extract was evaporated to 25 ml and was deposited on a plate with a fixed layer of silica gel in 0.18-ml portions. Chromatography, elution, and quantitative determination were performed as described above.

SUMMARY

A spectrophotometric method for determining glycyrrhizic acid in a thick licorice extract, dry licorice powder, and licorice roots after its chromatographic separation from the accompanying substances in a thin layer of sorbent has been proposed. The limit of detection of glycyrrhizic acid is 8 μg/ml. The concentration of glycyrrhizic acid in the thick extracts is ∿15.49%, in the dry powder ∿12%, and in licorice roots ∿4.3%.

LITERATURE CITED

1. I. A. Murav'ev and V. D. Ponomarev, Farmatsiya, 4, 46 (1966).
2. P. Braeckman and C. Van Hulle, Pharmac. Tijdschr. Belg., 43, 137 (1966).
3. C. Nieman, Ned. Chem. Ind., 4, No. 12, 226; No. 15, 249 (1962).
4. H. Thieme and U. Hartmann, Pharmazie, 29, 50 (1974).
5. J. Killacky, M. S. Ross, and T. D. Turner, Plants Medica, 30, 4 (1976).
6. B. Pasich, B. Adamczewski, and J. Wisniwski, Farm. Pol., 22, 35 (1966).
7. E. Stabrowska-Ostrowska, Farm. Pol., 30, 941 (1974).
8. H. Sakagami and I. Morishita, J. Agr. Chem. Soc. Jpn., 46, 443 (1972).
9. G. Kurono and S. Sasaki, J. Pharm. Soc. Jpn., 90, 497 (1970).
10. L. Michele, Ind. Aliment., 8, 56 (1969).

TRITERPENE GLYCOSIDES OF *Acanthophyllum gypsophiloides*

V. D-QUINOVOSE IN ACANTHOPHYLLOSIDES B AND C

Zh. M. Putieva, L. G. Mzhel'skaya, T. T. Gorovits, E. S. Kondratenko, and N. K. Abubakirov

UDC 547.918:547.914.4

As reported previously [1], in a study of the monosaccharide composition of acanthophyllosides B and C by gas–liquid chromatography (GLC) in the form of the trimethylsilyl derivatives, among the peaks belonging to D-xylose, L-arabinose, L-rhamnose, D-fucose, D-galactose, D-glucose, and D-glucuronic acid we found the peak of an unknown compound. It was impossible to determine its qualitative nature in thin-layer and paper chromatograms (TLC and PC) in various systems. By a comparison of various samples of monosaccharides by the GLC method, the unknown sugar has now been identified as D-quinovose.

An investigation of the fractions of individual monosaccharides obtained by separating a hydrolyzate of acanthophyllosides B and C on a column of cellulose has shown that D-quinovose was present in the fractions usually containing L-rhamnose. The complexity of the separation of the monosaccharides is apparently due to the closeness of their R_f values. It is possible to separate D-quinovose distinctly in a mixture with other sugars only by chromatographing the trimethylsilyl derivatives of the methyl glycosides. Where the acetates of the aldononitriles are used in GLC, the peak of D-quinovose is superimposed on the peak of D-fucose [2].

The difficulty in the identification and the separation of D-quinovose and L-rhamnose by the usual methods in natural mixtures has been discussed repeatedly in the literature. Various methods have been proposed for isolating these methylpentoses of similar structure [3-5]. We have attempted to use for this purpose a borate buffer on paper, but we were unable by this method, either, to obtain D-quinovose preparatively in the individual state.

In order to determine the position of the D-quinovose in the carbohydrate chains of acanthophyllosides B and C it was most desirable to start from an analysis of the methylated sugars. From a mixture of the methyl ethers of the monosaccharides, the separation on a column of silica gel and preparative separation on plates we obtained two substances one of which

