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# ACYLGLUCOSYL ISOFUCOSTEROL FROM CELL CULTURES OF *LYCOPERSICON ESCULENTUM*

SALVATORE DE ROSA,* ALFONSO DE GIULIO, GIUSEPPINA TOMMONARO and MARIA A. BELISARIO†

Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R., Via Toiano, 6, 80072 Arco Felice (Napoli), Italy; †Dipartimento di Biochimica e Biotecnologie Mediche, Università di Napoli "Federico II", Via S. Pansini, 5, 80131 Napoli, Italy

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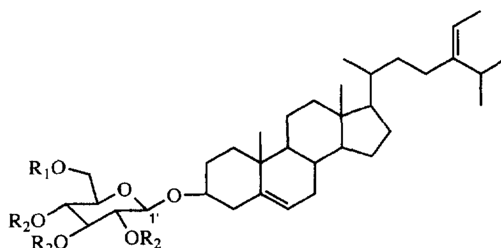
**Key Word Index**—*Lycopersicon esculentum*; Solanaceae; cell cultures; acylglucosylsterol; 3-*O*-[6′-*O*-palmitoyl-*β*-glucosyl]-isofucosterol; 3-*O*-[6′-*O*-stearyl-*β*-glucosyl]-isofucosterol; 3-*O*-[6′-*O*-oleyl-*β*-glucosyl]-isofucosterol; 3-*O*-[6′-*O*-linoleyl-*β*-glucosyl]-isofucosterol.

**Abstract**—A mixture of acylglucosyl isofucosterols has been isolated from the cell culture of *Lycopersicon esculentum*. The structures were elucidated from chemical and spectroscopic evidence. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Acylglucosylsterols have been isolated from both the animal and vegetable kingdoms. Within animals these compounds have been found in epidermal tissue of snake [1] and chicken [2], while from plants they were isolated from roots of *Caragana chamiagu* [3], from millet (*Pennisetum americanum*) [4] and wheat (*Triticum vulgare*) [5] seeds, from fruits of *Momordica charantia* [6] and from seedlings of *Cucumis sativus* [7]. In a preceding paper [8], we reported the isolation of sterol glucoside from cell suspension cultures of *Lycopersicon esculentum*. L. var. S. Marzano that have an important role in the economy of the Campania region of South Italy. This paper describes the isolation of acylglucosyl isofucosterols (**1a**–**1d**) from the cell cultures.




**1a** $R_1$ = palmitate, $R_2$ = H  **2a** $R_1$ = palmitate, $R_2$ = Ac
**1b** $R_1$ = stearate, $R_2$ = H  **2b** $R_1$ = stearate, $R_2$ = Ac
**1c** $R_1$ = oleate, $R_2$ = H  **2c** $R_1$ = oleate, $R_2$ = Ac
**1d** $R_1$ = linoleate, $R_2$ = H  **2d** $R_1$ = linoleate, $R_2$ = Ac

## RESULTS AND DISCUSSION

The diethyl ether-soluble fraction of the ethanol aqueous extract of a cell suspension culture of *L. esculentum* was chromatographed on a silica gel column to recover a fatty acids fraction. After methylation of the free fatty acids and repeated column chromatography this yielded a mixture of acylglucosyl sterols (**1a**–**1d**) which could not be separated into individual compounds.

