PREGNANE GLYCOSIDES OF Cynanchum sibiricum

III. THE STRUCTURE OF SIBIRICOSIDES D AND E

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We have previously [1] reported the isolation from a chloroform extract of the roots of Cynanchum sibiricum Willd. (family Asclepiadaceae) of five substances giving a positive xanthydrol reaction for 2-deoxysugars. These glycosides were named, in order of increasing polarity, sibiricosides A, B, C, D, and E. The present paper gives information on the structure of the most polar glycosides – sibiricosides D and E.

Sibiricoside D (II) is the main glycoside of the plant quantitatively. The strong maximum at 220 nm (log ε 3.85, ethanol) present in the UV spectrum of the compound can be assigned to an α,β-unsaturated carbonyl group.

Under the action of the pancreatic juice of the snail H. plectotropis the glycoside (II) readily hydrolyzes with the formation of desglucosibiricoside D (III) and D-glucose (IX). The presence of only one molecule of glucose in sibiricoside D was also confirmed by the gas–liquid chromatography of the silyl derivatives of the O-methyl glucosides. Judging from the difference in molecular rotations between sibiricoside D (II) and desglucosibiricoside D (III), it may be assumed that the D-glucose in the glycoside is connected by a β-glycosidic bond.

The mild acid hydrolysis of desglucosibiricoside D (III) with 0.05 N sulfuric acid led to the formation of a mixture of two aglycones which were identified as cynanchogenin (VII, ~45%) and sibirigenin (VIII, ~55%). The latter, as is known [2], is the 17α-H isomer of cynanchogenin [3]. In addition to the aglycones, the aqueous part of the hydrolyzate was found to contain D-cymarose (X) and D-oleandrose (XI). After their chromatographic separation, it was established with the aid of the xanthydrol reagent that these sugars were present in a ratio of 3 : 1 [4]. The presence in the molecule of sibiricoside D of four methylated sugars was also confirmed by an analysis of the glycoside for methoxy groups.

The positions of attachment of the sugars to the glycoside were determined by stepwise hydrolysis. When desglucosibiricoside D (III) was treated with 0.001 N sulfuric acid at 36°C, D-cymarose was first detected and only after a long time did D-oleandrose appear. This fact shows that the D-oleandrose is attached directly to the aglycone and a chain of three molecules of D-cymarose is attached to the oleandrose. Consequently, sibiricoside D is a pentoside of cynanchogenin or sibirigenin.

The formation of two aglycones on acid hydrolysis still does not show that we had isolated a mixture of glycosides, since as was confirmed by a special experiment, under the conditions of hydrolysis sibirigenin (VIII) readily isomerizes into cynanchogenin (VII). The predominance of sibirigenin in the hydrolysis products and its low stability with respect to isomerizing agents is, apparently, evidence in favor of the assumption that the 17α-H isomer is the native form.

It would appear to be easy to determine whether a glycoside belongs to the 17α-H or the 17β-H series: When the optical rotatory dispersion (ORD) curve of sibiricogenin was recorded, it showed a positive Cotton effect [2], while that of cynanchogenin showed a negative Cotton effect [5]. However, the ORD curve of sibiricoside D was indistinct, possibly because of the strong chromophoric effect caused by the α,β-unsaturated carbonyl group of the ikemoric (3,4-dimethylpent-2-enoic) acid.

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When sibiricoside D (II) was saponified with caustic soda in methanol in a current of nitrogen, compound (IV) was obtained which no longer showed in the UV spectrum the absorption at 220 nm due to the α,β-unsaturated carbonyl group of an acid. On saponification, the sugar moiety was retained, as was confirmed by the products of acid hydrolysis and by an analysis for methoxy groups.

The ORD curve of the deacylsibiricoside (IV) was fairly distinct and showed a negative Cotton effect. From this it should be concluded that sibiricoside D is a glycoside of cynanchogenin. But in this case the ORD curve does not have decisive value, since it is known that under the conditions of alkaline saponification the isomerization of the 17α-H into the 17β-H form also takes place [2], and in the isomeric pairs sibirigenin (VIII)–cynanchogenin (VII) and solineolone(VI) – lineolone (V) the second of these forms is the more stable.

