STEROID GLYCOSIDES OF THE SEEDS OF *Solanum melongena*. STRUCTURES OF MELONGOSIDES A, B, E, F, AND H

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Three chromatographically individual fractions, each containing tigogenin glycosides and diosgenin glycosides have been isolated by chromatography on a silica gel column from a methanolic extract of eggplant seeds. To separate the mixture of two difficultly separable glycosides into individual components, each fraction was acetylated and epoxidated, and the derivatives obtained were separated chromatographically. The tigogenin glycoside peracetates isolated were saponified, and the diosgenin epoxide glycoside acetates were de-epoxidated and saponified, to give the individual glycosides, melongosides A, B, E, F, and H. The complete chemical structure of each melongoside has been shown with the aid of acid hydrolysis, methylation, and periodate oxidation followed by a study of the products obtained.

It is known that eggplant seeds contain compounds of steroid nature [1, 2], but there is no information on the steroid glycosides of the eggplant, the methods for their isolation, and proofs of the chemical structures.

In the present paper we give information on the isolation from the seeds of *Solanum melongena* L. of a number of saponins which we have called melongosides and the determination of their complete chemical structures.

As the result of the repeated chromatographic separation of a methanolic extract on a column of silica gel, three chromatographically individual fractions of glycosides (1, 2, 3) giving a positive reaction with the Sannie reagent and a negative one with the Ehrlich reagent which showed their spirostanol nature, were isolated. The IR spectrum of the glycosides of each fraction contained absorption bands with the relationship 892 > 912, which is characteristic for a spiroketal chain of the (25R)-series. After acid hydrolysis, the presence of two genins was detected in each fraction. The isolated aglycones were separated in a thin layer of silica gel impregnated with 2% $AgNO_3$ and from their melting points and the results of GLC, TLC with monitors, IR spectroscopy, and mass spectrometry they were identified as tigogenin (I) and diosgenin (II). On the basis of the results obtained, it was assumed that each fraction consisted of a two-component mixture of tigogenin glycosides and diosgenin glycosides. To separate this mixture into individual compounds, each fraction was acetylated and the double bond of the diosgenin and the glycoside was epoxidated by the method of Grand and Weavers [3].

After chromatographic separation of the two glycoside derivatives, de-epoxidation [4], and saponification, individual glycosides were obtained. Fraction 1 yielded melongosides A (III) and B (IV), fraction 2 melongoside E (V) and F (VI), and fraction 3 melongosides G (VII) and H (VIII).

On complete acid hydrolysis of (III) and (IV), a genin was isolated with physicochemical constants identical with those of (I), while for (IV), (VI), and (VIII) the aglycone was identified as (II).

By paper chromatography and gas-liquid chromatography of the acetates of the aldononitrile derivatives of the sugars, glucose was detected in the oligosaccharide moieties of glycosides (III, IV, V, and VI), and glucose and rhamnose in a ratio of 2:1 in (VII).

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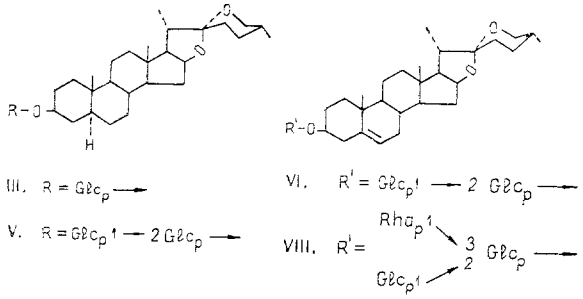
To determine the types of bonds between the monosaccharides, each melongoside was methylated by Hakomori's method [5], and the resulting permethylates were subjected to methanolysis. Using GLC in the presence of markers, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside was identified for the permethylates of (III) and (IV), methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4,6-tri-O-methyl-D-glucopyranoside for (IV) and (VI), and methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, and methyl 4,6-di-O-methyl-D-glucopyranoside for (VIII). The presence of the dimethyl glucose showed branching of the carbohydrate chain of (VIII).