The presence of pseudomolecular ions at *m/z* 839, 837, 835 and 811 $[M-H]^-$ and a fragment of *m/z* 411, in the negative-ion FAB mass spectrum, suggested the presence of at least four compounds in the mixture and a $C_{29}$-aglycone in the molecule. The IR bands at 1740, 1180 and 1050 $cm^{-1}$ were characteristic of an ester linkage. The presence, in the $^1H$ NMR spectrum of the mixture **1a**–**1d**, of a very intense broad signal at $\delta$ 1.26 (due to a long methylene chain) and of a triplet at $\delta$ 2.35 ($J$ = 7.5 Hz, —$CH_2$—COO), and of a signal at $\delta$ 174.5 (acribed to C=O ester) and an intense signal at $\delta$ 29.7–29.3 (many —$CH_2$'s) in the $^{13}C$ NMR spectrum, were indicative of the presence of a long chain fatty acid moiety. The GC/MS analysis of the fatty acid methyl esters obtained from the saponification, followed by methylation, of the acylglucosyl sterols confirmed the presence of palmitate, stearate, oleate and linoleate (ratio 4.4:1:1.6:3). The presence of two angular methyl proton signals at $\delta$ 0.68 and 0.96 (each *s*), an olefinic proton signal at $\delta$ 5.35 (*br d*, $J$ = 4.8 Hz) and a multiplet at $\delta$ 3.55 (1H), in the $^1H$ NMR spectrum of the mixture **1a**–**1d**, sug-

* Author to whom correspondence should be addressed.

gested the presence of a $\Delta^5$-3$\beta$-hydroxy sterol. The sterol side chain signals appeared at $\delta$ 0.83 (3H, *d*, $J$ = 6.4 Hz, H-21), 0.95 (6H, *d*, $J$ = 7.0 Hz, H-26 and H-27), 1.57 (3H, *d*, $J$ = 6.8 Hz, H-24$^2$), 5.10 (1H, *q*, $J$ = 6.8 Hz, H-24$^1$), suggesting that the aglycone was a stigmasta-5,24(24$^1$)-diene-3$\beta$-ol. The configuration of the trisubstituted double bond in the side chain was assigned as *Z* by the $^1$H NMR and $^{13}$C NMR chemical shifts ($\delta$ 1.57 and 12.7 for $^1$H and $^{13}$C, respectively) of the vinyl methyl and by $^{13}$C NMR chemical shifts ($\delta$ 27.2 and 28.6 for C-23 and C-25, respectively) of the vinyl carbon atoms. These data were consistent with those of isofucosterol and $\beta$-D-glucosyl isofucosterol previously reported [8, 9]. The identity of the sterol moiety was confirmed by the comparison of spectral data of the free sterol obtained from the acid hydrolysis of mixture (**1a**–**1d**) with those of isofucosterol [9]. The water soluble residue of the hydrolysate was analysed by high-performance anion exchange (HPAE) chromatography giving D-glucose. Acetylation of **1a**–**1d** with acetic anhydride in pyridine at room temperature gave a tri-acetyl derivative, indicating that only one molecule of fatty acid was ester linked per glucosylsterol. The position of the ester linkage was established on the basis of the acetylation shift [10] in the $^{13}$C chemical shifts of the carbon atoms of the sugar moiety. The carbon signals due to the sugar moiety of acetylated glucosyl sterols (**2a**–**2d**) were consistent with published data for acetylated glucosyl sterol [6, 8], as well as of an anomeric proton signal at $\delta$ 4.58 ($J$ = 7.9 Hz) and confirmed the presence of a $\beta$-D-glucopyranoside moiety. Comparison of the $^{13}$C NMR signals of **1a**–**1d** attributed to the glucosyl moiety with those of isofucosterylglucoside previously reported [8] indicated that the fatty acids were ester linked to the hydroxyl group at the C-6′ of glucose. In fact the deshielding of C-6′ ($\delta$ 63.8) and the shielding of C-5′ ($\delta$ 73.8) are in agreement with acylation on C-6′. Thus, the structures of the components of the mixture (**1a**–**1d**) were identified as 3-*O*-[6′-*O*-palmitoyl-$\beta$-glucosyl]-stigmasta-5,24(24$^1$)*Z*-diene; 3-*O*-[6′-*O*-stearyl-$\beta$-glucosyl]-stigmasta-5,24(24$^1$)*Z*-diene; 3-*O*-[6′-*O*-oleyl-$\beta$-glucosyl]-stigmasta-5,24(24$^1$)*Z*-diene; 3-*O*-[6′-*O*-linoleyl-$\beta$-glucosyl]-stigmasta-5,24(24$^1$)*Z*-diene.

