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A 24β-ETHYL-Δ⁷-STERYL GLUCOPYRANOSIDE FROM *CUCURBITA PEPO* SEEDS*

HANS-WILLI RAUWALD, MICHAEL SAUTER† and HEINZ SCHILCHER†‡

Institut für Pharmazeutische Biologie der Universität, Im Neuenheimer Feld 364, D-6900 Heidelberg 1, West Germany; †Institut für Pharmakognosie und Phytochemie der Freien Universität, Königin-Luise-Straße 2-4, D-1000 Berlin 33, West Germany

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Key Word Index—*Cucurbita pepo*; Cucurbitaceae; pumpkin; seeds; steryl glucosides; 3-*O*-(β-D-glucopyranosyl)-24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol; 3-*O*-(β-D-glucopyranosyl)-24β-ethyl-5α-cholesta-7,*trans*-22,25(27)-trien-3β-ol; spinasteryl-β-D-glucopyranoside.

Abstract—In the seeds of *Cucurbita pepo* three closely related 24-ethyl-Δ⁷-steryl glucosides were identified by hydrolytic studies and spectral analysis as spinasteryl-β-D-glucopyranoside, the new 3-*O*-(β-D-glucopyranosyl)-24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol and the corresponding $\Delta^{22E,25(27)}$-trienol. Except for its occurrence in cucumber seeds the latter is so far unknown as a natural product.

INTRODUCTION

The major sterols of the seeds of *Cucurbita pepo* are 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol(aglycone of **1a**), 24β-ethyl-5α-cholesta-7,*trans*-22,25(27)-trien-3β-ol (aglycone of **2a**) and the 24α-ethylsterol spinasterol (aglycone of **3a**) [1–4]. The configuration at C-24 was established by 270 MHz ^{1}H NMR spectroscopy [1] and further by stereospecific synthesis of the corresponding two C-24 epimers [1, 3, 4], recently confirmed by ^{13}C NMR spectroscopy [5, 6]. There has been no report on the occurence of glycosylated Δ⁷-sterols in pumpkin seeds. As part of an ongoing chemical and pharmacognostical analysis of *Cucurbita pepo* seeds [7–9] we now describe the identification of the three main steryl glycosides **1a**–**3a**, which represent the β-D-glucopyranosides of the above-mentioned 24-ethyl-Δ⁷-sterols. Compound **1a** is a new glycoside from natural sources, **2a** has so far been detected with the aid of GLC only from the seeds of *Cucumis sativus* [10] and **3a** (spinasteryl glucoside) is also reported for the first time from pumpkin seeds.

RESULTS AND DISCUSSION

In the course of an isolation procedure [7] the presence of the steryl glucosides **1a**–**3a** in the seeds of *Cucurbita pepo* was detected by TLC of an EtOAc percolate on silica gel using $CHCl_3$–MeOH (85:10) as eluant. This showed a purple-blue colouration with the Liebermann–Burchard reagent and a single spot at R_f 0.52 on TLC. The fraction (95 mg) was isolated by column chromatography on silica gel. Acetylation gave a tetraacetate, whose ^{1}H NMR spectrum showed signals due to 18-Me (δ0.53, *s*), 19-Me (0.76, *s*) of Δ⁷-sterol [1], four methyls (each *s*, 1.95, 2.00, 2.02, 2.05) of acetyls, the anomeric proton (4.62, *d*, J = 8 Hz) and 6'$_{1,2}$-H (4.25, *dd*, J = 12 and 5 Hz; 4.10, *dd*, J = 12 and 2.5 Hz) of the β-D-glucopyranosyl moiety. In the noise decoupled ^{13}C NMR spectrum of the free Δ⁷-steryl glucosides, for which so far no data are given in the literature, six distinct signals were assigned to glucose,

