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STEROID GLYCOSIDES FROM *Asparagus officinalis* ASPARAGOSIDES F AND H

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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

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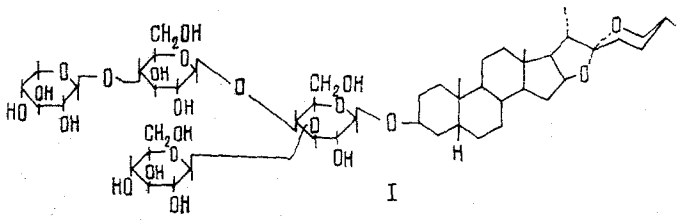
products (XI and XII). The acetylation and methylation with diazomethane of compound (XI) yielded the tetraacetylglycoside of methyl δ-hydroxy-γ-methylvalerate. The mass spectrum of this compound had peaks characteristic for tetraacetylglucose with m/e 331, 243, 242, 200, 169, 157, 145, 141, 140, 115, 109, 103, and 98, and also those of fragments with m/e 129 ($C_7H_{13}O_2$) and 97 (129 − MeOH) [5]. The acid hydrolysis of (XII) gave 3β-hydroxy-5β-pregn-16-en-20-one, which, after acetylation, was identified by IR and UV spectroscopy in the form of 3β-acetoxy-5β-pregn-16-en-20-one.

The configurations of the glycosidic centers of the monosaccharides were determined by the molecular rotation differences of the sapononins and their progenins, and agreed with those given by Klyne's rule [8].

On the basis of the results obtained, the following structures may be proposed for asparagosides F and H:



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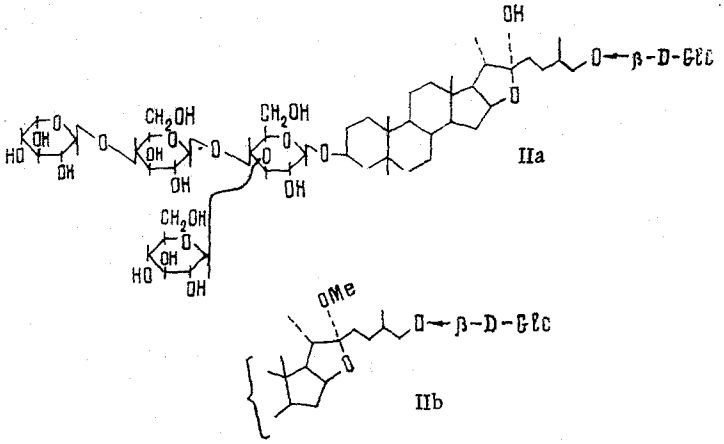

IIa

IIb

EXPERIMENTAL

Isolation of Saponins F and H from *Asparagus officinalis* L. The comminuted plant raw material (hypogeal organs) (500 g) was extracted with methanol in a Soxhlet apparatus for 48 h. After evaporation, an extract containing the total saponins was obtained which was purified on Sephadex G-25. The total saponins so obtained (20 g) were deposited on a column of silica gel and were eluted with the chloroform—methanol—water (65:30:10) system. In this way we isolated asparagoside F, $C_{50}H_{82}O_{22}$, with mp 271-273°C, $[\alpha]_D^{20}$ − 70° (c 1.5; MeOH), and saponin H with mp 146-150°C, $[\alpha]_D^{20}$ − 200° (c 0.6; H_2O).

Gas—Liquid Chromatography. Acetates of the aldononitriles and the methyl glycosides of the methylated sugars were identified on a "Chrom-4" instrument using a 2-m glass column filled with 5% of XE-60 [9] and 3% of XE-60 [10].

Hydrolysis of the Saponins. Asparagoside F or H (20 mg) was dissolved in 2 ml of 5% H_2SO_4 and the solution was heated at 100°C for 24 h. The aglycone that deposited was crystallized from ethanol. In both cases, sarsasapogenin was obtained with mp 198-199°C, $[\alpha]_D^{20}$ − 73° (c 0.9; $CHCl_3$); mass spectrum: m/e 416 (M^+). ν_{max}^{KBr}, cm^{-1}: 960, 912 > 892, 852 (25S spiroketal), 2300 (OH). Glucose and xylose were detected by paper and gas—liquid chromatography; for asparagoside F in a ratio of 3:1 and for asparagoside H in a ratio of 4:1 (GLC).