Guevara *et al.* [6] reported that similar acylglucosylsterols are able to protect *in vivo* against the mutagenic effect of mitomycin C. We have evaluated the antimutagenic potential of acylglucosyl-isofucosterols **1a**–**1d** using the *Salmonella typhimurium* assay [11]. In particular, their ability to effect the mitomycin C-induced mutagenicity in TA 102 *S. typhimurium* tester strain was assessed. In the range of tested concentrations (1–500 $\mu$g/plate) the acylglucosyl isofucosterols **1a**–**1d** were not toxic. Furthermore, they were not mutagenic, as no increase of the spontaneous revertant number (298 ± 59) was observed at all the doses tested. Mitomycin C-induced mutagenicity (8000 revertants/$\mu$g) was not affected by the addition of the compounds **1a**–**1d** up to 500 $\mu$g/plate concentration.

Our results do not confirm the antimutagenic potential of acylglucosyl sterols. The discrepancy with Gueavara *et al.* [6] could be ascribed either to the different chemical structures of the tested compounds or to the different biological assay employed for assessing the antimutagenic properties.

## EXPERIMENTAL

*General and cell cultures.* As described in Ref. [12].

*Extraction and isolation of compounds.* Tissue (50 g dry weight) was extracted with 70% aq. EtOH (3 × 500 ml). The combined extracts were concd *in vacuo* and the aq. residue extracted with $Et_2O$. The $Et_2O$-soluble fr. (4.9 g) was chromatographed on silica gel column, eluted with a solvent gradient from $CHCl_3$ to $CHCl_3$-MeOH (9:1). Frs with the same TLC profile were combined. The fr. containing fatty acids was methylated with $CH_2N_2$ and then chromatographed on a silica gel column, eluted with petrol–$Et_2O$ (4:1) recovering compounds **1a**–**1d**, as a mixt., showing a single spot on TLC. All efforts to separate this mixt. were unsuccessful.