The sequence of attachment of the monosaccharide residues in the melongosides was determined with the aid of partial acid hydrolysis. In the case of melongoside E (V), the progenin (IX), which decomposed on hydrolysis into glucose and tigogenin, was obtained, in the case of melongoside F (VI) the progenin (X), giving diosgenin and glucose on hydrolysis, and in the case of melongoside H (VIII) the three progenins (XI), (XII), and (XIII). Progenin (XII) contained diosgenin, glucose, and rhamnose in a ratio of 1:1:1, and (XIII) contained diosgenin and glucose. From their physicochemical constants, progenin (IX) was identical with melongoside A, (X) and (XI) with melongoside B, and (XIII) with melongoside (F).

The methylation and methanolysis of (XII) gave methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and methyl 2,4,6-tri-O-methyl-D-glucopyranoside, while (XIII) gave methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4,6-tri-O-methyl-D-glucopyranoside. Consequently, in melongoside H the terminal rhamnose is attached to the C_3 atom of the terminal glucose to the C_2 atom of a glucose residue attached directly to the aglycone.

An additional confirmation of the results of the methylation of (VIII) was obtained after its periodate oxidation followed by acid hydrolysis. Under these conditions, glucose was detected in the hydrolysate.

The configurations of the glycosidic centers were shown by means of Klyne's rule [6], taking into account the molecular rotation of the glycosides and their progenins. On the basis of what has been stated above, melongoside B was shown to be trillin, isolated previously from *Trillium erectum* [7], and the other glycosides presented the following structures:



EXPERIMENTAL

For chromatography we used silica gels L 100/160 μm and L 5/40 μm and FN-12 paper, with the following solvent systems: 1) chloroform—methanol (9:1); 2) chloroform—methanol (4:1); 3) benzene—ethanol (9:1); 4) chloroform—acetone (93:7); 5) methylene chloride—acetone (49:1); and 6) butanol—benzene—pyridine—water (5:1:3:3). Melting points were determined on a Boetius stage. Specific rotations were measured on SM polarimeter, IR spectra were recorded on a Specord-IR spectrophotometer. Mass spectra were taken on a MKh-1310 instrument, GLC analysis was performed on a Chrom-5 chromatograph using for the sugar derivatives a steel column 2.4 m long filled with 5% of XE-60 on Chromaton N-AW-HMDS, and for the genins a glass column 1.2 m long filled with 3% of QF-1 on Chromaton Super.

Isolation of Chromatographically Individual Fractions. The air-dry eggplant seeds (1 kg) were comminuted, defatted with diethyl ether and extracted with 70% methanol. The extract obtained was chromatographed repeatedly on silica gel in solvent systems 1 and 2. The separation of the saponins was monitored by TLC in system 2. Three chromatographically individual fractions were obtained: 1) 860 mg; 2) 720 mg; and 3) 900 mg.

Acetylation of the Individual Fractions. Each fraction (700 mg) was acetylated with acetic anhydride (12 ml) in the presence of pyridine (8 ml) at room temperature for 24 h. The reaction mixture was diluted with water and the reaction product was extracted with chloroform. The completeness of acetylation was monitored with the aid of the IR spectra, in which the absorption band characteristic for a hydroxy group was absent.

Epoxidation of Fractions 1, 2, and 3. To 600 mg of the mixture of peracetates of diosgenin glycoside and tigogenin glycosides of the first fraction, dissolved in 11 ml of freshly distilled chloroform, was added 162 mg of m-chloroperbenzoic acid. The reaction mixture was left at room temperature for 2.5 h and was then diluted with 30 ml of diethyl ether and 40 ml of water. The ethereal layer was treated with 2% NaOH (2 × 10 ml), washed with water to neutrality, dried over anhydrous Na_2SO_4, and evaporated. The glycosides were separated by preparative thin-layer chromatography in system 4. In this way, 180 mg of the tigogenin glycoside peraceatte (XIV) with mp 97°C, $[\alpha]_D^{20}$ −35° (c 1.0; $CHCl_3$), and 290 mg of the peracetate of the glycoside of the epoxide derivative of diosgenin (XVII) with mp 118°C, $[\alpha]_D^{20}$ −72° (c 1.0; $CHCl_3$), were isolated.

