TRITERPENE GLYCOSIDES OF *Scabiosa soongorica*.

IV. PARTIAL SYNTHESIS OF SONGOROSIDE A AND OF BISGLYCOSIDES OF OLEANOLIC ACID

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Partial syntheses of glycosides of oleanolic acid — the 3-O-β-D-xylopyranoside (songoroside A), the 3,28-bis-O-β-D-xylopyranoside, and the 28-O-β-gentiobioside-3-O-β-D-xylopyranoside — and also the formation of 3-O-β-D-xylopyranosyloleanolic acid 13,28-lactone are described.

As we have reported previously [1], five new triterpene glycosides have been isolated from the plant *Scabiosa soongorica* — songorosides C, G, J, M, and O. A progenin of all the glycosides isolated is oleanolic acid 3-O-β-D-xylopyranoside (songoroside A), which is also found in the native form in the plant itself. This glycoside has also been described as a product of the degradation of patrinoside D, one of the main glycosides of *Patrinia intermedia* Roem et Schult [2].

In the plant that we studied, oleanolic acid glycosides of low polarity are found in very small amounts and are difficult to isolate. At the same time, they possess a considerable physiological activity [3] which is possibly even different from that of glycosides rich in carbohydrates.

The aim of the present work was the partial synthesis of a number of glycosides of oleanolic acid in order, on the basis of model compounds, to identify the fraction of minor substances in the plant.

Several syntheses of glycosides of oleanolic acid (I) [4-6] and of its esters [7] have been described in the literature. In particular, there is a report [4] on the preparation of the 3,28-bisxylopyranoside of oleanolic acid by condensing the acid (I) with benzoylated xylopyranosyl bromide. On the basis of these facts, we have attempted to obtain the same compound in boiling dichloroethane in the presence of "active" mercuric oxide and mercuric bromide, with the difference that in place of benzoylated xylopyranosyl bromide we used the acetylated analog. However, after performing condensation and saponification of the reaction mixture the expected product (III) was not obtained. The main product of the reaction proved to be the glycoside (VIII). On acid hydrolysis this glycoside gave an aglycone far less polar than oleanolic acid.

The IR spectrum of compound (VIII) showed the presence of a lactone grouping (1758 cm^{-1}) and the PMR spectrum did not contain the signal of the C-12 olefinic proton. All these facts show that the aglycone of the xylopyranoside obtained was the 28 → 13 lactone of oleanolic acid. The formation of substances of this type from triterpene compounds in acid media has been reported in the literature [8] (Scheme 1).

The presence in the PMR spectrum of product (VIII) of the signal of an anomeric proton at 4.65 ppm with a spin—spin coupling constant (SSCC) of 7.5 Hz shows the β-configuration of the glycosidic bond and the C1 conformation of the carbohydrate residue [9]. Thus, compound (VIII) was the 3-O-β-D-xylopyranoside of the 28 → 13-lactone of oleanolic acid.

We obtained the hexaacetate of oleanolic acid 3,28-bisxylopyranoside (II) in fairly good yield by the reaction of oleanolic acid (I) with acetobromoxylose (IX) under the conditions used by Albrecht in the synthesis of cardiac glycosides.

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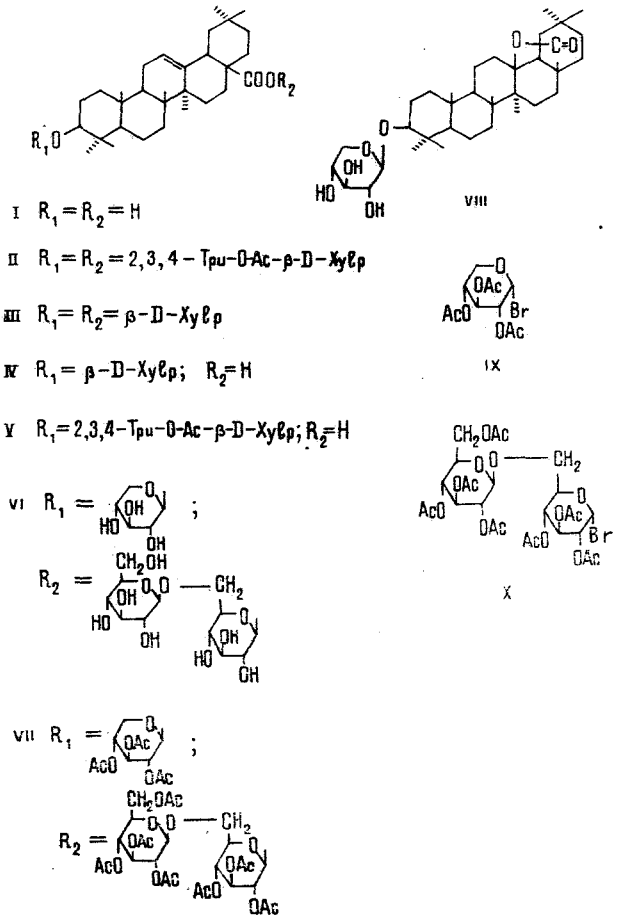