The size of the oxide rings and the positions of the mutual attachment of the component sugars of sibiricoside D have not been demonstrated strictly, but if one assumes the pyranose form, as in pachybiose, asclepobiose and other sugars found in plants of the family Asclepiadaceae [6], only positions C_1 and C_4 remain free for the bonds between the monosaccharides. Consequently, structure (II) can be proposed for sibiricoside D.

Sibiricoside E (I) has properties very close to those of sibiricoside D. In the enzymatic cleavage of the glycoside by the pancreatic juice of the snail H. plectotropis after only a few minutes, even at room temperature, sibiricoside D (II) and D-glucose (IX) were detected. Subsequent fermentation at 36°C led to the formation of desglucosibiricoside D (III). Consequently, sibiricoside E contains two residues of D-glucose molecules and has the structure corresponding to formula (I).

EXPERIMENTAL METHOD

For thin-layer chromatography we used type KSK silica gel with 5% of gypsum. The identification and the checking of the purity of the pregnane glycosides and aglycones were performed in a thin layer by the method of wedge-shaped bands [7] in the following systems: 1) benzene–chloroform–methanol (5 : 5 : 2); 2) benzene–acetone–butan-1-ol (2 : 2 : 4); 3) methylene chloride–methanol–formamide (80 : 19 : 1); 4) chloroform–isopropanol (4 : 1); and 5) ethyl acetate–n-hexane (1 : 1). The sugars were identified in the following systems: 6) butan-1-ol saturated with water; 7) toluene–butan-1-ol–water (4 : 1 : 5); in the case of paper chromatography and 8) butan-1-ol–acetone–water (4 : 5 : 1); and 9) chloroform–methanol (9 : 1) for TLC. The chromogenic agents were a saturated solution of antimony trichloride in chloroform for the pregnane glycosides and aglycones, a mixture of equal amounts of a 3% solution of perchloric acid and a 1% solution of vanillin in ethanol for the 2-deoxysugars, and a solution of salicyclic acid and O-toluidine in ethanol for D-glucose.

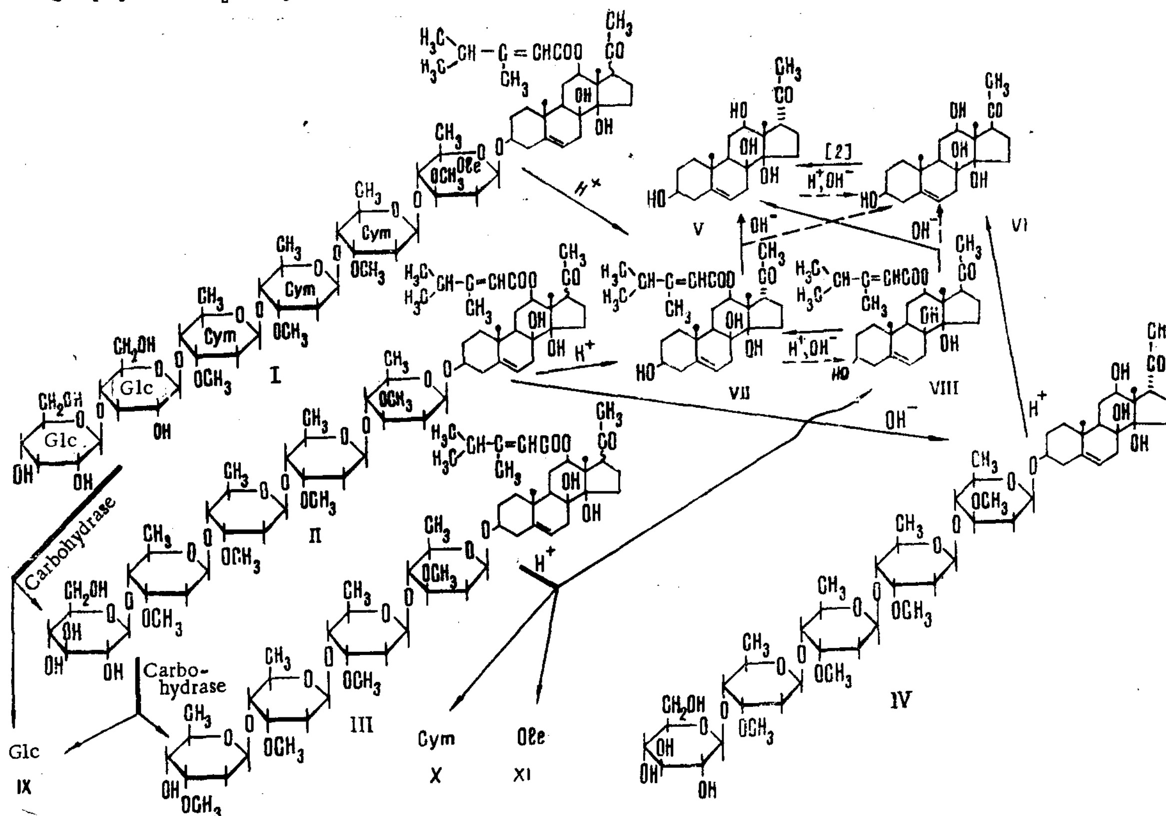
The glycosides (I-IV) have similar color reactions; with concentrated H_2SO_4 they give a brown coloration and with antimony trichloride on heating to 110°C a violet coloration changing to grayish blue. The xanthydrol reaction was positive for them.