3-*O*-[6′-*O*-*acyl*-$\beta$-D-glucosyl]-stigmasta-5,24(24$^1$)Z-diene (**1a**–**1d**). Amorphous solid, 150 mg: mp 134–136°; $[\alpha]_D$ = −24° ($CHCl_3$; *c* 0.9); IR $\nu_{max}^{film}$ cm$^{-1}$: 3600–3080, 2950, 1740, 1455, 1360, 1180, 1050. neg-FABMS *m/z* 839, 837, 835 and 811 $[M-H]^-$, 411 [M-Glc-fatty acid]$^-$. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 5.35 (1H, *br d*, $J$ = 4.8 Hz, H-6), 5.10 (1H, *q*, $J$ = 6.8 Hz, H-24$^1$), 4.50 (1H, *dd*, $J$ = 12, 4.5 Hz, H-6), 4.37 (1H, *d*, $J$ = 7.8 Hz, H-1′), 4.25 (1H, *dd*, $J$ = 12, 2.9 Hz, H-6′), 3.55 (2H, *m*, H-3 and H-5′), 3.50 (1H, *m*, H-2′), 3.45 (1H, *m*, H-4′), 3.30 (1H, *m*, H-3′), 2.83 (1H, *q*, $J$ = 7.0 Hz, H-25), 2.32 (1H, *dd*, $J$ = 12.9, 2.6 Hz, H-4eq), 2.20 (1H, *dd*, $J$ = 12.9, 8.6 Hz, H-4ax), 2.05 (1H, *m*, H-12), 1.78 (1H, *m*, H-2), 1.75 (2H, *m*, H-1), 1.68 (1H, *m*, H-7), 1.65 (1H, *m*, H-23), 1.58 (1H, *m*, H-15), 1.57 (3H, *d*, $J$ = 6.8 Hz, H-24$^2$), 1.49 (2H, *m*, H-11), 1.48 (1H, *m*, H-2), 1.40 (1H, *m*, H-7), 1.38 (2H, *m*, H-22), 1.30 (2H, *m*, H-8 and H-20), 1.16 (3H, *m*, H-16 and H-17), 1.10 (1H, *m*, H-15), 1.05 (1H, *m*, H-12), 0.96 (4H, *m*+*s*, H-14 and H-19), 0.95 (6H, *d*, $J$ = 7.0 Hz, H-26 and H-27), 0.90 (1H, *m*, H-9), 0.83 (3H, *d*, $J$ = 6.4 Hz, H-21), 0.68 (3H, *s*, H-18) signals due to fatty acid chains $\delta$ 5.38–5.30 (*m*), 2.75 (*dd*, $J$ = 6.4 Hz), 2.35 (*t*, $J$ = 7.5 Hz), 1.26 (*m*), 0.88 (*t*, $J$ = 6.6 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 145.9 (*s*, C-24), 140.5 (*s*, C-5), 122.1 (*d*, C-6), 116.5 (*d*, C-24$^1$), 101.4 (*d*, C-1′), 79.7 (*d*, C-3), 76.5 (*d*, C-4′), 73.8 (*d*, C-5′), 73.5 (*d*, C-2′), 70.4 (*d*, C-3′), 63.8 (*t*, C-6′), 56.8 (*d*, C-14), 56.0 (*d*, C-17), 50.2 (*d*, C-9), 42.4 (*s*, C-13), 39.8 (*t*, C-12), 38.8 (*t*, C-4), 37.4 (*t*, C-1), 36.8 (*s*, C-10), 36.2 (*d*, C-20), 36.0 (*t*, C-22), 32.0 (*t*, C-7), 31.9 (*d*, C-8), 29.3 (*t*, C-2), 28.6 (*d*, C-25), 27.9 (*t*, C-16), 27.2 (*t*, C-23), 24.3 (*t*, C-15), 21.1 (*t*, C-11), 21.0 (*q*, C-26 and C-27), 19.3 (*q*, C-19), 18.8 (*q*, C-21), 12.7 (*q*, C-24$^2$), 11.8 (*q*, C-18), signals due to fatty acid chains $\delta$ 174.5 (C=O), 130.8

(*d*), 130.3 (*d*), 130.0 (*d*), 129.9 (*d*), 128.1 (*d*), 127.9 (*d*), 34.4 (*t*, C-2″), 32.7 (*t*), 29.7–29.3 (*t*), 25.2 (*t*), 22.9 (*t*), 14.1 (*q*).

*Acetylation of* **1a–1d**. A soln of the mixt. of **1a–1d** (15 mg) in pyridine (3 mol) and $Ac_2O$ (0.5 ml) was kept at room temp. over night. The excess reagents were removed *in vacuo*, and the residue was partitioned between $H_2O$ and $Et_2O$. The ether extract was purified by silica gel column, petrol-$Et_2O$ (4:1) as eluent, to obtain **1a–1d** acetates (**2a–2d**, 12 mg).