Methylation of Asparagosides F and H. Saponins F and H (500 mg in each case) were meth-

ylated by Kuhn's method [7]. This gave the permethylated glycoside F with mp 80-82°C, $[\alpha]_D^{20}$ − 64° (c 1.5; $CHCl_3$), and permethylated H, with mp 76-78°C, $[\alpha]_D^{20}$ − 21° (c 1.4; $CHCl_3$). These products were subjected to methanolysis with a mixture of 72% $HClO_4$ and methanol (1:10) at 100°C for 5 h. After neutralization with the anion-exchange resin Dowex 1X8 and evaporation, four compounds (III-VI) were found by TLC [acetone–benzene (1:2) system]. All the methyl glycosides were separated on a column of silica gel in the same solvent system. Compounds (III-V) were identified by TLC and GLC in the presence of markers (see above). Compounds (V) and (VI) were characterized as methyl 2,3,6-tri-O-methyl-D-glucopyranoside and methyl 2,6-di-O-methyl-D-glucopyranoside, respectively, by mass spectrometry: m/e 71, 73, 75, 88, 101, 161 [11]; 91, 94, 104, 114, 134, 137, 162, 165, 176, 182, 190, (90), 193 (10), 211, 225 [12].

Partial Hydrolysis. 500 mg of compound (I) or (II) was heated in 15 ml of 2% H_2SO_4 at 90°C for 2 h. The hydrolyzate was diluted with water and extracted with butanol (3 × 30 ml). The residue was chromatographed on a column of silica gel [chloroform–ethanol–water (65:25:10) system]. In this way, asparagoside F yielded sarsasapogenin, progenin VII, $C_{33}H_{54}O_8$, with mp 243-245°C, $[\alpha]_D^{20}$ − 62° (c 2.0; CH_3OH) [1, 5]; progenin (VIII), $C_{39}H_{64}O_{13}$, with mp 278-280°C, $[\alpha]_D^{20}$ − 220° (c 1.5; CH_3OH); progenin IX, $C_{44}H_{72}O_{17}$, with mp 256-258°C, $[\alpha]_D^{20}$ − 151° (c 1.3; CH_3OH); and progenin X, $C_{45}H_{74}O_{18}$, with mp 246-250°C, $[\alpha]_D^{20}$ − 166° (c 1.5; CH_3OH). Asparagoside H yielded compounds (VII)-(X) and asparagoside F.

Enzymatic Hydrolysis. A solution of 200 mg of saponin (II) in 50 ml of H_2O was treated with 5 mg of the total preparation from *Helix pomatia*. After the mixture had been kept at room temperature for 12 h, it was extracted with butanol (3 × 20 ml). The butanolic extract was evaporated and the residue was chromatographed on a column of silica gel. This gave 150 mg of asparagoside F, of which the melting point, specific rotation, and ratio of monosaccharides after acid hydrolysis coincided with the corresponding characteristics of an authentic sample of asparagoside F.

Reduction of Asparagoside H with $NaBH_4$. A solution of 200 mg of asparagoside H in water was treated with 10 mg of $NaBH_4$ and the mixture was left overnight at room temperature, after which it was neutralized and evaporated. The residue was subjected to acid hydrolysis, and the aglycone was extracted with chloroform and was identified by means of its R_f value in TLC with a marker as dihydrosarsasapogenin.

The oxidation with CrO_3 of the peracetylated asparagoside H was performed by the method of Tschesche et al. [5], and gave 3β-acetoxy-5β-pregn-16-en-20-one and the tetraacetyl glycoside of methyl δ-hydroxy-γ-methylvalerate.

SUMMARY

The structures of two new steroid glycosides isolated from garden asparagus have been shown.

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