acetyl-3,4-di-O-methyl-α-xylopyranoside, and methyl 3-O-acetyl-2,4-di-O-methyl-α-xylopyranoside.

## CONCLUSION

1. The complete structure of a new triterpene hexaoside — stichoposide D — from the holothurian *Stichopus chloronotus* has been established. It is 23(S)-acetoxy-3β-{4'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-β-D-glucopyranosyl]-2'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl]-β-D-xylopyranosoloxy}holost-7-ene.

2. A hypothesis of the biosynthesis of the carbohydrate chains of the glycoside holothurians from bioside blocks has been performed.

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# TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS.

# IV. CYCLOSIEVERSIOSIDE E — A NEW DIGLYCOSIDE FROM *Astragalus sieversianus*


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A new glycoside of the cycloartane series has been isolated from the roots of the plant *Astragalus sieversianus* Pal.; it is cyclosieversigenin 3,6-di-O-β-xyloside.


We have previously [1] reported the isolation from the roots of the *Astragalus sieversianus* Pal. of cyclosieversigenin (III) — an isoprenoid of the cycloartane series. Continuing a study of the methylsteroids of this plant, we have isolated from a methanolic extract of the roots eight substances having a glycosidic nature. In order of increasing polarity they have been called compounds A, B, C, D, E, F, G, and H. In this paper we consider the determination of the structure of compound E, which we have called cyclosieversioside E.

It was shown with the aid of GLC [2] that cyclosieversioside E (I) contains two D-xylose residues. The presence in the PMR spectrum of glycoside (I) of a one-proton signal in the


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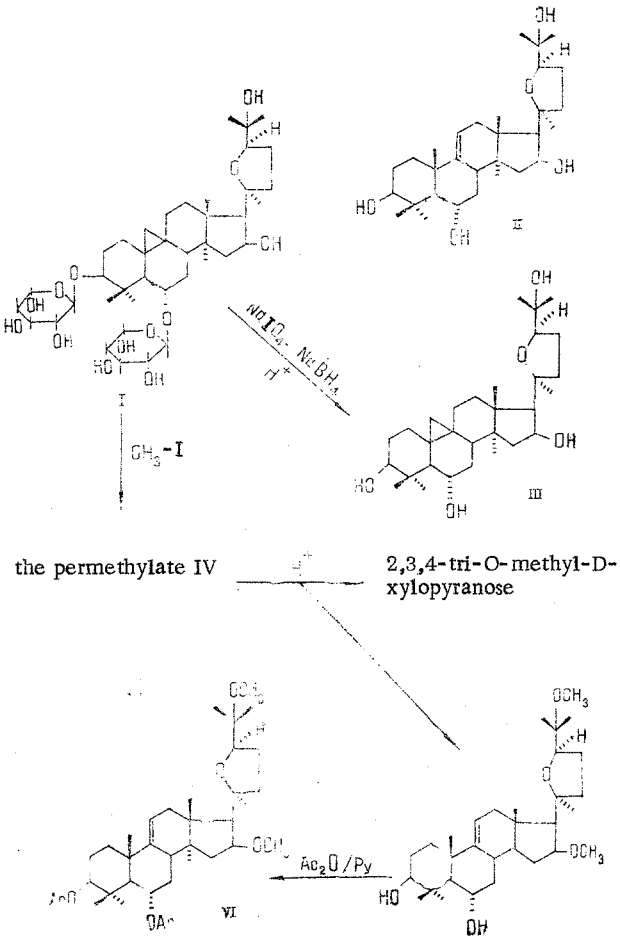
strong field at 0.46 ppm showing the presence of a cyclopropane ring permitted the assumption that the compound belonged to the cycloartane series. It is known [3] that under the action of acids substances of this type isomerize into derivatives of 9(11)-lanostene. In actual fact, in preparative experiments on the hydrolysis of bioside (I) with 7% sulfuric acid, cyclosieversigenin (III) and the product of its transformation that we have described previously [1], sieversigenin – 20(S),24(R)-epoxylanost-9(11)-ene-3β,6α,16β,25-tetraol (II) – were detected on TLC. Consequently, to obtain the native genin we subjected the diglycoside (I) to Smith degradation [4]. Cyclosieversigenin (III) was identified as the genin in the reaction products.