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

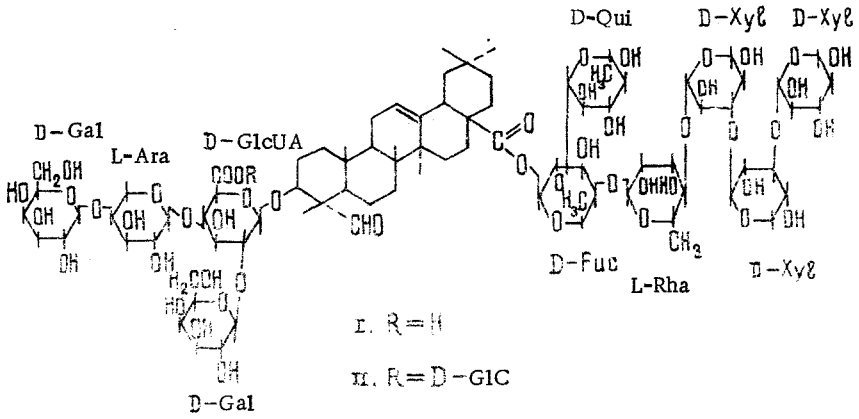
Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 806-809, November-December, 1977. Original article submitted July 1, 1977.

we previously [6] considered to be 2,3,4-tri-O-methyl-L-rhamnose and the other 2,3-di-O-methyl-L-rhamnose. After their demethylation by hydrobromic acid [7], the first product yielded a substance identical with D-quinovose and the second yielded L-rhamnose.

It must be observed that the full methyl ethers of D-quinovose and of L-rhamnose when deposited in the same spot are likewise not separated on PC and TLC in the systems that we used.

Thus, the carbohydrate chains of acanthophyllosides B and C contain D-quinovose in addition to the other sugars mentioned. The D-quinovose occupies the position of the terminal L-rhamnose in formulas given previously [6]. Its assignment to the D series follows on the basis of a determination of the optical rotation of 2,3,4-tri-O-methylquinovose.

Taking the new information into account, the refined formulas of acanthophyllosides B and C (I) and (II) are as follows:



Until now, D-quinovose was known in the vegetable kingdom as a carbohydrate component of triterpene glycosides only in two glycosides of cinchona bark [8]; however, this sugar is a common component of the saponins of marine echinodermata [9]. In the light of the new information, it becomes clear that D-quinovose is more widespread in nature than was previously considered. It is found particularly frequently in saponins of plant origin and, in particular, in oligosides of gypsogenin isolated from plants of the family Caryophyllaceae. Thus, by means of GLC D-quinovose has been detected in samples of saponoside D [10], trichoside D [11], gypsoside [12], and vacsegoside C [13] present in our collection.

EXPERIMENTAL

For chromatography we used type M (slow) paper, type KSK silica gel, cellulose powder obtained by treating absorbent cotton with hydrochloric acid, and the following solvent systems: 1) butanol—acetic acid—water (4:1:5); 2) butanol—ethanol—water (5:1:4); 3) butanol—pyridine—water (6:4:3); 4) butanol—methanol—water (5:3:1); 5) benzene—acetons (2:1); 6) chloroform—methanol (25:1); 7) toluene—ethanol (9:1).

The sugars were detected with an ethanolic solution of o-toluidine salicylate.

Gas—liquid chromatography was performed on a "Tsvet-4" chromatograph (flame-ionization detector) using for the TMS ethers a 2 m × 4 mm column containing 5% of G-30M silicone phase on Diaforit (0.2-0.315 μ), temperature 170°C, carrier gas helium, rate of flow of the helium 50 ml/min. For the aldononitrile acetates, the column contained 5% of neopentylglycol succinate on Chromaton and 5% of XE-60 on Chromaton.

Detection and Identification of D-Quinovose. The mixture of monosaccharides obtained after the sulfuric-acid hydrolysis of acanthophyllosides B and C [1] was analyzed in the form of the trimethylsilyl derivatives of the methyl glycosides by GLC. The D-quinovose was detected in the form of a doublet with relative retention times of 0.37 and 0.40. The addition of an authentic sample of D-quinovose to the mixture under investigation led to an increase in the intensity of the peak previously ascribed to an unknown sustance [1].

When a mixture of an authentic sample of D-quinovose and of the sugar from acanthophyl-

losides B and C in the form of the aldononitrile acetates was analyzed, the relative retention times of the two samples likewise coincided (0.36).

Separation of the Mixture of Monosaccharides. A mixture of monosaccharides (6.7 g) from acanthophyllosides B and C was separated on a column of cellulose (200 g) with elution by system 1. Fractions amounting to 50 ml were collected. In fractions 16-25, containing L-rhamnose according to TLC in system 4 and to PC in systems 1, 2, and 3, D-quinovose was also detected by the GLC method.