Isolation of Sibiricosides D and E. A chloroform extract of the roots of C. sibiricum was evaporated to dryness in vacuum. The residue was again dissolved in a small amount of chloroform, and the solution was poured into a fivefold volume of petroleum ether. The precipitate that deposited was separated off, and this reprecipitation was repeated another three times. The reprecipitated and vacuum-dried extract (10 g, corresponding to 1.5 kg of roots) was dissolved in 50 ml of methanol, the solution was diluted with a threefold volume of water, and the resulting suspension was extracted with benzene. The benzene fraction was not studied. The residual aqueous phase was extracted with butanol exhaustively – until the xanthydrol reaction was negative. After evaporation of the butanol in vacuum, a light brown powder (7 g) was obtained, which was mixed with a fivefold volume of silica gel and transferred to a chromatographic column containing 800 g of type KSK silica gel [8].

The contents of the column were first eluted several times with benzene and with benzene–chloroform (1 : 1) and, finally, with benzene–chloroform–methanol (5 : 5 : 1). The fractions collected amounted to 250 ml each, and after concentration in vacuum they were analyzed in systems 1-3. The benzene and benzene–chloroform eluates (a total of 11 fractions) contained no pregnane glycosides.

Fractions 12-36, extracted with the mixtures of benzene, chloroform, and methanol (5 : 5 : 1) contained a mixture of glycosides consisting of five substances; the fractions from 12 to 23 contained sibiricosides A, B, and C (because of their small amounts they were not studied); the fractions from 24 to 36 contained a mixture of sibiricosides D and E. These glycosides could be separated only by preparative chromatography

on a lyaer of silica gel fixed with 5% of gypsum on glass (33 × 33 cm) in system 1 and by ascending column chromatography on SiO_2 in system 2.



From 7 g of extractive substances we obtained 0.2 g of sibiricoside E and 0.5 g of sibiricoside D (0.013 and 0.033%, respectively, on the weight of the dry raw material).

Sibiricoside D (II). Sibiricoside D (II) formed a white amorphous powder with $[\alpha]_D^{22}$ −26.6 ± 3° (c 1.5; methanol). Readily soluble in alcohols, sparingly in water and chloroform, insoluble in ether; $\lambda_{max}^{C_2H_5OH}$ 220 nm (log ε 3.85); ν_{max}^{KBr} 3440 (OH), 1720 (C=O), 1645 (C=C), 1175 (C−O−C) cm^{-1}. $C_{62}H_{100}O_{23}$ (Mol. wt. 1213.48).

Found: OCH_3 10.16%. Calculated: 4 OCH_3 10.23%.

A weighted sample of the substance (3.31 mg) was dissolved in 2.5 ml of ethanol, 0.5 ml of this solution was added to 3 ml of xanthydrol reagent, and the optical density was determined [4, 9]. D-Digitoxose was used as standard.

Found: 2,6-dideoxyaldose 49.60%. Calculated: 2,6-dideoxyaldose 53.46%.