The methylation of cyclosieversioside E (I) by Hakomori's method [5] gave the permethylate (IV) ($M^+$ 866). The hydrolysis of compound (IV) with 7% sulfuric acid gave the dimethyl ether (V). 2,3,4-Tri-O-methyl-D-xylopyranose was identified as the carbohydrate component. The formation of these substances shows that cyclosieversioside E contains two terminal D-xylose residues and, consequently, is a bisdesmosidic glycoside.

The PMR spectrum of compound (V) shows at 5.21 ppm the signal of an olefinic proton at C-11. As was to be expected, resonance lines of the protons of a 9,19-cyclopropane ring are absent. Thus, when the permethylate (IV) was hydrolyzed the 9,19-ring opened with the formation of a 9(11)-double bond. Consequently, substance (V) must be considered to be a derivative of sieversigenin (II).




In the PMR spectrum of sieversigenin (II) [1], protons geminal to the hydroxy groups at C-3 and C-6 resonate at 3.40 and 4.34 ppm. Attention is attracted to the fact that in the spectrum of the dimethyl ether (V) one-proton multiplets are also observed in the spectrum of the dimethyl ether (V) at 3.44 and 4.31 ppm. The good agreement of these values permits the assumption that in compound (V) the hydroxy groups at C-3 and C-6 have remained free and, consequently, it is the 16,25-dimethyl ether of sieversigenin.

This assumption was confirmed by the acetylation of the ether (V). This gave the diacetate (VI), in the PMR spectrum of which the signals under consideration are shifted down-

field and appear at 4.54 and 5.47 ppm. The indices given agree well with the values of the chemical shifts of the protons at C-3 and C-6 (4.65 and 5.48 ppm, respectively) and the PMR spectrum of sieversigenin 3,6-diacetate [1].

Thus, compound (VI) is the 3,6-diacetate 16,25-dimethyl ether of 20(S),24(R)-epoxylanost-9(11)-en-3β,6α,16β,25-tetraol.

The experimental results given show that in cyclosieversioside E the two D-xylose residues must be attached to cyclosieversigenin through the hydroxy groups at C-3 and C-6.

In the PMR spectrum of the permethylate (V), the anomeric protons resonate at 4.42 and 4.56 ppm in the form of two partially superposed doublets with a spin-spin coupling constant of J = 7 Hz, which shows the β configurations of the glycosidic centers [6]. Consequently, cyclosieversioside E is the 3,6-di-O-β-D-xyloside of cyclosieversigenin.

## EXPERIMENTAL

For general observations, see [1]. PMR spectra were taken in $C_5D_5N$ on a JNM-4H-100/100 MHz instrument with HMDS as internal standard, δ scale. GLC was performed on a Chrome-5 chromatograph. Sugars were chromatographed in the form of the trimethylsilyl ethers of the methyl glycosides [2] on a column (3.7 m × 3 mm) containing Chromaton N-AW impregnated with 5% of the silicone phase SE-30. The thermostat temperature was 190°C and the carrier gas, here and below, was helium, at the rate of 45 ml/min. The methyl ethers of the sugars were identified in the form of their methyl glycosides [7]. They were chromatographed on a column (1.2 m × 3 mm) containing Celite impregnated with 20% of poly(butane-1,4-diyl succinate) at a thermostat temperature of 180°C, and on a column (1.2 m × 3 mm) containing Chromaton NAW impregnated with 10% of poly(phenyl ether) 5 F-4E at a thermostat temperature of 190°C.

Isolation of the Glycosides. The air-dry comminuted roots of *Astragalus sieversianus* Pal. (5 kg) collected in September, 1977 in the foothills of Mt. Chimgan (Chatkal'skii range, Western Tien Shan) were exhaustively extracted with methanol at room temperature. The extract was evaporated to two liters, diluted with 1.5 liters of water, and shaken with butanol. The solvent was evaporated off from the butanol solution to give 210 g of combined extractive compounds.