Solutions of 650 mg of the peracetates of fraction 2 in 80 ml of chloroform and of 600 mg of the peracetates of fraction 3 in 11.5 ml of chloroform were treated with 120 and 111 mg, respectively, of m-chloroperbenzoic acid. After 2.5 h, the reaction mixtures were worked up, and the glycosides were separated by the procedure described above.

From fraction 2 were isolated 160 mg of the tigogenin glycoside peracetate (XVI), mp 116°C, $[\alpha]_D^{20}$ −69° (c 1.0; $CHCl_3$), and 350 mg of the diosgenin epoxide glycoside peracetate (XVII), mp 124°C, $[\alpha]_D^{20}$ −9.0° (c 1.0; $CHCl_3$).

The epoxidated diosgenin glycoside peracetate (XVIII) isolated from fraction 3 (380 mg) had mp 145-146°C, $[\alpha]_D^{20}$ (c 1.0; $CHCl_3$).

De-epoxidation. To eliminate the epoxide groups, compounds (XV), (XVII), and (XVIII) were treated in the following way: To a stirred solution of 260,49 mmole of anhydrous NaI in 330 ml of dry CH_3CN was added 126.9 mmole of chlorotrimethylsilane (at room temperature). Then a solution of 86.8 mmole of the epoxide in 110 ml of dry CH_3CN was added, the mixture was stirred with a magnetic stirrer for 30 min, and then a saturated solution of $Na_2S_2O_3$ was added until the solution had become decolorized. The reaction products were extracted with diethyl ether, the extract was washed with water, treated with a saturated solution of sodium bicarbonate, washed with water again to neutrality, dried over anhydrous Na_2SO_4, and evaporated. The diosgenin glycoside peracetates obtained by the procedure described above were purified chromatographically on silica gel in system 4.

The de-epoxidation of compounds (XV) gave 210 mg of a diosgenin glycoside peracetate (XIX), mp 106°C, $[\alpha]_D^{20}$ −35° (c 1.0; $CHCl_3$ (XVII) gave 280 mg of the diosgenin glycoside peracetate (XX), with mp 126°C, $[\alpha]_D^{20}$ −70° (c 1.0; $CHCl_3$), and (XVIII) gave 260 mg of the acetylated diosgenin glycoside (XXI) with mp 132.5°C, $[\alpha]_D^{20}$ −43° (c 1.0; $CHCl_3$).

Saponification of the Melongoside Peracetates. Compounds (XIV, XVI, XIX, XX, and XXI) were saponified with 10% NaOH in methanol at 100°C for 4 h. In this way, (XIV) yielded 160 mg of an individual glycoside — melongoside A (III) with mp 273°C, $[\alpha]_D^{20}$ −62° (c, 1.0; CH_3OH), and (XIX) yielded 156 mg of melongoside B (IV) with mp 268°C, $[\alpha]_D^{20}$ −97° (c 1.0; CH_3OH). Literature figures for (IV): mp 269-271°C, $[\alpha]_D^{20}$ −103° (c 1.0; dioxane). Compound (XVI) yielded 143 mg of melongoside E with mp 237°C, $[\alpha]_D^{20}$ −53° (c 1.0 CH_3OH); (XX) yielded 180 mg of melongoside F, mp 233°C $[\alpha]_D^{20}$ −55° (c 1.0; CH_3OH); and (XXI) yielded 234 mg of melongoside H, with mp 289°C, $[\alpha]_D^{20}$ −80°C (c 1.0; CH_3OH).

