STEROID SAPONINS

X. GLYCOSIDES OF Allium narcissiflorum

THE STRUCTURE OF GLYCOSIDES A AND B

V. V. Krokhmalyuk and P. K. Kintya

UDC 547.918+547.597

The presence of steroid glycosides in various species of the genus Allium has been reported previously [1]; for detailed study we have selected Allium narcissiflorum Wells. The present paper gives exhaustive information on the structure of two saponins and also the partial characterization of other saponins from this plant.

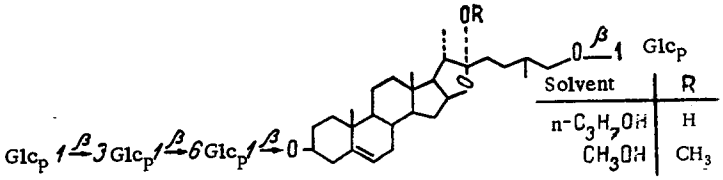
After the elimination of resinous impurities with ether, a methanolic extract was concentrated and separated from free sugars by elution with water through Sephadex G-25. The combined saponins were separated on a column of silica gel into five individual substances, which we have called in order of increasing polarity alliumosides A, B, C, D, and E. Glycoside A does not give the qualitative reaction with Ehrlich's reagent [2], and it therefore has a spirostan structure. The others do give a positive reaction and belong to the furostanol series. Furthermore, glycosides B, C, D, and E underwent enzymatic hydrolysis under the action of a complex of enzymes isolated from the snail Helix pomatia with the formation of the spirostan analogs [3]. The aglycone of all the saponins after hydrolysis was identified as diosgenin.

The quantitative determination of the monosaccharides obtained on hydrolysis was performed by a method that we have developed [4] (Table 1).

After methylation by Hakomori's method [5] followed by methanolysis saponin A gave only methyl 2,3,4,6-tetra-O-methyl-D-glucoside, i.e., it is a diosgenin monoside. In its physical properties and chromatographic mobility this monoside proved to be identical with an authentic sample of trillin.* According to its chromatographic mobility, glycoside B was more polar than dioscin* and gracillin, which gave us grounds for considering it to be at least a tetraoside of diosgenin. The methanolysis of permethylated alliumoside B gave methyl 2,3,4,6-tetra-O-methyl-D-glucoside, methyl 2,4,6-tri-O-methyl-D-glucoside, and methyl 2,3,4-tri-O-methyl-D-glucoside. The methyl glycosides were identified by means of thin-layer (TLC) and gas–liquid (GLC) chromatography in the presence of markers. Partial hydrolysis yielded trillin, a bioside, and a trioside.

Methanolysis of the permethylated bioside gave methyl 2,3,4,6-tetra-O-methyl-D-glucoside and methyl 2,3,4-tri-O-methyl-D-glucoside, which shows a 1–6 bond between the glucose molecules.

Methanolysis of a permethylate of the trioside gave the same methyl glycosides as for alliumoside B. The assignment of glycoside B to the furostanol series was also confirmed by its reduction with $NaBH_4$ followed by acid hydrolysis, which yielded dihydrodiosgenin. Spirostanols are not produced under such conditions [6]. When saponin B was subjected to periodate oxidation, the glucose was unaffected, which confirmed the methylation results. The configuration of the glycosidic centers are in harmony with Klyne's rule [7]. Thus, the structure of alliumoside B may be considered to have been demonstrated.



*The authors thank Professor Kowasaki (Japan) for kindly providing samples.

Institute of Chemistry, Academy of Sciences of the Moldavian SSR, Kishinev. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 55-58, January-February, 1976. Original article submitted November 25, 1974.