Part of the dry residue (60 g) was chromatographed on a column of silica gel using chloroform–ethanol (99:1–90:10) systems. The fractions obtained were rechromatographed on silica gel [chloroform–methanol–water (70:23:4)] to give the following compounds: A, 2.0 g (0.14%; yield here and below calculated on the air-dry raw material); B, 50 mg (0.0035%); C, 100 mg (0.007%); D, 85 mg (0.0059%); E, 3.5 g (0.24%); F, 4.5 g (0.31%); G, 1.3 g (0.09%); H, 700 mg (0.05%).

Cyclosieversioside E (I, Substance E). $C_{40}H_{66}O_{13}$, mp 218–220°C (from methanol), $[\alpha]_D^{20}$ +29.9 ± 2° (c 0.67; ethanol). $\nu_{max}^{KBr}$ ($cm^{-1}$): 3200–3600 (OH). PMR spectrum (δ, ppm): 0.46 (H at C-19, broadened singlet); 0.95 ($CH_3$, s); 1.15 ($CH_3$ × 3, s); 1.25 ($CH_3$, s); 1.45 ($CH_3$, s); 1.71 ($CH_3$, s). Two molecules of D-xylose were detected in a hydrolysate of cyclosieversioside E by the GLC method [2] with the addition of D-glucose as internal standard.

Cyclosieversigenin (III) from (I). A solution of 500 mg of cyclosieversioside E (I) in 100 ml of aqueous methanol (1:1) was treated with 2.1 g of sodium periodate, and the reaction mixture was stirred at room temperature for 6 h. The unconsumed oxidant was decomposed with ethylene glycol. The residue remaining after the methanol had been evaporated off was treated with 50 ml of water, and the mixture was extracted with chloroform. The chloroform was distilled off, and 30 ml of methanol and 1.5 g of sodium tetrahydroborate were added to the residue. The reaction mixture was heated at 80°C for 7 h, after which it was acidified to pH 2.0 and was left at room temperature for 17 h. The hydrolysis products were extracted with chloroform, the solvent was evaporated off, and the residue was chromatographed on a column of silica gel. Elution was carried out with ethyl acetate. In this way, 200 mg of cyclosieversigenin (III) was isolated with mp 239–242°C (from methanol), $[\alpha]_D^{20}$ +50.0 ± 2° (c 1.55; methanol), identical with an authentic sample [1] both from its IR characteristics and according to its mobility on TLC (ethyl acetate).

Cyclosieversioside E Permethylate (IV) from (I). A solution of 1.5 g of cyclosieversioside E (I) in 300 ml of dry dimethyl sulfoxide was treated with 1.5 g of sodium hydride. After 30 min, 18 ml of methyl iodide was added dropwise and the reaction mixture was left for 5 h. All the operations were performed at room temperature with stirring. The reaction products were poured into 400 ml of aqueous sodium hyposulfite solution and exhaustively

extracted with chloroform. The residue obtained after the evaporation of the combined chloroform extracts was chromatographed on a column of silica gel with elution first by benzene and then by a mixture of benzene and ethyl acetate (1:1).

This led to the isolation of 600 mg of the permethylate (IV), $C_{48}H_{32}O_{13}$, mp 172-174°C from methanol), $[\alpha]_D^{20}$ +30.8 ± 2° (c 1.09; chloroform). $\nu_{max}^{Nujol}$ ($cm^{-1}$): 3040 (>$CH_2$ of a cyclopropane ring), no absorption in the region of the hydroxy groups. PMR spectrum (δ, ppm): 0.47 (H at C-19, d, J = 4.5 Hz); 0.98 ($CH_3$, s); 1.10 ($CH_3$ × 2, s); 1.16 ($CH_3$, s); 1.26 ($CH_3$, s); 1.32 ($CH_3$, s); 1.44 ($CH_3$, s); 2.97-3.59 ($OCH_3$ × 8, s), 4.42 and 4.56 (2 H, anomeric protons, d, J ≈ 7.5 Hz); $M^+$ 866.

