Phytochemistry, Vol 24, No 7, pp 1567–1569, 1985
Printed in Great Britain

0031-9422/85 $3 00 + 0 00

MELONGOSIDES N, O AND P: STEROIDAL SAPONINS FROM SEEDS OF *SOLANUM MELONGENA*

PAVEL K. KINTIA and STEPAN A. SHVETS

Department of Plant Genetics, Academy of Sciences of the Moldavian S.S.R., Grosul Str. 1, Kishinev 277028, U.S.S.R

(*Revised received* 19 *September* 1984)

Key Word Index—*Solanum melongena*; Solanaceae; furostanol glycosides; melongoside N; melongoside O; melongoside P.

Abstract—Three new saponins, melongosides N, O and P, have been isolated from the methanolic extract of seeds of *Solanum melongena* and their structures elucidated. Melongoside N is 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-*O*-(β-D-glucopyranosyl)-(25*R*)-5α-furostan-3β,22α,26-triol, whereas melongoside O is 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-*O*-(β-D-glucopyranosyl)-(25*R*)-furost-5-en-3β,22α,26-triol and melongoside P is 3-*O*-{[β-D-glucopyranosyl-(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-(25 *R*)-5α-furostan-3β,22α,26-triol.

INTRODUCTION

Tigogenin and diosgenin have previously been reported to be present in seeds of *Solanum melongena* [1]. The structures of three furostanol glycosides isolated from this plant are presented in this paper.

RESULTS AND DISCUSSION

Three chromatographically individual fractions giving a positive colour with Ehrlich's reagent [2] have been isolated from the methanolic extract of *Solanum melongena* seeds by chromatography on a silica gel column. Glycosides present in these fractions were identified as furostanol compounds.

Acid hydrolysis of each individual fraction resulted in the formation of two genins which were separated on 2% silver nitrate-impregnated silica gel plates. Aglycones were identified by their physico-chemical constants as tigogenin and diosgenin, but as the glycosides were furostanol ones the native aglycones occurred as (25*R*)-5α-furostan-3β,22α,26-triol and (25*R*)-furost-5-en-3β,22α,26-triol.

The data obtained suggested that these fractions were a two-component mixture of glycosides with various aglycones. To separate this mixture into individual compounds, each fraction was acetylated with subsequent epoxidation using the technique described by Grant and Weavers [3]. These compounds were then separated on a column of silica gel to obtain melongoside N peracetate (**4**) from the first fraction, melongoside P peracetate (**5**) from the second, and an epoxide peracetate of the derivative of melongoside O (**6**) from the first. As a result of saponification of **4** and **5**, and deepoxidation [4] and saponification of **6**, individual glycosides, namely melongoside N (**1**), melongoside O (**2**) and melongoside P (**3**), were obtained. Complete acid hydrolysis of **1** led to the formation of tigogenin and glucose, compound **2** gave diosgenin and glucose and, from **3**, tigogenin, glucose and rhamnose were obtained in the ratio of 1:3:1.