TABLE 1

Saponins	mp, °C	$[\alpha]_D^{18}$, deg	Monosaccharides, composition	Molar ratio	Mol. wt. calc.	Mol. wt. theor.
A	260–262	−104(*c* 0,5; CH_3OH)	Glc	1	565	576
B	270–272	− 70(*c* 0,7; CH_3OH)	Glc	4	1042	1062
C	290–291	− 66(*c* 0,6; CH_3OH)	Rha, Gal, Glc	3:1:2	1325	1338
D	268–269	− 88(*c* 0,85; CH_3OH	Rha, Glc	2:4	1334	1354
E	286–288	− 33(*c* 0,6; CH_3OH)	Rha, Glc	2:5	1497	1516

EXPERIMENTAL

Type FN-3 paper, type KSK silica gel, neutral alumina, and the following solvent systems were used: 1) butanol–ethanol–water (10:2:5); 2) chloroform–methanol–water (65:35:10); 3) butanol–benzene–pyridine–water (5:1:3:3); 4) chloroform–methanol (9:1); 5) benzene–ethanol (9:1); and 6) benzene–acetone (2:1). Gas–liquid chromatography was performed on a Khrom-4 instrument, FID, glass columns (200 × 0.35 cm) filled with 5% of XE-60 on Chromaton N-AW-HMDS; programmed temperature 180–225°C; V_{prog} = 3 deg/min; carrier gas helium, V_{He} = 45 ml/min.

Isolation of the Individual Saponins. A concentrated methanolic extract (100.0 g) was deposited on a column of Sephadex G-25 and eluted with water. The total saponins freed from sugars was chromatographed on a column of silica gel, elution being performed first with system 1. The enriched fraction consisting of saponins A and B was then separated in system 2 and the combined saponins B, C, D and E in system 1.

Hydrolysis of the Individual Saponins A and B. The saponin A or B (30.0 mg) was dissolved in 5% H_2SO_4 and the solution was heated in a sealed tube at 100°C for 20 h. After cooling, the hydrolyzate was diluted with water and filtered. The precipitate was dissolved in hot methanol. The crystals of aglycone that deposited, with mp 196–197°C, had an IR spectrum identical with that of an authentic sample of diosgenin. On TLC in system 4, the aglycone had the same R_f value as the authentic sample. The mixture of sugars was neutralized and, after evaporation, was analyzed by paper chromatography in system 3. The remaining part of the sugars was converted into the acetates of the aldononitriles as described previously [4] and was used for analysis by the GLC method. The amounts of sugars for each saponin are given in Table 1.

Methylation of Glycosides A and B. To 10 ml of freshly redistilled dimethyl sulfoxide was added 30 mg of NaH, and the mixture was heated at 45°C with stirring in a current of nitrogen. Then it was separated into two portions, to each portion was added 50 mg of saponin A or saponin B dissolved in the minimum amount of dimethyl sulfoxide, and heating was continued in a current of nitrogen for another 45 min, after which 5 ml of CH_3I was added to each reaction mixture. The completeness of methylation was checked by chromatography in a thin layer of silica gel in system 5. After purification on a column of silica gel, methylated saponin A with mp 151–152°C, $[\alpha]_D^{18}$ +0.8° (c 0.9; CH_3OH) and methylated saponin B with mp 147–149°C, $[\alpha]_D^{18}$ +0.5° (c 2.0; CH_3OH) were obtained. These permethylates were heated in sealed tubes with a solution of 72% $HClO_4$ in methanol (1:10) at 100°C for 5 h. After neutralization with an anion-exchange resin (HCO_3 form), the mixtures of methyl glycosides were chromatographed in system 6. Using the GLC method in the presence of markers, only methyl 2,3,4,6-tetra-O-methyl-D-glucoside was found in the case of glycoside A, and in the case of glycoside B methyl 2,4,6-tri-O-methyl-D-glucoside and methyl 2,3,4-tri-O-methyl-D-glucoside were found in addition (1.8:1:1).

Periodate Oxidation of Saponins A and B. In each case, 20 mg of the saponin was dissolved in 40 ml of aqueous methanol and 30 mg of $NaIO_4$ was added. After complete dissolution, the contents of the flask were left in the dark at room temperature for 48 h. Then two drops of ethylene glycol were added and after 1 h the mixture was extracted with butanol, the extract was evaporated, and the residue was subjected to hydrolysis with 5% H_2SO_4 at 100°C for 15 h. Glucose was detected in the hydrolyzate of glycoside B by paper chromatography.

In the case of glycoside A, 2 moles of $NaIO_4$ were consumed and in the case of glycoside B, 6 moles.