Separation of a Mixture of L-Rhamnose and D-Quinovose. A mixture of L-rhamnose and D-quinovose from the preceding experiment was separated preparatively on paper impregnated with borate buffer (38.12 g of $Na_2B_2O_7 \cdot 10H_2O$ + 8.08 g of NaOH in 2 liters of water) in the butanol—ethanol—borate buffer (5:1:4) system. The running time was 7 days. Separation was monitored by GLC. No individual sugars were obtained.

Separation of 2,3,4-Tri-O-methyl-D-quinovose and 2,3-Di-O-methyl-L-rhamnose. An aqueous solution (1.7 g in 20 ml of water) of a mixture of methylated monosaccharides [1] was extracted with chloroform (5 × 20 ml). The chloroform extracts were washed with water (3 × 5 ml), and evaporated to a syrupy consistency. The residue of permethylated monosaccharides so obtained was separated on plates (20 × 10 cm) with a fixed layer of silica gel (5 g) in system 5. The zones were detected by means of a heated nichrome wire and were cut out and extracted with chloroform. The chloroform was evaporated, to give 2,3,4-tri-O-methyl-D-quinovose (GLC) in the form of a syrup with $[\alpha]_D^{20}$ + 45.9° (c 1.45; water). Literature information: $[\alpha]_D^{20}$ + 49.2° (c 1.2; water) [3].

The aqueous residue containing the partially methylated sugars was evaporated to dryness (1 g) and separated on a column of silica gel (100 g) in system 6. Fractions with a volume of 10 ml were collected. The separation was monitored by TLC in systems 5, 6, and 7. Fractions 35-59 yielded 2,3-di-O-methyl-L-rhamnose.

Demethylation of 2,3,4-Tri-O-Methyl-D-quinovose. A small amount (10 mg) of syrupy 2,3,4-tri-O-methyl-D-quinovose was placed in a 2-ml tube, 0.5 ml of 45% HBr was added, and the solution was saturated with gaseous HBr at −10°C (0.5 g) until HBr vapors were evolved copiously from the tube [7]. The tube was then sealed and was left in the refrigerator at +5°C for 4 days. Then the contents of the tube were evaporated in vacuum, the residual HBr was neutralized with freshly prepared Ag_2CO_3, and the resulting precipitate of silver bromide was filtered off and washed with water. The filtrate and the wash-waters were concentrated, and then TLC in system 4 and PC is systems 1, 2, and 3 showed a spot at the level of D-quinovose and L-rhamnose. GLC unambiguously showed the presence of D-quinovose alone.

Further Methylation and Demethylation of 2,3-Di-O-methyl-L-rhamnose. 2,3-Di-O-L-rhamnose in the form of a syrup (50 mg) was methylated by Hakomori's method [14]. The reaction was monitored by TLC in system 5. The reaction mixture was worked up in the usual way. A substance was obtained which, according to TLC in systems 5 and 7, PC in system 2, and GLC corresponded to 2,3,4-tri-O-methyl-L-rhamnose.

The substance obtained in the preceding experiment (10 mg) was demethylated as described above. The presence of L-rhamnose in the reaction product was shown by GLC.

SUMMARY

It has been established that the carbohydrate moiety of acanthophyllosides B and C contain D-quinovose. Corrected structural formulas of acanthophyllosides B and C have been put forward. The hypothesis has been expressed that D-quinovose is present in a number of glycosides isolated from plants of the family Caryophyllaceae.

LITERATURE CITED

1. Zh. M. Putieva, L. G. Mzhel'skaya, T. T. Gorovits, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 177 (1975).
2. T. T. Gorovits and N. K. Abubakirov, Khim. Prirodn. Soedin., 241 (1975).
3. H. Ripperger and K. Schreiber, Chem. Ber., 101, 2450 (1968).
4. H. Kaufmann, P. Mühlardt, and T. Reichstein, Helv. Chim. Acta, 50, 2287 (1967).

