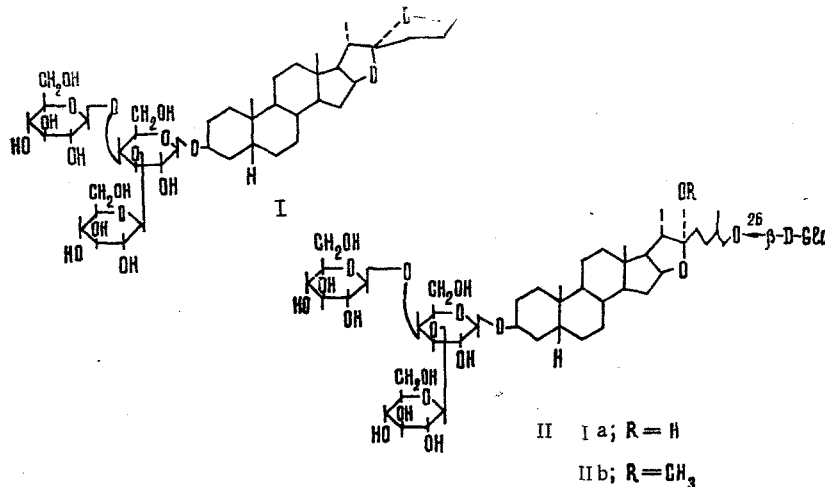


The structures of asparagosides A, B, C, and E have been shown previously [1, 2]. In the present paper we give the results of a chemical study and a proof of the structures of two new steroid glycosides — asparagosides D (I) and G (II). With Ehrlich's reagent [3], asparagosome G gives a positive reaction, while asparagosome D does not react. On silica gel plates in ethanolic systems, asparagosome G behaves like all glycosides of this furostanol series [4] and, consequently, is a mixture of 22-hydroxy- and 22-methoxyfurostanol glycosides (IIa and IIb). The IR spectrum of asparagosome G contains an absorption band in the $\sim 900\text{ cm}^{-1}$ region which is typical for compounds of the furostanol series, and its NMR spectrum shows the signal of a methoxy group at 3.15 ppm, which is characteristic for a C-22 methyl ketal [5] and is absent from the NMR spectrum of asparagosome D.

The enzymatic hydrolysis of asparagosome G with the enzyme complex from *Helix pomatia* gave asparagosome D. After acid hydrolysis, both glycosides formed an aglycone which, from its specific rotation, melting point, and IR and mass spectra, and also its chromatographic mobility on TLC in the presence of a marker was identical with sarsasapogenin. However, in view of the fact that asparagosome G belongs to the furostanol series, it can be stated that its aglycone is actually (25S)-5 β -furostan-3 β ,22 α ,26-triol. Thus, asparagosome G is the prototype of asparagosome D. In both cases, hydrolyzates were found by paper and gas-liquid chromatography to contain glucose. When asparagosides D and G were methylated by Kuhn's method [6] followed by methanolysis of the products obtained, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,6-di-O-methyl-D-glucopyranoside, identified by TLC and GLC in the presence of markers, were obtained. The latter compound was also identified mass-spectrometrically. Partial hydrolysis of asparagosome D led to sarsasapogenin and asparagosides A and C while asparagosome G gave asparagosome D, in addition. The presence of a molecule of glucose attached at the C₂₆ position of the aglycone in asparagosome G was shown by the oxidation of its peracetate with CrO₃ [4] with the formation of the tetraacetylgluco-



Kishinev State Medical Institute. Institute of Chemistry, Academy of Sciences of the Moldavian SSR, Kishinev. Translated from *Khimiya Prirodnykh Soedinenii*, No. 6, pp. 762-765, November-December, 1976. Original article submitted June 29, 1976.

side of methyl δ -hydroxy- γ -methylvalerate. The mass spectra of the latter had characteristic peaks with m/e 331, 243, 242, 200, 169, 157, 145, 141, 140, 109, 103, and 98 for tetra-acetylglucose and also fragments with m/e 129 ($C_7H_{13}O_2$) and 97.

The configurations of the glycosidic centers were determined by means of Klyne's rule.

On the basis of the results presented above, asparagosides G and D have the structures presented on the previous page.

EXPERIMENTAL

Chromatography was performed on FN-3 chromatographic paper and L 40/100 μ and L 5/100 μ silica gels. GLC was performed on a "Khrom-4" instrument (Czechoslovakia) with helium as the carrier gas in glass columns containing 5% of XE-60 on chromaton N-AW-HMDS (0.125-0.250 mm), V_{He} = 50 ml/min.

The following solvent systems were used: 1) chloroform-methanol-water (65:30:10), 2) chloroform-methanol (96:5), 3) benzene-acetone (2:1), 4) benzene-pyridine-butanol-water (1:3:5:3) and 5) benzene-ethanol (9:1).

The silica gel thin-layer chromatograms were revealed with concentrated sulfuric acid, and the paper chromatograms with aniline phthalate.