Partial Hydrolysis of Glycoside B. A mixture of 100 mg of saponin B and 1% H_2SO_4 was heated at 100°C for 40 min. Traces of diosgenin were detected in the hydrolyzate and trillin was isolated with mp 260–262°C. $[\alpha]_D^{18}$ −103° (c 0.5; dioxane). Literature information for trillin [8]: mp 261–263°C, $[\alpha]_D^{18}$ −105° (c 0.5; dioxane). Also isolated was a bioside with mp 230–232°C, $[\alpha]_D^{18}$ −86° (c 0.75; methanol). The methanolysis of a permethylate of the bioside yielded methyl 2,3,4,6-tetra-O-methyl-D-glucoside and methyl 2,3,4-tri-O-methyl-D-glucoside. A trioside was also obtained with mp 235–237°C, $[\alpha]_D^{18}$ −77° (c 0.9; methanol). Hydrolysis of a permethylate of the trioside gave the same methyl glycosides as alliumoside B but in a ratio of 1:1:1.

SUMMARY

From Allium narcissiflorum Wells have been isolated for the first time trillin and a glycoside B, which proved to be 3-O-[β-O-D-glucopyranosyl-(1 → 3)-O-β-D-glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 →]-26-O-[β-D-glucopyranosyl-(1 →]-25R-furost-5-ene-3β,22 α, 26-triol.

LITERATURE CITED

1. V. V. Krokhmalyuk, P. K. Kintya, and V. Ya. Chirva, Izv. Akad. Nauk MSSR No. 3, 92 (1974).
2. S. Klyosawa and M. Huton, Chem. Pharm. Bull., 16, 1162 (1968).
3. Y. Tomita and A. Uomori, Phytochem., 13, 729 (1974).
4. V.V. Krokhmalyuk, P. K. Kintya, and V. Ya. Chirva, Izv. Akad. Nauk MSSR, No. 1, 103 (1975).
5. S. Hakomori, J. Biochem., (Tokyo), 55, 205 (1964).
6. R. Tschesche, A. Har, and I. Petričic, Chem. Ber., 107, 53 (1974).
7. W. Klyne, J. Biochem. 47, xli (1950).

GLYCOSYLATION OF CARDENOLIDES

III. PARTIAL SYNTHESES OF CONVALLATOXIN, EVOMONOSIDE, AND PERIPLORHAMNOSIDE

N. Sh. Pal'yants, A. F. Bochkov, N. K. Abubakirov

UDC 547.918:547.926+615.711.5

Together with the various modifications of the synthesis of glycosides by the Koenigs–Knorr method, the orthoester method is the most widely used method of glycosylation [1, 2], enabling 1,2-trans-glycosides of various structures to be obtained stereospecifically and with high yields. In particular, it has been used for the glycosylation of cholesterol [3] and other steroid and triterpene aglycones [4].

There were certain misgivings concerning the suitability of the reaction with orthoesters for the glycosylation of cardenolides. The presence of tertiary hydroxy groups readily undergoing dehydration in the latter, and also the extremely labile lactone ring, required a particularly attentive approach to the choice of the reaction conditions.

Continuing work on the glycosylation of cardenolides [5], we have studied the reaction of 3,4-di-O-acetyl-1,2-O-(1-methoxyethylidene)-β-L-rhamnopyranose with cardenolide aglycones of various structures, namely strophanthidin, digitoxygenin, and periplogenin. Convallatoxin, evomonoside, and periplogenin α-L-rhamnoside were obtained in fairly good yields. All these compounds have been isolated previously from plant materials and have been thoroughly investigated. Partial syntheses of convallatoxin [6] and of evomonoside [7] by the Koenigs–Knorr method are known.

It is obvious that under the standard conditions that we use the side reactions that have been observed in the glycosylation of other steroids also take place; however, the fact that the orthoester method has proved suitable for the glycosylation of cardiac aglycones is in itself of considerable interest.

EXPERIMENTAL

For paper chromatography (PC) we used paper of the "medium" type and for thin-layer chromatography (TLC) silica gel of type KSK with 5% of gypsum. Nitromethane was purified by a method described previously [3]. The 3,4-di-O-acetyl-1,2-O-(1-methoxyethylidene)-β-L-rhamnopyranose was obtained under the conditions described by Mazurek and Perlin [8]. The UV spectra were taken on an SF-4 spectrophotometer and the IR spectra on a UR-20 instrument.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSSR, Tashkent. N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 58-60, January-February, 1976. Original article submitted December 4, 1974.