Pergamon

PII: S0031-9422(96)00657-7

Phytochemistry, Vol. 44, No. 5, pp. 861–864, 1997

0031-9422/97 $17.00+0.00

TRITERPENOIDS AND STEROL GLUCOSIDE FROM CELL CULTURES OF *LYCOPERSICON ESCULENTUM*

SALVATORE DE ROSA,* ALFONSO DE GIULIO and GIUSEPPINA TOMMONARO

Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R., Via Toiano, 6, 80072 Arco Felice (Napoli), Italy

(*Received in revised form* 3 *September* 1996)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; cell cultures; lupeol; cylcoartenol; isofucosterol; isofucosteryl glucoside.

Abstract—Lupeol, cycloartenol, isofucosterol and the new β-D-glucosyl isofucosterol, have been isolated from the cell cultures of *Lycopersicon esculentum*. The structures were elucided from chemical and spectroscopic evidence. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In a preceding paper [1], we reported the isolation of aliphatic and aromatic glycosides from cell cultures of *Lycopersicon esculentum*. L. var. S. Marzano that have an important rule in the economy of the Campania region of southern Italy. This paper describes the isolation from cell cultures and the structural elucidation of lupeol (**1**), cycloartenol (**2**), isofucosterol (**3a**) and the new β-D-glucosyl isofucosterol (**3b**) on the basis of chemical and spectroscopic evidence.

RESULTS AND DISCUSSION

Silica gel chromatography of the chloroform-soluble fraction of the ethanol aqueous extract of cell suspension cultures of *L. esculentum* yielded two main fractions. The less polar fraction was further purified by HPLC to give three compounds that, in order of polarity, were: lupeol (**1**), cycloartenol (**2**), and isofucosterol (**3a**). The polar fraction after crystallization from chloroform gave **3b**.

Compound **1** had a molecular formula $C_{30}H_{50}O$ (HR-mass spectrum), the structure was established from its spectral data. The ^1H and ^{13}C NMR spectra and the two-dimensional NMR experiments (COSY, HETCOR and HMBC) suggested that **1** was lupeol. The spectral data of **1** were in excellent agreement with published data [2–4]. Lupeol is one of the most widespread of pentacarbocyclic triterpenes of plants [2, 5] and was also isolated from seeds of *L. esculentum* [6].

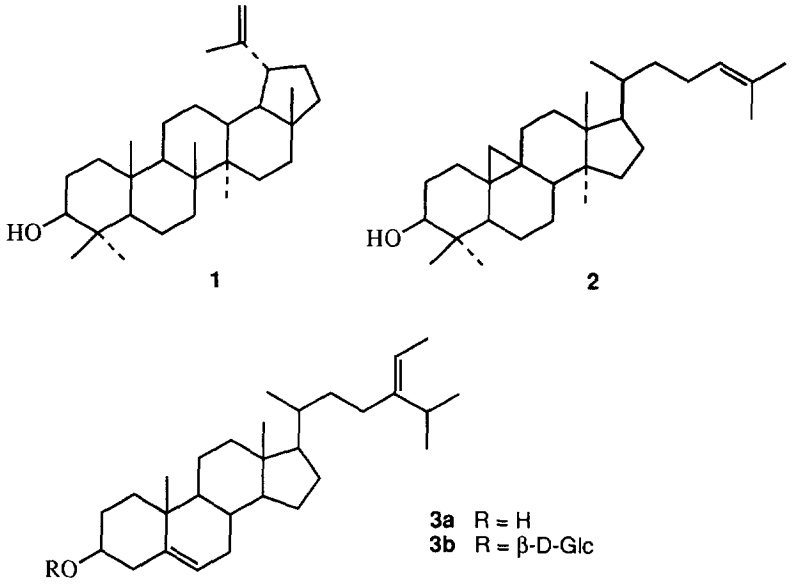
Compound **2** with a molecular formula $C_{30}H_{50}O$, is an isomer of **1**. The presence, in the ^1H NMR spectrum, of signals at δ 0.33 and 0.56 (2H, AB doublets, $J = 4.3$ Hz), characteristic of a cyclopropane ring, suggested a cycloartane skeleton. Besides, the only double bond was localized on C-24, based in the presence in the ^1H NMR spectrum of two vinylic methyl signals at δ 1.68 and 1.60 (3H, each), long range coupled with the olefinic proton at δ 5.10 (from COSY). The ^{13}C NMR spectrum and the two-dimensional NMR experiments (COSY, HETCOR and HMBC) confirmed that compound **2** was cycloart-24-en-3β-ol. The spectral data of **2** were in excellent agreement with published data [7].

Compound **3a** had a molecular formula $C_{29}H_{48}O$ (HR-mass spectrum), the structure was established from its spectral data. The NMR data suggested that compound **3a** was stigmasta-5,24(24′)*Z*-3β-ol. The spectral data of **3a** were in excellent agreement with published data [8].

Cycloartenol (**2**) and isofucosterol (**3a**), the 24(24′)-diastereoisomer of the more common fucosterol, are typical phytosterols of higher plants [9–11]. Isofucosterol has also been isolated from algae [12] and from some marine organisms [13, 14].