Preparation of Individual Glycosides. The combined glycosides were passed through Sephadex G-25 and were eluted with water. Then the pure mixture was chromatographed on a column of silica gel in solvent system 1. Separation was checked by TLC in the same system. The fractions containing the individual glycosides were evaporated to dryness, and the fractions containing several glycosides simultaneously were rechromatographed. This gave asparagoside D with mp 246-250°C, $[\alpha]_D^{20}$ -166° (c 0.43; MeOH) and asparagoside G with mp 170-174°C, $[\alpha]_D^{20}$ -200° (c 0.6; H_2O).

Acid Hydrolysis. Asparagosides D and G (50 mg each) in separate tubes were hydrolyzed with 2.5% H_2SO_4 at 110°C for 18 h and, after cooling, the reaction mixtures were diluted with water and filtered. The precipitates were recrystallized from methanol. Each glycoside yielded 20 mg of the genin - sarsasapogenin with mp 198-199°C, $[\alpha]_D^{20}$ -73° (c 0.9; $CHCl_3$); M^+ 416; IR spectrum $912 > 896\text{ cm}^{-1}$. In a thin layer of silica gel, the R_f values were the same as that of an authentic sample. The filtrates were passed through Dowex 1 \times 8 and evaporated. Glucose was identified by chromatography in system 4. Glucose was also detected in the form of the acetate of the aldonitrile by GLC.

Methylation of Asparagosides. Each glycoside (300 mg) was methylated by Kuhn's method, and the permethylated products obtained were purified on a column of silica gel with monitoring by TLC in system 5. This gave 320 mg of permethylated asparagoside D with mp 78-80°C, $[\alpha]_D^{20}$ -72° (c 1.0; $CHCl_3$), and 310 mg of permethylated asparagoside G with mp 84-86°C, $[\alpha]_D^{20}$ -50° (c 1.5; $CHCl_3$). The permethylated saponins were heated with 70% $HClO_4$ in methanol (1:10) for 5 h, and then the reaction mixtures were diluted with water and the precipitates that deposited were filtered off. The filtrates were neutralized and evaporated, and the methyl glycosides were separated on silica gel in system 3. In both cases methyl 2,3,4,6-tetra-O-methyl-D-glucoside and methyl 2,6-di-O-methyl-D-glucoside were obtained, being identified by TLC and GLC in the presence of authentic samples. Methyl 2,6-di-O-methyl-3,4-di-O- CD_3 -D-glucoside was subjected to mass-spectrometric analysis [8] after its preparation by Hakomori's method [9] from methyl 2,6-di-O-methyl-D-glucoside.

Partial Hydrolysis of (I) and (IIa + IIb). A sample of 200 mg of saponin D or G in 20 ml of 1% H_2SO_4 was heated in the water bath for 6 h, and then the mixture was diluted with water and extracted with butanol. The extract was evaporated and chromatographed on silica gel in system 1. In addition to traces of sarsasapogenin, asparagoside D gave a monoside with mp 243-245°C, $[\alpha]_D^{20}$ -62° (c 1.0; MeOH) and a bioside with mp 287-290°C, $[\alpha]_D^{20}$ -220° (c 1.5; MeOH), identical with asparagosides A and C, respectively. Asparagoside G gave sarsasapogenin and asparagosides A, C, and D.

Reduction of Asparagoside G with $NaBH_4$. A solution of 300 mg of (IIa + IIb) in 50 ml of H_2O was treated with 200 mg of $NaBH_4$, and the mixture was left overnight at room temperature. Then concentrated HCl to an acidity of 2 N was added and it was boiled for 5 h. After extraction with chloroform and evaporation, the product was chromatographed on a column of silica gel. Among other substances, 55 mg of dihydrosarsasapogenin [4] was obtained.

Enzymatic Cleavage of Asparagoside G. A solution of 300 mg of asparagoside G in 50 ml of H₂O was treated with 20 mg of the enzyme complex from *Helix pomatia* and was left at room temperature for 24 h. Then a small amount of methanol was added and the mixture was boiled for 30 min. The precipitate that deposited was filtered off, and the filtrate was extracted with butanol (3 × 30 ml). The butanolic extracts were evaporated and the residue was deposited on a column of silica gel and eluted with system 1. This gave 200 mg of asparagoside D.

Oxidative Cleavage of Asparagoside G. Peracetylated glycoside G (2.0 g) was oxidized with CrO₃ by Tschesche's method [4]. The oxidation products were extracted with chloroform and, after evaporation of the solvent, were hydrolyzed by tert-butanol containing KOH. After dilution with water, the mixture was extracted with butanol, and aqueous and butanolic phases were obtained. The aqueous phase was acidified to pH 3 and extracted with butanol, and after evaporation the product was subjected to acetylation and permethylation with diazomethane. The mass spectrum of the compound obtained showed m/e 331, 243, 242, 200, 169, 157, 145, 141, 140, 115, 109, 103, 98 (completely acetylated glucose) and also m/e 129, 97, 89, 81 for the acid residue. The butanolic phase was washed several times with water and evaporated to dryness. The residue was subjected to acid hydrolysis with 4 N HCl at 80°C for 3 h. The pregnenolone obtained was acetylated and was then chromatographed on a column of silica gel. 3 β -Acetoxy-5 β -pregn-16-en-20-one [10] was isolated.

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