Desglucosibiricoside D (III). A mixture of 50 mg of glycoside (II) in 1 ml of ethanol and 1 ml of the pancreatic juice of the snail *H. plectotropis* in 150 ml of water was kept in a thermostat at 36°C for three days, and then the solution was evaporated in vacuum at 45–50°C to a volume of 50 ml and was extracted with chloroform. The residue after evaporation of the chloroform was reprecipitated several times from ethanol with ether until a substance giving a single spot in systems 1–3 was obtained. Yield 30 mg. The desglucosibiricoside D (III), $C_{56}H_{90}O_{18}$, formed a white amorphous powder, $[\alpha]_D^{23}$ −15.0 ± 3° (c 1.08; chloroform). Its IR and UV spectra and color reactions were similar to those for sibiricoside D.

The aqueous residue after the separation of the glycoside (III) was evaporated in vacuum to a volume of 5 ml and was analyzed for the presence of sugars. D-Glucose was found in systems 6 and 8.

Deacylsibiricoside D (IV). A solution of 200 mg of glycoside II in 5 ml of a 5% methanolic solution of caustic potash was boiled in a current of nitrogen for 2 h. The cooled solution was diluted with 5 ml of water and extracted with chloroform. The residue obtained after the evaporation of the chloroform was dissolved in 10 ml of ethanol, and the solution was passed through a column of silica gel. A fivefold volume of ether was added to the ethanolic eluate, and the resulting precipitate was filtered off. Reprecipitation from ether was repeated another three times. Yield: 120 mg. The deacylsibiricoside D (IV) formed a

white amorphous powder appearing as an individual substance on chromatography in systems 1, 2, and 4; $[\alpha]_D^{24}$ −52.1 ± 4° (c 1.4; methanol); $\lambda_{max}^{C_2H_5OH}$ 202 nm (log ε 3.66), $C_{55}H_{90}O_{22}$ (Mol. wt. 1103.3).

Found: OCH_3 11.13%. Calculated: 4 OCH_3 11.20%.

ORD curve in CH_3OH, 24°C: $[M]_{589}$ −572, $[M]_{545}$ −718, $[M]_{467}$ −990, $[M]_{428}$ −1280 (at c 2.11); $[M]_{372}$ −2210, $[M]_{335}$ −2920, $[M]_{324}$ −3450, $[M]_{309}$ −3800, $[M]_{298}$ −3980, $[M]_{279}$ −3540, $[M]_{271}$ −3270, $[M]_{275}$ −3900, $[M]_{246}$ −4700 (at c 0.24).

Acid Hydrolysis of Desglucosibiricoside D. A solution of 100 mg of glucoside (III) in 5 ml of methanol was treated with 5 ml of 0.1 N H_2SO_4, and the mixture was boiled for 40 min. Then 5 ml of water was added and the solution was evaporated in vacuum to remove the methanol. The aqueous residue was extracted with chloroform, and the chloroform extracts were washed with water, dried over Na_2SO_4, and evaporated in vacuum. The resulting amorphous mass (40 mg) was separated preparatively on a thin layer of silica gel deposited on glass (12 × 30 cm) in the benzene–acetone (2 : 1) system. The zones corresponding to sibirigenin and cynanchogenin were scraped off and were extracted separately with ethanol.

The ethanolic eluates were evaporated to dryness, and the residues were treated with a mixture of ethyl acetate and hexane. The sibirigenin $C_{28}H_{42}O_6$ (VIII) and cynanchogenin $C_{28}H_{42}O_6$ (VII) obtained in the individual state had properties identical with those given in our preceding paper [2].

The acid aqueous solution after the separation of the aglycones was neutralized with freshly precipitated barium carbonate. The precipitate of barium sulfate was separated off, and the filtrate was evaporated to a syrupy consistency and analyzed in systems 7 and 9. D-Oleandrose (XI) and D-cymarose (X) were detected.

Acid Hydrolysis of Deacylsibiricoside D. A mixture of 60 mg of the glycoside (IV) in 3 ml of methanol and 2 ml of 0.1 N H_2SO_4 was boiled under the conditions described above. The aglycone fraction was separated preparatively in a thin layer of silica gel in system 5. This gave lineolone, $C_{21}H_{32}O_5$, mp 238-240°C; the amount of isolineolone was small, and it was identified only chromatographically in system 5.

Stepwise Hydrolysis of Desglucosibiricoside D. A solution of 7 mg of the glycoside (III) in 0.5 ml of a 0.001 N aqueous dioxane (1 : 1) solution of H_2SO_4 was left to stand at 36°C. After 24 h, D-cymarose was detected in system 7. After three days, in addition to the D-cymarose, the spot of D-oleandrose appeared. After the reaction solution had been kept for a week, no other sugars were found.