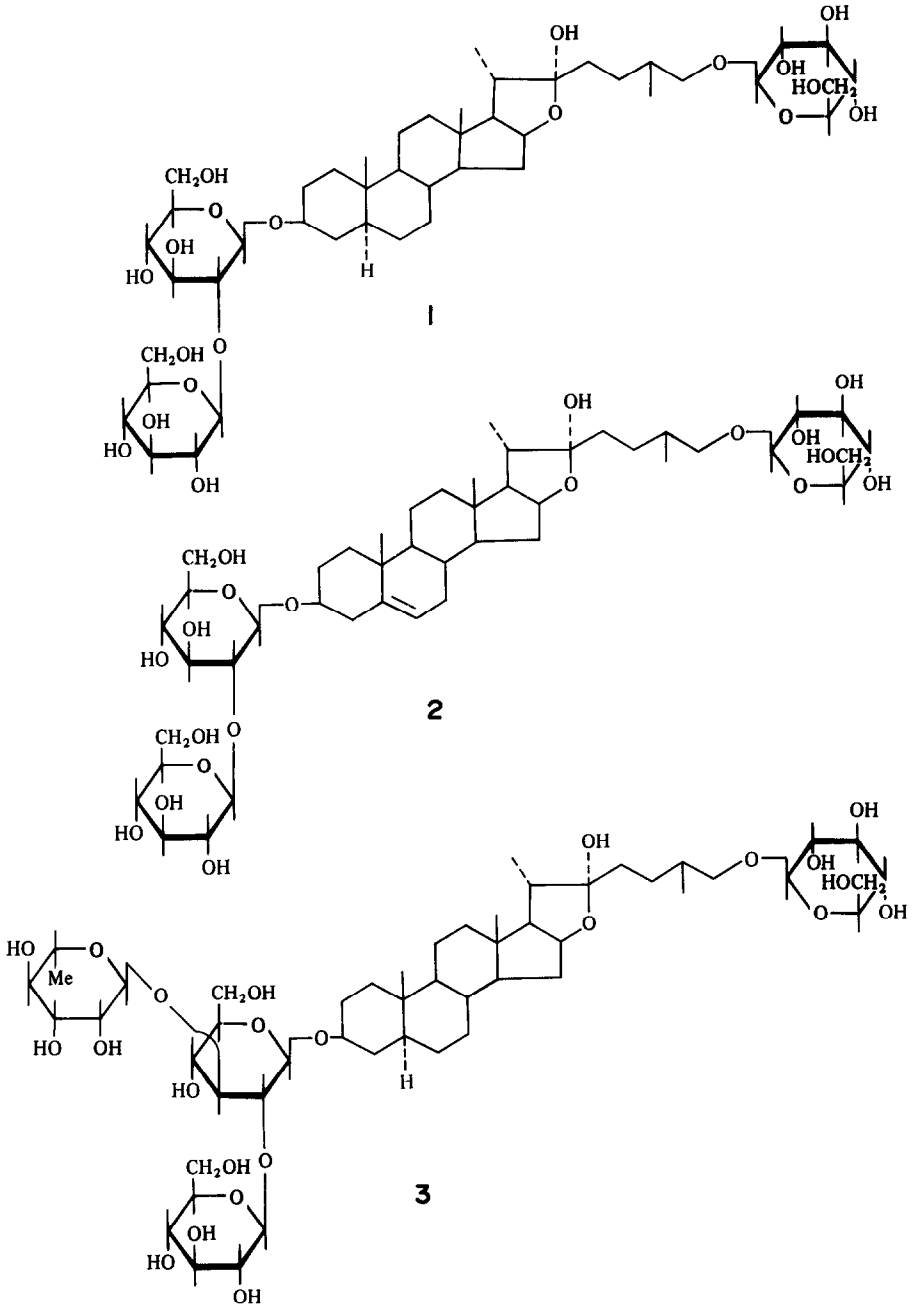
The type of glycosidic linkages in each melongoside was determined using Hakomori's methylation technique [5] with subsequent methanolysis. Methylglycosides were identified by TLC and GC with the aid of authentic samples. From permethylated **1** and **2** were obtained methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (**7**) and methyl-3,4,6-tri-*O*-methyl-D-glucopyranoside (**8**), respectively. From **3** were obtained compound **7**, methyl-2,3,4-tri-*O*-methyl-L-rhamnopyranoside (**9**) and methyl-4,6-di-*O*-methyl-D-glucopyranoside (**10**).

The sequence of monosaccharides in the carbohydrate chain was proved by partial hydrolysis. Tigogenin monoside (**11**) and tigogenin bioside (**12**) were obtained from **1**, the same products and additionally tigogenin trioside (**13**) from **3**, and diosgenin monoside (**14**) and diosgenin bioside (**15**) from **2**. Acid hydrolysis of **11**, **12**, **14** and **15** led to the formation of glucose and **13** gave rhamnose and glucose in the ratio of 1:2. After methylation and methanolysis of permethylated **11** and **14** compound **7** was identified by GC. In addition, **7** and **8** were obtained from **12** and **15** in the ratio of 1:1 while **7**, **9** and **10** were obtained from **13**.

Enzymic hydrolysis of **1** with β-glucosidase yielded a spirostanol glycoside coinciding in its physico-chemical constants with **12**. Enzymic hydrolysis of **2** and **3** led to **15** and **13**, respectively.

To prove the furostanol nature of **1**–**3** they were subjected to reduction by sodium borohydrate with subsequent hydrolysis to obtain dihydrotigogenin from melongosides N and P, and dihydrodiosgenin from melongoside O.