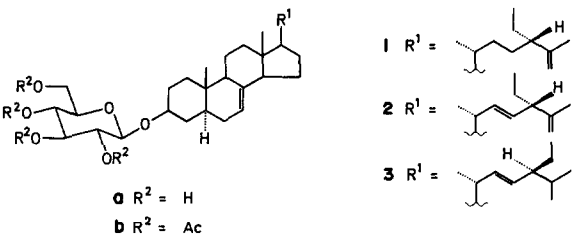

Table 1. ^{13}C NMR chemical shifts of C-20/C-29 for steryl glucosides **1a**, **2a** and **3a** (20.4 MHz; pyridine-d_5; δ/ppm; TMS as internal standard)

Carbon No.	1a	2a	3a
20	40.7	40.7	40.7
21	18.8	22.0*	22.0*
22	34.2	137.5	138.6
23	29.8	130.6	129.9
24	49.8	52.3	51.5
25	148.4	148.6	32.2
26	19.0	20.2	21.9*
27	111.9	110.2	21.9*
28	26.7	25.8	25.8
29	12.0	12.3	12.6

*Assignments may be interchangeable.

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‡To whom correspondence should be addressed.

indicating its β-configuration (δ102.4) and the pyranose form [11]. The placement of the glucosyl residue at C-3 of the aglycones followed from the observed downfield shift (7.5 ppm) of the C-3 signal and the highfield shifts (2.3 resp. 3.9 ppm) of the C-2/C-4 signals relative to their corresponding shifts in 5α-cholestan-3β-ol [11]. Thus, the expected δ_c values of the aglycone 78.0, 30.1/35.2 are in agreement with the experimental values 78.7, 30.1/34.9, whereas δ73.8, 26.0 and 35.1 would be expected for the corresponding 3α-yl-isomer [11].

From the mass spectra and the NMR spectra the fraction was assumed to be a mixture of 24-ethyl-Δ^7-steryl-β-D-glucopyranosides. Since the chromatographic separation of such closely related steryl glucosides was not possible and has not yet been reported, the steryl components were identified according to the method previously described for this type of compound [12]. Either careful acid hydrolysis or enzymatic cleavage with β-glucosidase yielded D-glucose and a steryl mixture. The former was identical with D-glucose as regards TLC examination and enzymatic test with glucose oxidase. After acetylation of the latter sterols, it was suggested to be a mixture of three components by GLC examination. The peaks were assigned to the acetylated aglycones of **1a**, **2a** and **3a** by comparison with pure authentic samples in concordance with published data [5, 13] and the ratio of their content was determined as approximately 1:3:2 from the results of the GLC analysis. Preparative separation of the acetate mixture was achieved by TLC on $AgNO_3$–silica gel, which showed three bands at R_f 0.23, 0.53 and 0.86. This afforded 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-yl acetate (aglycone of **1a**), 24β-ethyl-5α-cholesta-7,*trans*-22,25(27)-trien-3β-yl acetate (aglycone of **2a**) and spinasteryl acetate with the 24α-configuration (aglycone of **3a**). The identity of these compounds, including the assignment of the configuration at C-24, was obtained by mmp examination, IR and ^{1}H NMR comparison with authentic steryl acetates. Furthermore the experimental ^{13}C NMR shifts of the side chain carbons in **1a**–**3a** were in good agreement with the described values of the corresponding aglycone acetates [5], as shown in Table 1. Summarizing all the data obtained, the main steryl glycosides of the seeds of *Cucurbita pepo* are **1a**, **2a** and **3a**.

The free 24-ethyl-Δ^7-sterols are typical for the seeds of some Cucurbitaceae [14], whereas reports of glucosylated Δ^7-sterols are very rare [15], except for spinasteryl glucoside [4, 12]. Possibly this also may be due to the difficult chromatographic separation of this type of glycosides. The significance of this awaits further investigation in other genera of the Cucurbitaceae.

EXPERIMENTAL

The origin of the seeds of *Cucurbita pepo* L. convar. citrullinina I. GREB. var. styriaca I. GREB. was reported previously [8]. GLC was carried out on an OV-1701 SCOT glass capillary column (15 m × 0.25 mm, isothermal 240°, FID det., He at 35 cm/sec, split ratio 30:1).