The solution was deposited quantitatively on paper and, after chromatography in system 7, the zones corresponding to D-oleandrose and to D-cymarose were cut out. The zones of the sugars, without eluting them from the paper, were finely cut up, transferred to a flask, and covered with an equal volume (5 ml) of the xanthydrol reagent. The contents of the flask were heated at 100°C for 5 min, and cooled, and the colored solutions were separated from the paper and transferred to cells, and their optical densities were determined (see Experimental Method of [4]). The optical density of the solution containing the cymarose (D = 752) was 2.5 times greater than the density of the solution of oleandrose (D = 300).

Sibiricoside E. This had the formula $C_{68}H_{110}O_{28}$ and formed a white amorphous powder, $[\alpha]_D^{22}$ −18.5 ± 3° (c 1.4; methanol). The solubility, color reactions with concentrated H_2SO_4 and $SbCl_3$, and the UV and IR spectra were similar to those for sibiricoside D.

Acid Hydrolysis of Sibiricoside E. A mixture of 10 mg of the glycoside (I) in 3 ml of methanol and 0.5 ml of 0.1 N H_2SO_4 was boiled under the conditions described for the glycoside (III). After the appropriate working up, in the aglycone fraction sibirigenin (VIII) and cynanchogenin (VII) were detected in systems 1 and 5, and in the sugar fraction D-glucose (IX), D-oleandrose (XI), and D-cymarose (X) were detected in systems 6, 7, and 9.

Stepwise Enzymatic Hydrolysis of Sibiricoside E. At room temperature, 10 mg of the glycoside (I) in 0.5 ml of ethanol was mixed with 0.5 ml of the pancreatic juice of the snail H. plectotropis in 50 ml of water. After 10 min, chromatography in systems 1-3 showed the presence of sibiricoside D (II) in the hydrolyzate, and chromatography in systems 6 and 8 showed the presence of D-glucose. After 50 min from the beginning of fermentation, traces of desglucosibiricoside D (III) were detected in the hydrolyzate (system 1). Then the reaction mixture was placed in a thermostat at 36°C. After 18 h, only desglucosibiricoside D (III) was found in the hydrolyzate.

Isomerization of Sibirigenin. A solution of 40 mg of sibirigenin (VIII) in 5 ml of methanol was mixed with 2 ml of a 0.1 N methanolic solution of H_2SO_4, and the mixture was boiled at 100°C. The course of iso-

merization was monitored in system 5. After 30 min from the beginning of heating, the spot of cynanchogenin (VII) appeared on a chromatogram together with that of sibirigenin (VIII). Boiling was continued for another 4 h. Analysis showed that although the amount of cynanchogenin increased during this time, a certain amount of unisomerized sibirigenin remained in the reaction mixture.

The solution was treated with 10 ml of water, and the methanol was evaporated somewhat in vacuum. The residue was extracted with chloroform, and the extract was dried. After the evaporation of the chloroform, the residue was separated preparatively on plates with a fixed layer of silica gel in system 5. The zones corresponding to the sibirigenin and cynanchogenin were scraped off, transferred to columns, and eluted with a mixture of chloroform and ethanol (3 : 1). After evaporation of the solvents, sibirigenin (13 mg) with mp 207-208°C and cynanchogenin (10 mg) with 164-165°C were obtained.

Isomerization of Cynanchogenin. The whole of the cynanchogenin obtained in the preceding experiment was subjected to isomerization under the conditions described above. After the reaction mixture had been heated for four hours, TLC in system 5 showed a scarcely detectable spot of sibirigenin.

SUMMARY

The structures of two pregnane glycosides from Cynanchum sibiricum Willd. have been established. Sibiricoside D (II) is sibirigenin (cynanchogenin) 3-D-glucosido-D-cymarosido-D-cymarosido-D-cymarosido-D-oleandroside. Sibiricoside E (I) is sibirigenin (or cynanchogenin) 3-D-glucosido-D-glucosido-D-cymarosido-D-cymarosido-D-cymarosido-D-oleandroside.

The hypothesis has been put forward that glycosides of isolinoleone are present in the native state which are isomerized into linoleone derivatives in the process of isolation and treatment with acid and alkaline agents.

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