To further verify the structures of **1**–**3** they were acetylated, refluxed with acetic anhydride for isomerization into $\Delta^{20(22)}$-compounds and then oxidized with chromium trioxide in acetic acid (Marker's degradation) [6]. After treatment of the oxidized products with alkali, **1** and **3** were finally decomposed into 3β-hydroxy-5α-pregn-16-en-20-one glycosides, and **2** into 3β-hydroxypregna-5,16-dien-20-one glycoside. After such decomposition, all three glycosides gave a product which was obtained from the steroidal side chain which, upon acetylation followed by methylation with diazomethane yielded the tetra-



acetylglucoside methyl ester of δ-hydroxy-γ-methyl-*n*-valeric acid (**16**) which showed the characteristic mass spectral peaks for acetylated glucose, as well as fragment peaks at *m/z* 129 ($C_7H_{13}O_2$) and 97 $[129 - MeOH]^+$ for the acidic residue [6]. The structure of **16** was thus supported by the mass spectrum.

The configurations of the anomeric centres of glucose and rhamnose were revealed as β and α, respectively, by application of Klyne's rule [7] of molecular rotation. Consequently, melongoside N is 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-*O*-(β-D-glucopyranosyl)-(25*R*)-5α-furostan-3β,22α,26-triol, melongoside O is 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-*O*-(β-D-glucopyranosyl)-(25*R*)-furost-5-en-3β,22α,26-triol and melongoside P is 3-*O*-{[β-D-glucopyranosyl-(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-2*R*)-5α-furostan-3β,22α,26-triol.

EXPERIMENTAL

Isolation of saponins. Commercial dried seeds (1 kg) were extracted with refluxing MeOH (3 × 2 l.) at 65° for 4 hr and the extract was concd under red. pres. to give a mixture of saponins (40 g). Part (20 g) of the residue was placed on a silica gel (400 g) column. Successive elution with $CHCl_3$ and $CHCl_3$–MeOH (90:10, 85:15 and 8:2) afforded different saponins and their mixtures. Fractions eluted with $CHCl_3$–MeOH–H_2O (65:30:10)

yielded mixtures of the furostanol saponins. These were re-chromatographed over silica gel. The chromatography was monitored by TLC. Three chromatographically individual fractions were obtained, each of which was separately acetylated with subsequent epoxidation following the method described in ref. [3]. The reaction mixtures were separated on a silica gel column, eluting the columns with $CHCl_3$–Me_2CO (95:5), to give compounds **4**–**6**. After saponification of **4** and **5**, the following were obtained: melongoside N, mp 187–189°, $[\alpha]_D^{20}$ −15° (MeOH; *c* 1.0) and melongoside P, mp 179–180°, $[\alpha]_D^{20}$ −75° (H_2O; *c* 1.0).

After de-epoxidation of **6** and saponification of the product obtained, melongoside O was isolated, mp 183–184°, $[\alpha]_D^{20}$ −19° (CH_3OH; *c* 1.0).

Hydrolysis of **1**–**3**. Compounds **1** (40 mg), **2** (30 mg) and **3** (50 mg) were hydrolysed with 2.5% H_2SO_4 at 110° for 6 hr. Tigogenin was obtained from **1** and **3** and purified by TLC (CH_2Cl_2–Me_2CO, 49:1), MS *m/z*: 416 $[M]^+$. The IR spectrum showed 892 > 912 cm^{-1}, which is characteristic of the (25*R*)-configuration. Diosgenin was obtained from **2** and purified by TLC, mp 208°, $[\alpha]_D^{20}$ −120° ($CHCl_3$; *c* 1.0). The IR spectrum showed 892 > 912 cm^{-1} [(25*R*)-configuration]; MS *m/z*: 414 $[M]^+$ Glucose and rhamnose were identified in the hydrolysate from the glycosides by PC (BuOH–C_6H_6–pyridine–H_2O, 5:1:3:3). GC of the aldonenitryl derivatives of the sugars [8] showed the presence of glucose in **1** and **2**, and glucose and rhamnose (3·1) in **3**.