Isolation. Dried and ground seeds (3 kg) of *C. pepo* were percolated with petrol (5 l.), CH_2Cl_2 (5 l) (1.45 kg pumpkin seed oil [7]) and EtOAc (5 l.). The residue (5.7 g) after removal of EtOAc *in vacuo* was washed with *n*-hexane and partitioned between MeOH–CH_2Cl_2–H_2O (40:40:10). After evaporation of the organic phase (2.5 g residue) further purification by CC on silica gel using CH_2Cl_2–*n*-hexane–MeOH (49:50:1) yielded 95 mg of the Δ^7-steryl glucoside fraction, consisting of **1a**, **2a** and **3a**.

Acid hydrolysis. Steryl glucosides **1a**–**3a** (35 mg) were hydrolysed with $CHCl_3$–MeOH–HCl 37% (42:42:16 = 1.65 N) under reflux for 1 hr. The resulting mixture was concd *in vacuo* after H_2O had been added, and the residue was extracted with Et_2O. The Et_2O extract was washed with H_2O, dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The H_2O layer on Et_2O extraction was neutralized with Amberlite 400 and evaporated to dryness *in vacuo*. The presence of D-glucose was shown by TLC at R_f 0.35 in Me_2CO–*n*-BuOH–H_2O (50:40:10) as well as by an enzymatic test with glucose oxidase (available from Fa. Roth, Karlsruhe) [9].

Enzymatic hydrolysis. To the aq. acetate buffer soln. (pH 5) of fraction **1a**–**3a** (not completely soluble) β-glucosidase was added, and the mixture kept at 37° for 2 days. Aglycones were extracted with EtOAc; on TLC the aq. layer showed a spot corresponding to that of glucose.

Sterol analysis. The sterols obtained by acid hydrolysis were acetylated with Ac_2O–pyridine (1:1) in the usual manner. The steryl acetates were fractionated by TLC on $AgNO_3$–silica gel [5] with $CHCl_3$. This gave the aglycone acetates of **1a** (R_f 0.53), **2a** (R_f 0.23) and **3a** (R_f 0.85). Each steryl acetate was shown to be identical with the corresponding authentic samples by mmp (aglycone acetates of **1a**, **2a**, **3a**: mp 155–158°; 168–171°; 170–172°), IR (KBr), ^{1}H NMR and GLC comparison with the Δ^7-sterols obtained from the unsaponifiable lipids of *C. pepo* seeds. Further details can be obtained by us [7, 9]. Furthermore, the steryl acetates were separated by GLC. The RR_1s were (cholesteryl acetate = 1.00) 1.77 (aglycone of **1a**), 1.61 (aglycone **2a**) and 1.58 (aglycone **3a**). The amounts of the different sterols were calculated from these GLC results.

