose was identified by paper chromatography[8] and medicagenic acid by TLC.[2] Quantitative determination of glucose was carried out with the 3,5-dinitrosalicylic acid reagent[9] and that of medicagenic acid with a modified Lieberman–Burchard reagent.[10]

Methylation of the saponin. This was carried out by the procedure of Kuhn *et al.*[11] The course of methylation was followed by IR spectrometry. After two successive methylations, no absorption at 3200–3700 cm^{-1} could be observed. Methanolysis was carried out in *ca.* 4% (w/w) methanolic HCl, for 6 hr.

Gas chromatography. This was performed on 2 m × 0·32 cm columns of Chromosorb W, AW 60/80 coated either with (a) 5% by weight of neopentyl glycol adipate polyester (operating temperature 150°) or (b) 15% by weight of ethylene glycol adipate polyester (175°).[12] Retention times (T) are quoted relative to methyl, 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (Table 1).

Enzymic hydrolysis. Yeast α-glucosidase at pH 6·8, in phosphate buffer and almond β-glucosidase at pH 5·0, in acetate buffer were used. The enzymes were from Sigma Chemicals Co. 1 mg of the enzyme and 5 mg of the saponin were dissolved in 2 ml of the respective buffer (0·01 M), covered with toluene, and shaken in a water bath at 37° for 72 hr. Aliquots were withdrawn at different time intervals and analysed chromatographically[2,8] for liberated sugars and sapogenins.

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