3-*O*-[6′-*O*-*acyl*-β-D-glucosyl]-stigmasta-5,24(24[1])Z-diene acetate (**2a–2d**): mp 172–174°; IR $\nu_{max}^{film}$ $cm^{-1}$: 3055, 2950, 1740, 1455, 1360, 1240, 1140, 1080. neg-FABMS *m/z* 965, 963, 961 and 937 [M-H]$^-$, 411 [M-Glc(Ac)$_3$-fatty acid]$^-$. $^1$H NMR (500 MHz, $CDCl_3$): δ 5.35 (1H, *br d*, *J* = 4.8 Hz), 5.20 (1H, *dd*, *J* = 9.5, 9.3 Hz), 5.10 (1H, *q*, *J* = 6.8 Hz), 5.04 (1H, *dd*, *J* = 9.5, 8.1 Hz), 4.95 (1H, *t*, *J* = 8.7 Hz), 4.58 (1H, *d*, *J* = 7.9 Hz), 4.22 (1H, *dd*, *J* = 12.1, 5.1 Hz), 4.10 (1H, *dd*, *J* = 12.1, 2.6 Hz), 3.67 (1H, *m*), 3.48 (1H, *m*), 2.83 (1H, *q*, *J* = 7.0 Hz), 2.77 (*dd*, *J* = 6.4 Hz), 2.32 (1H, *dd*, *J* = 12.9, 2.6 Hz), 2.23 (1H, *dd*, *J* = 12.9, 8.6 Hz), 2.05 (3H, *s*), 2.02 (3H, *s*), 2.00 (3H, *s*), 1.59 (3H, *d*, *J* = 6.8 Hz), 1.26 (*m*), 0.96 (3H, *s*), 0.95 (6H, *d*, *J* = 7.0 Hz), 0.90 (1H, *m*), 0.83 (3H, *d*, *J* = 6.4 Hz), 0.88 (*t*, *J* = 6.6 Hz), 0.68 (3H, *s*). $^{13}$C NMR (125 MHz, $CDCl_3$): δ 173.5 (*s*), 170.4 (*s*), 169.4 (2*s*), 145.8 (*s*), 140.3 (*s*), 130.8 (*d*), 130.3 (*d*), 130.0 (*d*), 129.9 (*d*), 128.1 (*d*), 127.9 (*d*), 122.1 (*d*), 116.4 (*d*), 99.6 (*d*), 80.1 (*d*), 72.9 (*d*), 71.8 (*d*), 71.5 (*d*), 68.7 (*d*), 62.0 (*t*), 56.7 (*d*), 56.0 (*d*), 5.1 (*d*), 42.3 (*s*), 39.7 (*t*), 38.9 (*t*), 37.2 (*t*), 36.7 (*s*), 36.1 (*d*), 35.9 (*t*), 34.4 (*t*), 32.7 (*t*), 31.9 (*t*), 31.8 (*d*), 29.7–29.3 (*t*), 28.6 (*d*), 27.9 (*t*), 27.2 (*t*), 25.2 (*t*), 24.3 (*t*), 22.9 (*t*), 21.2 (*t*), 21.1 (*q*), 21.0 (*q*), 19.3 (*q*), 18.8 (*q*), 14.1 (*q*), 12.7 (*q*), 11.8 (*q*).

*Acid hydrolysis of mixture* **1a–1d**. The mixt. **1a–1d** (20 mg) was heated in 2N HCl (0.5 ml) at reflux for 30 min. The reaction mixture was extracted with EtOAc and the solvent evaporated to dryness under $N_2$. The water-soluble residue of the hydrolysate was analysed by HPAE-PAD giving D-glucose. The EtOAc extract was subjected to prep HPLC to isolate the free sterol (5 mg).

*Saponification of mixture* **1a–1d**. A soln of the mixt. **1a–1d** (15 mg) in 3 ml of MeOH-3M NaOH (1:1) was kept at 50° and under stirring over night. The reaction mixt., after acidification with 2N HCl, was extracted with $Et_2O$ and the solvent evapd to dryness under $N_2$. The $Et_2O$ extract was methylated with $CH_2N_2$ and then purified by silica gel column, petrol–$Et_2O$ (19:1) as eluent. The resulting fatty acid methyl esters were analysed by GC/MS, using a Fisons Trio 2000 spectrometer connected to a Fisons GC 8000 gas chromatography, equipped with a Supelco SPB-1 fused silica column 30 m × 0.25 mm, 0.25 μm film thickness at 60° for 2 min then 10°/min to 280°, giving palmitate, linoneate, oleate and stearate methyl esters at a ratio of 4.4:3:1.6:1.

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