The identification of the three components was verified by the following spectral analysis of the fraction of free and acetylated glucosides. UV (**1a**–**3a**) λ_{max}^{MeOH} nm: 210. EIMS (**1b** – **3b**) 80 eV, *m/z* (rel. int.): 742 $[M_1]^+$ (4.5), 740 $[M_2]^+$ (2), 700 $[M_1 - COCH_2]^+$ (2), 698 $[M_2 - COCH_2]^+$ (1.5), 601 $[M_{1,2}$ – side chain$]^+$ (7), 559 $[601 - COCH_2]^+$ (3.5), 454 (2), 412 $[M_1^+$ – gluc. ac.] (7), 410 $[M^+{}_2$ – gluc. ac.] (8), 397 $[412 - Me]^+$ (11), 395 $[410 - Me]^+$ (28), 331 [gluc. tetraac.]$^+$ (81), 273 $[410 -$ side chain$]^+$ (10), 271 $[331 - HOAc]^+$ (34), 139 [side chain]$^+$ (12), 137 [side chain]$^+$ (51), 43 (100). ^{1}H NMR (assignment by comparison with published data for aglycones [1–4]) (250 MHz, shifts in DMSO-d_6 for **1a**–**3a**/$CDCl_3$ for **1b**–**3b**) **1a**/**1b**: δ3.55/3.54 (1H, *m*, H-3), 5.11/5.18 (1H, *m*, H-7), 0.49/0.51 (*s*, 18-Me), 0.74/0.76 (3H, *s*, H-19), 0.92/0.90 (*d*, 21-Me), 1.53/1.55 (*s*, 26-Me), 4.70/4.69 (*dd*, 27-CH_2), 0.78/0.82 (*t*, 29-Me). **2a**/**2b**: δ3.55/3.54 (1H, *m*, H-3), 5.11/5.18 (1H, *m*, H-7), 0.51/0.53 (*s*, 18-Me), 0.74/0.76 (3H, *s*, H-19), 1.00/1.01 (*d*, 21-Me), 5.19–5.23/5.18–5.23 (*dd*, H-22/23), 1.61/1.63 (*s*, 26-Me), 4.70/4.69 (*dd*, 27-CH_2), 0.79 (*t*, 29-Me). **3a**/**3b**: 3.55/3.54 (1H, *m*, H-3), 5.11/5.18 (1H, *m*, H-7), 0.51/0.53 (*s*, 18-Me), 0.74/0.76 (3H, *s*, H-19), 1.00/1.01 (*d*, 21-Me), 5.19–5.23/5.18–5.23 (*dd*, H-22/23), 0.85/0.85 (*br s*, 26-Me), 0.80/0.80 (*br s*, 27-Me), 0.78 (*t*, 29-Me). Glucosyl-H (**1a**–**3a**) δ4.90/4.94 (2H, *d*, *J* = 5.1 Hz, 1H, *d*, *J* = 5.5 Hz, 2'-, 3'-, 4'-OH), 4.44 (1H, *t*, *J* = 5.1 Hz, 6'-OH); (DMSO-d_6 + CF_3 COOD): 4.22 (1H, *d*, *J* = 7.7 Hz, H-1'), 2.92 (1H, *dd*, *J* = 7.7 and 9 Hz, H-2'), 3.2–3.08 (3H, *m*, H-3', 4', 5'), 3.65 (1H, *br d*, *J* = 10.3 Hz, H-6'$_1$), 3.45 (1H, *dd*, *J* = 10.3 and 6 Hz, H-6'$_2$). Glucosyl-H (**1b**–**3b**): δ4.60 (1H, *d*, *J* = 8 Hz, H-1'), 4.94 (1H, *dd*, *J* = 8 and 9 Hz, H-2'), 5.19 (1H, *dd*, *J* = 9 and 9.5 Hz, H-3'), 5.10 (1H, *dd*, *J* = 9.5 and 9.7 Hz, H-4'), 3.68 (1H, *m*, H-5'), 4.25 (1H, *dd*, *J* = 12 and 4.8 Hz, H-6'$_1$), 4.10 (1H, *dd*, *J* = 12 and 2.5 Hz, H-6'$_2$), 1.95, 2.00, 2.02, 2.05 (each 3H, *s*, OAc). ^{13}C NMR of **1a**–**3a** (20.4 MHz, pyridine-d_5): see Table 1, except: δ26.1 (C-1), 30.1 (C-2), 78.7 (C-3), 34.9 (C-4), 40.4 (C-5), 29.9 (C-6), 117.9 (C-7), 139.7 (C-8), 49.8 (C-9), 34.9

(C-10), 21.9 (C-11), 39.8 (C-12), 43.6 (C-13), 55.4 (C-14), 23.5 (C-15), 28.2 (**1a**) and 28.8 (**2a**/**3a**) (C-16), 56.4 (C-17), 12.3 (C-18), 13.1 (C-19), 40.7 (C-20). Glucosyl-C: 102.4 (C-1'), 75.4 (C-2'), 78.3 (C-3'), 72.0 (C-4'), 77.4 (C-5'), 63.1 (C-6').