Scheme 1

The fact that the expected hexaacetate (II) had actually been obtained was shown by its PMR and mass spectra. Mass-spectrometric fragmentation took place in agreement with literature information [11]. The anomeric protons of the sugar residues at C-3 and C-28 resonated, respectively, at 4.70 and 6.07 ppm with an SSCC of 7.5 Hz, which indicates the β-configuration of the glycosidic bonds and the C1 conformation of the sugar residues. Consequently, compound (II) was the hexaacetate of oleanolic acid 3,28-bis-β-D-xylopyranoside.

The action on the hexaacetate (II) of a methanolic solution of sodium methanolate yielded oleanolic acid 3,28-bis-β-D-xylopyranoside (III). The physicochemical constants of this compound differed from those of oleanolic acid 3-O-β-D-xylopyranoside 28-O-α-D-xylopyranoside synthesized by a different method [4].

Under the action of a 10% solution of caustic soda, the acetate (II) lost not only the acetate groups but also the xylose molecule attached to the oleanolic acid via an acyloside bond. As a result, we obtained oleanolic acid 3-O-β-D-xylopyranoside (IV). In its physicochemical constants, this compound was identical with natural songoroside A [1].

On alkaline hydrolysis, the main glycosides of *Sc. soongorica* — songorosides M and O — were converted, respectively, into songorosides G and J, which have also been found in the plant in the native form [1]. A feature of their structure is that glycosides M and O contain gentiobiose in the acyloside moiety.

In this connection, it was interesting to obtain from the synthetic songoroside A (IV) an acyloside containing a gentiobiose residue at the carboxy group of the genin. In view of the fact that the triterpene glycosides of *Sc. soongorica* are closely connected with one another genetically, it is not excluded that this type of compound may be found in the plant itself.

To put this idea into effect, the monoglycoside (IV) was acetylated with acetic anhydride in pyridine to give the triacetate of oleanolic acid 3-O-β-D-xylopyranoside (V). The condensation of the triacetate (V) with acetobromogentiobiose (X) under the conditions of the

Koenigs–Knorr method (in V. T. Chernobai's modification [12]) followed by deacetylation with a methanolic solution of sodium methanolate led to oleanolic acid 28-O-gentiobioside 3-O-β-D-xylopyranoside (VI). The presence in the PMR spectrum of the signal of an anomeric proton at 6.01 ppm with $W_{1/2}$ Hz showed the β-configuration of the glycosidic bond.

The acetylation of the trioside (VI) gave the decaacetate of oleanolic acid 28-O-β-gentiobioside 3-O-β-D-xylopyranoside (VII). The mass-spectrometric fragmentation of this compound confirmed the correctness of the structure suggested for it and, consequently, that of the structure of the trioside (VI).

EXPERIMENTAL

For thin-layer chromatography (TLC) we used type KSK silica gel with 5% of gypsum and the following solvent systems: 1) benzene–ether (97:3); 2) chloroform–methanol (50:1); 3) chloroform–methanol (25:1); 4) chloroform–methanol (10:1); and 5) chloroform–methanol–water (80:35:7).