Acid Hydrolysis of the Permethylate (IV). A solution of 600 mg of the permethylate (IV) in 100 ml of methanol was treated with 100 ml of 15% methanolic sulfuric acid, and the reaction mixture was heated in the boiling water bath for 7 h. After cooling, 20 ml of water was added, the methanol was distilled off, the precipitate that had deposited was separated off, and the aqueous solution was extracted with a small amount of chloroform. The residue obtained by the distillation of the chloroform was combined with the precipitate that had been separated off previously and the material was chromatographed on a column of silica gel with elution by benzene—acetone (3:1). This led to the isolation of 46 mg of the 16,25-dimethyl ether of sieversigenin (V), $C_{32}H_{54}O_5$, mp 221-223°C (from methanol): $[\alpha]_D^{20}$ +125.7 ± 2° (c 0.61; chloroform); $\nu_{max}^{KBr}$ ($cm^{-1}$): 3440-3480 (OH). PMR spectrum (δ, ppm): 0.69 ($CH_3$, s); 0.94 ($CH_3$, s); 1.08 ($CH_3$, s); 1.11 ($CH_3$, s); 1.16 ($CH_3$, s); 1.29 ($CH_3$, s); 1.42 ($CH_3$, s); 1.83 ($CH_3$, s); 3.00 and 3.15 ($OCH_3$ ± 2, s); 3.44 (H at C-2, m, $W_{1/2}$ ≈ 20 Hz); 5.21 (H at C-11, broadened singlet). Mass spectrum, m/z (%): $M^+$ 518(20), 503(8), 500(4), 486(26), 445(26), 427(33), 413(46), 395(66), 377(26), 359(13), 157(100).

The aqueous solution was boiled for 7 h, after which it was cooled and was neutralized with barium carbonate, the precipitate was filtered off, and the filtrate was evaporated. The residue was found by TLC in the benzene—acetone (2:1) system, and also by GLC [7] in the presence of a marker, to contain 2,3,4-tri-O-methyl-D-xylopyranose.

3β,6α-Diacetoxy-16β,25-dimethoxy-20(S),24(R)-epoxyanost-9(11)-ene (VI) from (V). The dimethyl ether (V) (70 mg) was acetylated with 3 ml of acetic anhydride in 4 ml of pyridine at room temperature for 4 h. The reaction products were poured into ice water, and the precipitate that deposited was filtered off. This gave 30 mg of the diacetate (VI), $C_{36}H_{58}O_7$, mp 190-192°C (from methanol): $[\alpha]_D^{20}$ +174.9 ± 2° (c 0.30; chloroform; $\nu_{max}^{KBr}$ ($cm^{-1}$): 1735, 1255 (ester group). There was no absorption in the region of hydroxy groups. PMR spectrum (δ, ppm): 0.62 ($CH_3$, s); 0.85 ($CH_3$, s); 0.96 ($CH_3$, s); 1.00 ($CH_3$, s); 1.05 ($CH_3$, c); 1.09 ($CH_3$, s); 1.14 ($CH_3$, s); 1.25 ($CH_3$, s); 1.93 and 1.99 (OAc × 2, s); 2.97 and 3.13 ($OCH_3$ × 2, s); 3.73 (2 H at C-24 and C-16, m); 4.54 (H at C-3 m, $W_{1/2}$ ≈ 17 Hz); 5.10 (H at C-11, broadened singlet, 5.47 (H at C-6, m, $W_{1/2}$ ≈ 20 Hz. Mass spectrum, m/z (%): $M^+$ 602(13), 587(5), 570(7), 555(8), 529(7), 510(8), 469(100), 437 (33), 377 (53), 157(60).

## CONCLUSION

A new glycoside of the cycloartane series has been isolated from the roots of the plant *Astragalus sieversianus* Pal. and has proved to be cyclosieversigenin 3,6-di-O-β-D-xylopyranoside.

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