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REFERENCES

1. Sucrow, W., Slopianka, M. and Kircher, H. W. (1976) *Phytochemistry* **15**, 1533.
2. Sucrow, W. and Reimerdes, A. (1968) *Z. Naturforsch.* 23b, 42.
3. Sucrow, W. and Girgensohn, B. (1970) *Chem. Ber.* **103**, 750.
4. Sucrow, W., Schubert, B., Richter, W. and Slopianka, M. (1971) *Chem. Ber.* **104**, 3689.
5. Itoh, T., Kikuchi, Y., Tamura, T. and Matsumoto, T. (1981) *Phytochemistry* **20**, 761.
6. Itoh, T., Yoshida, K., Tamura, T. and Matsumoto, T. (1982) *Phytochemistry* **21**, 727.
7. Sauter, M., Schilcher, H. and Segebrecht, S. (1984) *Pharm. Ztg.* **130**, 73.
8. Schilcher, H. (1980) *Acta Hortic.* **96**, 151.
9. Sauter, M. (1984) Dissertation, Freie Universität, Berlin.
10. Mashchenko, N. E., Kintya, P. K. and Lazurévskii, G. V. (1975) *Khim. Prir. Soedin. (Chem. Nat. Prod.)* **5**, 660.
11. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y. (1977) *Tetrahedron Letters* **2**, 179.
12. Konoaka, M., Yoshizaki, M. and Fujino, H. (1982) *Chem. Pharm. Bull.* **30**, 2570.
13. Itoh, T., Tani, H., Fukushima, K., Tamura, T. and Matsumoto, T. (1982) *J. Chromatogr.* **234**, 65.
14. Nes, W. R., Krevitz, K., Joseph, J., Nes, W. D., Harris, B., Gibbons, G. F. and Patterson, G. W. (1977) *Lipids* **12**, 511.
15. Axelos, M. and Péaud-Lenoel, C. (1982) in *Plant Carbohydrates I (Steryl Glycosides)* (Loewus, F. A. and Tanner, W., eds) p. 613. Springer, Berlin.

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A FUROSTANOL GLUCURONIDE FROM *SOLANUM LYRATUM**

SHOJI YAHARA, NAOMI MURAKAMI, MASAKI YAMASAKI,† TOSHIYUKI HAMADA, JUN-EI KINJO and TOSHIHIRO NOHARA

Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan; †Department of Biochemistry, Medical School, Kumamoto University, Honjo, Kumamoto 860, Japan

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Abstract—A new furostanol glucuronide and three known glycosides, SL-0, aspidistrin and methyl proto-aspidistrin, were isolated from the fresh immature berries of *Solanum lyratum*. The structure of the new compound was characterized as 26-*O*-β-D-glucopyranosyl-(22ξ,25*R*)-3β,22,26-trihydroxyfurost-5-ene 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucuronopyranoside.

INTRODUCTION

It was previously reported that a furostanol (SL-0), a spirostanol (SL-1) and two steroidal alkaloid glycosides (SL-c, SL-d) were obtained from the stems of *Solanum lyratum* Thunb. and their structures were elucidated [1, 2]. Our continuing study of the fresh immature berries of this plant has led to the isolation of a new steroidal glucuronide (**1**), which was a major component (*ca* 2.8%), along with three known glycosides, SL-0 [1], aspidistrin [3] and methyl proto-aspidistrin [4]. This paper deals with the structural elucidation of compound **1**.

*Part 6 in the series "Studies on the Constituents of *Solanum* Plants". For Part 5 see ref. [2].

RESULTS AND DISCUSSION

Compound **1**, an amorphous powder, $[\alpha]_D - 61.4°$, showed strong absorptions in the IR spectrum due to a carboxyl group (1600 cm^{-1}) and a hydroxyl group (3400 cm^{-1}), but not for a spiroketal function [5, 6] and it was positive to the Ehrlich reagent [7], suggesting a furostanol glycoside structure. Enzymic hydrolysis with almond emulsin gave a spirostanol glycoside (**2**) and D-glucose. Compound **2**, colourless needles, mp > 300°, $[\alpha]_D - 83.4°$, showed absorptions due to a carboxyl group (1600 cm^{-1}) and a characteristic spiroketal ring (920, 900, 865 cm^{-1}) in the IR spectrum, and in the FD mass spectrum the peak at *m/z* 937 originated from $[M + K]^+$. Acid hydrolysis of compound **2** yielded diosgenin together with rhamnose, glucose and glucuronic acid. The EI mass spectrum of the acetate of **2** showed the peaks