5. S. Singh and B. E. Stacey, Analyst, 97, No. 1161, 977 (1972).
6. Zh. M. Putieva, M. G. Mzhel'skaya, T. T. Gorovits, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 728 (1975).
7. L. Hough and R. S. Theobald, in: Methods of Carbohydrate Chemistry, Vol. 2 (ed. by R. L. Whistler and M. L. Wolfrom), Academic Press, New York (1963).
8. R. Tschesche, J. Duphorn, and G. Shatzke, Ann. Chem., 667, 151 (1963).
9. I. Kitagawa and M. Kobayashi, Tetrahedron Lett., 859 (1977).
10. V. Ya. Chirva and P. K. Kintya, Khim. Prirodn. Soedin., 214 (1970).
11. V. N. Luchanskaya, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 69 (1972).
12. N. K. Kochetkov, A. Ya. Khorlin, and Yu. S. Ovodov, Zh. Obshch. Khim., 32, 782 (1962).
13. R. T. Baeva, M. O. Karryev, and N. K. Abubakirov, Khim. Prirodn. Soedin., 658 (1975).
14. S. Hakomori, J. Biochem. (Tokya), 55, 205 (1964).

STEROID GLYCOSIDES FROM *Asparagus officinalis* ASPARAGOSIDES F AND H

G. M. Goryanu and P. K. Kintya

UDC 547.917+547.918

Asparagosides A, B, C, D, E, and G have been isolated from garden asparagus and their structure has been demonstrated [1-3]. The present work was devoted to determining the structure of new steroid glycosides isolated from the hypogeal organs of this plant.

By column chromatography on silica gel we obtained asparagosides F (I) and H (II) in the individual state. Only compound (II) gave a positive reaction with Ehrlich's reagent [4], which shows that it belongs to the furostanol series. In the IR spectrum of asparagoside H there is an absorption band at 900 cm^{-1} which is typical for compounds of the furostanol series. Asparagoside H was subjected to reduction with $NaBH_4$ followed by hydrolysis, giving dihydrosarsasapogenin, as in the case of sarsaporaloside [5]. On plates coated with silica gel in methanol-containing systems substance (II) behaved in the same way as glycosides of the furostanol series [5] and gave two compounds, (IIa) and (IIb). The NMR spectrum contained the signal of a methoxy group at 3.15 ppm which is characteristic for a C_{22}-methyl ketal [6]. The enzymatic cleavage of asparagoside H with the complex enzyme from *Helix pomatia* led to asparagoside F. The acid hydrolysis of glycosides (I) and (II) gave an aglycone identical in its melting point, specific rotation, IR and mass spectra, and chromatographic mobility with sarsasapogenin. The gas–liquid chromatography of the acetates of the aldononitriles of the sugars of compounds (I) and (II) showed the presence in them of glucose and xylose in ratios of 3:1 and 4:1, respectively. To determine the positions of connection between the monosaccharides, asparagosides F and H were methylated by Kuhn's method [7], and the permethylated products obtained were subjected to methanolysis with perchloric acid in methanol. The methyl glycosides formed were chromatographed on a column of silica gel. Four individual substances (III-VI) were obtained. By GLC in the presence of markers, substance (III) was identified as methyl 2,3,4-tri-O-methyl-D-xyloside, and substance (IV) as methyl 2,3,4,6-tetra-O-methyl-D-glucoside. According to GLC and mass spectroscopy, substance (V) was methyl 2,3,6-tri-O-methyl-D-glucopyranoside, and (VI) methyl 2,6-di-O-methyl-D-glucopyranoside.

To determine the sequence of monosaccharide residues in the carbohydrate chains, glycosides (I) and (II) were hydrolyzed under mild conditions, as a result of which (I) yielded sarsasapogenin and four progenins (VII-X), and (II) yielded asparagoside F, in addition.

Only glucose was identified in hydrolyzates of (VII), (VIII), and (X), while in the case of (IX) glucose and xylose were found in a ratio of 2:1. When the methylated progenin (VII) was subjected to methanolysis, methyl 2,3,4,6-tetra-O-methyl-D-glucospyranoside was detected; in the case of permethylated (VIII), methyl 2,3,4,6-tetra-O-methyl-D-glucoside; in the case of (IX) — (III) and (V); and in the case of permethylated (X) — (IV) and (VI). Peracetylated asparagoside H was subjected to oxidative cleavage [5] followed by hydrolysis, which gave two

Kishinev State Medical Institute. Institute of Chemistry, Academy of Sciences of the Moldavian SSR, Kishinev. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 810-813, November-December, 1977. Original article submitted May 10, 1977.

 0009-3130/77/1306-0682$07.50