Acid Hydrolysis of the Melongosides. Compounds (III, IV, V, VI, and VIII) (20 mg each) were hydrolyzed with 2.5% H_2SO_4 at 110°C for 8 h. The reaction mixtures were diluted with water and, if the result of the reaction required it, the aglycones were extracted with ether. The hydrolysis of substances (III) and (IV) gave as the aglycone tigogenin (I), R_f 0.46 on plates coated with silica gel impregnated with 2% of $AgNO_3$ in system 5, mp 202-203°C, $[\alpha]_D^{20}$ −65° (c 1.0; $CHCl_3$), M^+ 416, while in the case of (IV, V, and VIII) diosgenin (II) was isolated with R_f 0.42 on $AgNO_3$-impregnated plates in the same system, mp 208°C, $[\alpha]_D^{20}$ −120° (c 1.0; $CHCl_3$), M^+ 414. Paper chromatography in system 6 and GLC of the acetates of the aldonitrile derivatives of the sugar [8] revealed the presence of glucose in the hydrolysates of the glycosides (III, IV, V, and VI) and of glucose and rhamnose in a reaction of 2:1 in the case of (VIII).

Methylation of the Melongosides and the Progenins. A solution of a glycoside or progenin (20 mg) in 4 ml of methylsulfinyl anion (prepared from 700 mg of NaH and 30 ml of DMSO) was stirred at 50°C in an atmosphere of argon for 1 h. Then CH_3I (4 ml) was added to the reaction mixture and it was left at room temperature (in the dark) for 12 h. After this it was diluted with water and extracted with chloroform, and the extract was washed with saturated $Na_2S_2O_3$ solution and with water and was concentrated in vacuum. The methylation product was purified by column chromatography on silica gel in system 3.

The methanolysis of the permethylates obtained was carried out with 72% $HClO_4$ in methanol (1:10) at 100°C for 6 h. The hydrolysates were neutralized with Dowex anion-exchange resin and evaporated. The methyl glycosides referred to in the discussion were detected by gas–liquid chromatography in the presence of markers.

Partial Hydrolysis of the Melongosides. Compounds (V) and (VI) (100 mg each) and (VIII) (150 mg) were dissolved in 1% H_2SO_4 in methanol (30 ml) and the solutions were heated on the water bath for 1.5 h. The course of the reaction was monitored every 30 min by TLC in systems 1 and 2. The final reaction mixtures were diluted with water and extracted with butanol. The butanolic extracts were evaporated and chromatographed on a column of SiO_2 with systems 1 and 2 successively. Compound (V) yielded tigogenin and the progenin (IX) (57 mg), mp 273°C $[\alpha]_D^{20}$ −62° (c 1.0; CH_3OH); (VI) yielded diosgenin and the progenin (X) (49 mg) with mp 268°C $[\alpha]_D^{20}$ −97° (c 1.0; CH_3OH); and (VIII) yielded diosgenin and the three progenins (XI), (XII), and (XIII). Progenin (XI) was identical with (IV), and (XIII) with (X).

After the hydrolysis of 30 mg of progenin (XII), mp 239°C, $[\alpha]_D^{20}$ −59° (c 1.0; CH_3OH), the oligosaccharide fraction contained glucose and rhamnose in a ratio of 1:1 (GLC), while for the other progenins only glucose was detected in the hydrolysates after hydrolysis.

Periodate Oxidation. A solution of 25 mg of melongoside H in 10 ml of methanol was treated with 10 ml of 2% $NaIO_4$ solution. The mixture was kept at room temperature for 48 h, and then several drops of ethylene glycol were added and after an hour it was extracted with n-butanol. The butanolic extracts were evaporated and the residues were hydrolyzed with 2.5% H_2SO_4 at 110°C for 8 h. Glucose was identified in the hydrolysate of melongoside H by paper chromatography.

CONCLUSIONS

Five glycosides of steroid nature have been isolated for the first time from *Solanum melongena* L. and their complete chemical structures have been established.

It has been shown that melongoside A is (25R)-5α-spirostan-3β-ol 3O-β-D-glucopyranoide; melongoside B is trillin; melongoside E is (25R)-5α-spirostan-3β-ol 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside]; glycoside F is (25R)-spirost-5-en-3β-ol 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside]; and melongoside H is (25R)-spirost-5-en-3β-ol 3-O-{[O-β-D-glucopyranosyl-(1→2)][O-α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside}.

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