Crystalline 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide (IX) and hepta-O-acetyl-α-gentiobiosyl bromide (X) were obtained under conditions given in the literature [13, 14], and silver carbonate was prepared by Becker's method immediately before use [15]. Before use, the dichloroethane was distilled twice over $CaCO_3$ and twice over $CaCl_2$, and the toluene twice over sodium. "Active" mercuric oxide was obtained by keeping yellow mercuric oxide over HCl for 10 min followed by drying over concentrated H_2SO_4 [4]. Mercury cyanide was obtained by the reaction of hydrocyanic acid with mercuric oxide [16]. Before use, 4 Å molecular sieves were heated at 300-400°C for 3 h.

IR spectra were taken on a IK-20 spectrophotometer (KBr), PMR spectra on a JNM-4H-100 instrument (100 MHz, O — HMDS, δ, ppm), and mass spectra on a MK-1310 instrument.

Conventional symbols: s, singlet; d, doublet; m, multiplet; single primes denote the protons of the sugar residue at C-3 and double primes those at C-28.

Oleanolic Acid 28 → 13-Lactone 3-O-β-D-Xylopyranoside (VIII). A mixture of 3.0 g of oleanolic acid (I), 11.0 g of acetobromoxylose (IX), 7.4 g of "active" mercuric oxide, 0.1 g of mercuric bromide, and 20.0 g of anhydrous calcium sulfate in 150 ml of dichloroethane was boiled for 4 h. The course of the reaction was followed by chromatography in system 1. The reaction mixture was poured into chloroform (1.5 liter) and the solution was washed successively with 1% potassium bromide solution (3 × 300 ml) and with water (2 × 300 ml) and was evaporated in vacuum to dryness. The residue was dissolved in 100 ml of absolute methanol and was mixed with 50 ml of a 0.1 N solution of sodium methanolate in methanol.

After 20 h, KU-2 cation-exchange resin was added to the solution to neutralize it. Then the solution was filtered from the resin and the latter was washed several times with methanol. The combined filtrate was evaporated and the residue was chromatographed on silica gel (500 g). The column was eluted by the gradient method with chloroform containing increasing amounts of methanol. The separation was monitored by TLC in systems 2 and 3. This gave 1.04 g (26.8%) of the individual compound (VIII). The monoglycoside (VIII), $C_{35}H_{56}O_7$, had mp 261-265°C (decomp; from methanol), $[\alpha]_D^{20}$ +32.7 ± 2° (c 0.55; chloroform). ν_{max}^{KBr}, cm^{-1}: 3450-3300 (OH), 1758 (C=O of a lactone ring). PMR spectrum (C_5D_5N), ppm: 0.71, 0.88, 1.02, 1.11, 1.21 (21 H at C-23, C-24, C-25, C-26, C-27, C-29, and C-30; the signals partially overlapped one another); 3.20 (H at C-3, m); 3.50-4.40 (5 H at C-2', C-3', C-4', C-5', m); 4.65 (H at C-1', d, J = 7.5 Hz).

Oleanolic Acid 3,28-Bis-O-β-D-xylopyranoside 2',2",3',3",4',4"-Hexaacetate (II). A solution of 3.0 g of oleanolic acid (I) in 84 ml of dichloroethane was treated with 8.3 g of acetobromoxylose (IX), 8.0 g of mercury cyanide, and 4.4 g of 4 Å molecular sieve. The reaction mixture was stirred at room temperature in a gentle current of nitrogen for 6 h. The course of the reaction was followed by TLC in systems 2 and 3. The solution was filtered, diluted with 500 ml of chloroform, and washed successively with 20% potassium iodide solution (2 × 100 ml), sodium carbonate solution, and water, and was evaporated in vacuum. The crystalline product that deposited was recrystallized from methanol. The yield of substance (II) amounted to 3.8 g (59.5%). The hexaacetate (II), $C_{52}H_{76}O_{17}$, had mp 140-143°C, $[\alpha]_D^{20}$ +11.6 ± 2° (c 1.38; chloroform); ν_{max}^{KBr}, cm^{-1}: 1760, 1260-1220 (absorption of ester groups); PMR spectrum (C_5D_5N), ppm: 0.78, 0.82, 0.94, 1.12 (21 H at C-23, C-24, C-25, C-26, C-27, C-29, and C-30; the signals partially overlapped one another); 1.86, 1.88, 1.93, 2.00, 2.02, 2.07 (6