Methylation and methanolysis of permethylated products. Each melongoside and progenin (40 mg) was methylated by Hakomori's method [5] to yield permethylated products which were hydrolysed with 72% $HClO_4$ in MeOH (1:10) for 5 hr at 110°. After neutralization by anionic Dowex 1 × 8, TLC on silica gel (C_6H_6–Me_2CO, 1.2) of permethylated **1** and **2** gave **7** and **8**. Compounds **7**, **9** and **10** were obtained from **3**; **7** from **11** and **14**; **7** and **8** from **12** and **15**; **7**, **9** and **10** from **13**. Compounds **7**–**10** were identified by GC with the aid of authentic samples.

Partial hydrolysis of **1**–**3** Each melongoside (100 mg) was heated in 50 ml 1% H_2SO_4 at 90° for 2 hr. The reaction mixture was then diluted with H_2O and extracted with BuOH (3 × 30 ml). The BuOH extracts were chromatographed on silica gel to obtain **11**, mp 273°, $[\alpha]_D^{20}$ −62° (MeOH; *c* 1.0), and **12**, mp 237°, $[\alpha]_D^{20}$ −55° (MeOH; *c* 1.0) from **1**; **11**–**13**, mp 270°, $[\alpha]_D^{20}$ −70° (MeOH; *c* 1.0) from **3**; **14**, mp 267°, $[\alpha]_D^{20}$ −97° (MeOH; *c* 1 0), and **15**, mp 233°, $[\alpha]_D^{20}$ −65° (MeOH; *c* 1 0) from **2**.

Enzymic hydrolysis with β-glucosidase from Helix pomatia. Each melongoside (100 mg) in 50 ml H_2O was incubated with the enzyme for 24 hr at room temp. and the products checked by TLC ($CHCl_3$–MeOH–H_2O; 65:25:10). After 24 hr, a mixture was extracted with 3 × 50 ml BuOH and the extract chromatographed on a column of silica gel to yield **12** from **1**; **15** from **2**; **13** from **3**. Acid hydrolysis of **12** and **15** yielded glucose and hydrolysis of **13** yielded glucose and rhamnose in the ratio of 2:1 and also tigogenin.

Oxidation of melongosides N, O and P. Acetylated **1**–**3** (300 mg of each), obtained by reaction with HOAc, were dissolved in 10 ml HOAc and 100 mg NaOAc was added [6]. The oxidation was carried out as described in ref. [6] to produce the tetra-acetylglucoside methyl ester of δ-hydroxy-γ-methyl-*n*-valeric acid (**16**), which showed the characteristic MS peaks at *m/z* 331, 243, 242, 200, 169, 157, 145, 141, 109 and 98.

The 3β-hydroxy-5α-pregn-16-en-20-one-3-glycoside (20 mg) and 3β-hydroxy-pregna-5,16-dien-20-one-3-glycoside (15 mg) were hydrolysed in 3 ml 4 M HCl and 3 ml C_6H_6 for 3 hr at 80° The pregnenolones obtained were acetylated in 4 ml Ac_2O–pyridine (2:3), then purified on silica gel to give 3β-acetoxy-5α-pregn-16-en-20-one and 3β-acetoxypregna-5,16-dien-20-one, IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 1735, 1660 (characteristic of Δ^{16}-20-one [9]) 958, 920, 895 and 820.

REFERENCES

1. Apsomatova, R. A., Denikejeva, M. F. and Koshoev, K. K. (1976) *Organic Chemistry and Ways of Development of Chemical Industries in Kirgizia*, p. 55.
2. Kiyosawa, S. and Masakaru, H. (1968) *Chem. Pharm. Bull.* **16**, 1162.
3. Grant, P. K. and Weavers, R. T. (1974) *Tetrahedron* **30**, 2385.
4. Caputo, R., Mangoni, L., Neri, O. and Polumbo, G. (1981) *Tetrahedron Letters* **22**, 3551.
5. Hakomori, S. (1964) *J. Biochem. Tokyo* **55**, 205.
6. Tschesche, R., Lüdke, G. and Wulff, G. (1969) *Chem. Ber.* **102**, 1253.
7. Klyne, W. (1950) *Biochem. J* **41**, 47.
8. Krohmalyuk, V. V., Kintia, P. K. and Tschirva, V. Y. (1975) *Izv Acad. Nauk. M.S.S.R., Ser Biol. Khim.* **1**, 85.
9. Jones, R. N., Humphries, P and Dobriner, K. (1949) *J. Am. Chem. Soc* **71**, 241.