Ac at C-2', C-3', C-4', C-2", C-3", and C-4", s); 3.05 (H at C-3, m); 3.40-3.75 (2 H at C-5', m); 4.05-4.20 (2 H at C-5", m); 4.70 (H at C-1', d, J = 7.5 Hz); 5.00-5.70 (7 H at C-2', C-3', C-4', C-2", C-3", C-4", and C-12, m); 6.07 (H at C-1", d, J = 7.5 Hz). Mass spectrum, m/e (%): 972 (0.2; M^+), 713 (2), 697 (1), 669 (41), 465 (8), 439 (40), 438 (38), 395 (12), 393 (23), 259 (100), 248 (47), 203 (25), 199 (99), 157 (86), 139 (71).

Oleanolic Acid 3,28-Bis-O-β-D-Xylopyranoside (III) from (II). The hexaacetate (II) (0.71) was dissolved in 25 ml of absolute methanol, and 10 ml of a 0.1 N solution of sodium methanolate in methanol was added. Deacetylation was monitored in system 4. After 4 h, the reaction mixture was neutralized with KU-2 cation-exchange resin and was then filtered from the resin, and the filtrate was evaporated in vacuum. The residue was crystallized from ethanol. This gave 0.50 g of substance (III) (95.0%, calculated on the (II)). The bisglycoside (III), $C_{40}H_{64}O_{11}$, had mp 204-205°C (decomp.) $[\alpha]_D^{20}$ +19.6 ± 3° (c 1.12; chloroform–methanol); ν_{max}^{KBr}, cm^{-1}: 3350-3450 (OH), 1730 (C=O group).

Literature information for oleanolic acid 3-β-O-D-xylopyranoside 28-O-α-D-xylopyranoside [4]: amorphous, $[\alpha]_D^{20}$ +16.2°.

Oleanolic Acid 3-O-β-D-Xylopyranoside (IV) from (II). A suspension of 2.0 g of the hexaacetate (II) in 150 ml of 10% aqueous methanolic (1:1) caustic soda was heated in the boiling water bath for 6 h. For neutralization, the mixture was passed through a column of carboxymethylcellulose (H^+ form). The column was washed with solvent system 5, the eluate was evaporated to dryness, and the residue was crystalized from ethanol to give 1.1 g of compound (IV) (91.6%, calculated on the (II)). The monoglycoside (V), $C_{35}H_{56}O_7$, had mp 228-231°C, $[\alpha]_D^{20}$ +40 ± 3° (c 1.32; chloroform–methanol). ν_{max}^{KBr}, cm^{-1}: 3450-3300 (OH), 1690 (C=O group). PMR spectrum (C_5D_5N), ppm: 0.76, 0.86, 1.20 (21 H and C-23, C-24, C-25, C-26, C-27, C-29, and C-30; the signals partially overlapped one another); 3.20 (H at C-3, m); 3.60-4.35 (5 H at C-2', C-3', C-4', and C-5', m); 4.66 (H at C-1', d, J = 7.5 Hz); 5.32 (H at C-12).

Literature information [1]: mp 230-234°C; $[\alpha]_D^{20}$ +35 ± 2°.

Oleanolic Acid 3-O-β-D-Xylopyranoside 2',3',4'-Triacetate (V) from (IV). A solution of 1.3 g of glycoside (IV) in 12 ml of pyridine was treated with 10 ml of acetic anhydride and the mixture was left at room temperature. After 12 h it was poured into water containing ice. The crystalline precipitate was filtered off, giving 1.4 g of compound (V), $C_{41}H_{62}O_{10}$, mp 170-172°C (from methanol); $[\alpha]_D^{20}$ +23 ± 2° (c 1.89; chloroform). ν_{max}^{KBr}, cm^{-1}: 3500 (OH), 1760, 1250-1220 (ester groupings). Mass spectrum, m/e (%): 714 (1, M^+), 669, (3), 465 (3), 439 (3), 438 (3), 395 (3), 393 (3), 259 (50), 248 (97), 203 (100), 199 (62), 189 (77), 139 (96).

Oleanolic Acid 28-O-β-Gentiobioside 3-O-β-D-xylopyranoside (VI) from (V). A solution of 0.7 g of the triacetate (V) in 200 ml of dichloroethane–toluene (5:1, by volume) was heated to the boil with stirring. After the first 10 ml of solvents had been distilled off, 0.7 g of calcium oxide and 3.0 g of silver carbonate were added to the reaction mixture. Then a solution of 2.1 g of acetobromogentiobiose (X) in dichloroethane was added dropwise at such a rate that the volume of the reaction mixture remained constant (one hour). Then the mixture was boiled for another 7 h. The course of the reaction was monitored by TLC in systems 1 and 2. The solution was filtered, the solid matter was washed with chloroform, and the filtrate was evaporated in vacuum to dryness. The residue was saponified with a methanolic solution of sodium methanolate and was treated in the same way as described above in the preparation of the bisglycoside (III). The reaction products were chromatographed on a column of silica gel with elution by chloroform–methanol with gradually increasing concentrations of the latter. When the ratio of chloroform and methanol reached 8:1, 0.25 g of the crystalline compound (VI) (28%, calculated on the (V)) was eluted. The trioside (VI), $C_{47}H_{76}O_{17}$, had mp 158-160°C (from ethanol); $[\alpha]_D^{20}$ +30.7 ± 2° (c 1.08; methanol). ν_{max}^{KBr}, cm^{-1}: 3350-3450 (OH), 1730 (C=O group).

Oleanolic Acid 28-O-β-Gentiobioside 3-O-β-D-xylopyranoside Decaacetate (VII) from (VI). The trioside (VI) (0.1 g) was acetylated and the product was worked up in the same way as described above for compound (IV). This gave 1.1 g of the acetate (VI), $C_{67}H_{96}O_{27}$, with mp 125-127°C (from methanol); $[\alpha]_D^{20}$ +60 ± 2° (c 1.29; chloroform). ν_{max}^{KBr}, cm^{-1}: 1750, 1240-1225 (ester groupings). Mass spectrum, m/e (%): 1332 (0.1; M^+), 1057 (1), 987 (1), 713 (6), 669 (40), 654 (4), 619 (44, heptaacetylgentiobiosyl), 465 (3), 439 (7), 438 (7), 395 (2), 393 (2), 331 (15), 259 (20, triacetylxylosyl), 248 (92), 203 (100), 199 (24), 189 (40), 139 (100)

SUMMARY

Partial syntheses have been performed of glycosides of oleanolic acid at the C-3 hydroxyl and at the C-28 carboxy group: the 3-O-β-D-xylopyranoside (songoroside A), the 3,28-bis-O-β-D-xylopyranoside, and the 28-O-β-gentiobioside 3-O-β-D-xyloside of oleanolic acid.

The possibility has been shown of the formation of oleanolic acid 13,28-lactone 3-O-β-D-xylopyranoside in the glycosylation of oleanolic acid with acetobromoxylose.

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POLAROGRAPHIC DETERMINATION OF ALKALOIDS OF THE PYRROLIZIDINE SERIES IN PLANT RAW MATERIAL

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A polarographic method has been developed for the determination of platiphylline, sarracine, and seneciphylline in the epigeal part of the groundsel *Senecio platyphylloides* at a dropping mercury electrode in 0.5 M tetraethylammonium iodide solution.

Methods are known for the quantitative determination of platyphylline hydrogen tartrate in medicinal preparations which are based on processes of nonaqueous titration or of extraction followed by colorimetry or spectrophotometry [1], and also known are methods for the quantitative determination of platyphylline and seneciphylline in plant raw material which consist in repeated extraction of the alkaloids with ether from an alkaline medium followed by repeated extraction with a solution of hydrochloric acid and fractional titration [2] and a method for the quantitative determination of the alkaloids using extraction, chromatography, and photocolorimetry [3].

The disadvantages of these methods for the quantitative determination of alkaloids is the necessity for separating the combined alkaloids, the use of toxic and expensive organic solvents, the long times of analysis, and the unavoidable losses of alkaloids at all stages of the process.

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