Compound **3b** had $[\alpha]_D$ −40.8° and a molecular formula $C_{35}H_{58}O_6$ suggested from FAB-mass spectral and NMR data. The presence of a pseudomolecular ion at *m/z* 573 $[M-H]^-$ and a fragment at *m/z* 411 $[M-Glc]^-$, in the negative-ion FAB-mass spectrum, suggested the presence of a sugar and a C-29 aglycone in the molecule. The presence of two angular methyl proton signals at δ 0.59 and 0.91 (each *s*), an olefinic proton signal at δ 5.27 (*br d*, $J = 4.8$ Hz) and a multiplet at δ 3.48 (1H), in the ^1H NMR spectrum of **3b**, suggested the presence of a Δ^5-3β-hydroxy sterol. The side chain signals appeared at δ 0.85 (3H, *d*, $J = 6.4$ Hz, H-21), 0.87 (6H, *d*, $J = 7.0$ Hz, H-26 and H-27),

* Author to whom correspondence should be addressed.



1.49 (3H, *d*, $J = 6.8$ Hz, H-29), 5.0 (1H, *q*, $J = 6.8$ Hz, H-28), suggesting that the aglycone was a stigmasta-5,24(24′)-diene-3β-ol.

The configuration of the trisubstituted double bond in the side chain was assigned as *Z* by ^{1}H NMR and ^{13}C NMR chemical shifts (δ 1.49 and 12.3 for ^{1}H and ^{13}C, respectively) of the vinyl methyl and by ^{13}C NMR chemical shifts (δ 27.5 and 28.3 for C-23 and C-25, respectively) of the vinyl carbon atoms. Acetylation of **3b** with acetic anhydride in pyridine at room temperature gave a tetra-acetyl derivative. The identity of the sterol moiety was confirmed by the comparison of spectral data of the free sterol obtained from the acid hydrolysis of **3b** with those of isofucosterol (**3a**). The carbon signals due to the sugar moiety of **3b**, as well as of an anomeric proton signal at δ 4.3 ($J = 7.8$ Hz) indicated the presence of a β-D-glucopyranoside moiety. Acid hydrolysis of **3b** yielded D-glucose, identified by high-performance anion exchange (HPAE). Thus, the structure, of **3b** was formulated as 3-*O*-[β-D-glucosyl]-stigmasta-5,24(24′)*Z*-diene.

Steroidal saponins bearing a 24-ethylidene group are rare in nature. While a fucosteryl-rhamnoside has been isolated from *Cleome viscosa* [15], the compound **3b** is the first glucoside of this class of steroids.

EXPERIMENTAL

General and cell cultures. As described in ref. [1].

Extraction and isolation of compounds. Tissue (25 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 $CHCl_3$. The $CHCl_3$-soluble fr. (4.9 g) was chromatographed on a silica gel column, eluted with a solvent gradient from $CHCl_3$ to $CHCl_3$–MeOH (4:1) yielding two main frs. The less polar fr. was further purified on HPLC (Spherisorb S5W; *n*-hexane–EtOAc (4:1; flow 3 ml min^{-1}) recovering three compounds, **1**–**3**. The polar fraction, after crystallization from $CHCl_3$, gave **3b**.

Lupeol (**1**). Yield 10 mg; mp 213–214°C (from MeOH); $[\alpha]_D = 26.0°$ ($CHCl_3$; *c* 0.01). EI-MS *m/z* 426.3864 $[M]^+$ ($C_{30}H_{50}O$ requires 426.3862).

Cycloartenol (**2**). Yield 12 mg; mp 113–114°C (from MeOH); $[\alpha]_D = 53.1°$ ($CHCl_3$; *c* 0.01). EI-MS *m/z* 426.3867 $[M]^+$ ($C_{30}H_{50}O$ requires 426.3862).

Isofucosterol (**3a**). Yield 36 mg; mp 128–130°C (from MeOH); $[\alpha]_D = -36.8°$ ($CHCl_3$; *c* 0.02). EI-MS *m/z* 412.3700 $[M]^+$ ($C_{29}H_{48}O$ requires 412.3705) (6), 394 $[M-H_2O]^+$ (18), 296 $[M-C_7H_{14}]^-$ (95), 281 (25), 213 (29), 147 (60), 145 (70), 81 (100).

3-O-[β-D-*Glucosyl*]-*stigmasta*-5,24(24′)*Z*-*diene* (**3b**). Crystals, 64 mg; mp 266–268°C; $[\alpha]_D = -40.8°$ ($CHCl_3$; *c* 0.05). IR ν_{max}^{Film} cm^{-1}: 3600–3080, 2950, 1455, 1360, 1140. Negative-FAB-MS *m/z* 573 $[M-H]^-$, 411 $[M-Glc]^-$, positive-FAB-MS *m/z* 597 $[M+Na]^+$. ^{1}H NMR and ^{13}C NMR data see Table 1.

Acetylation of **3b**. A soln of **3b** (15 mg) in pyridine (3 ml) and Ac_2O (0.5 ml) was kept at room temp. over night. The excess reagents were removed *in vacuo*, and the residue was partioned between H_2O and Et_2O. The Et_2O extract was purified on a silica gel column, petrol–Et_2O (4:1) as eluent, to obtain **3b**-acetate, that was crystallized from MeOH (10 mg).

3-O-[β-D-*Glucosyl*]-*stigmasta*-5,24(24′)*Z*-*diene acetate*. Crystals; mp 177–179°C. IR ν_{max}^{Film} cm^{-1}: 3055, 2950, 1740, 1455, 1360, 1240, 1140, 1080. Negative-FAB-MS *m/z* 741 $[M-H]^-$, 411 $[M-Glc(Ac)_4]^-$; positive-FAB-MS *m/z* 765 $[M+Na]^+$. ^{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 (2H, *m*), 4.96 (1H, *dd*, $J = 9.5, 8.1$ Hz), 4.58 (1H, *d*, $J = 7.9$ Hz), 4.25 (1H, *dd*, $J = 12.1, 4.7$ Hz), 4.10 (1H, *dd*, $J = 12.1, 2.6$ Hz), 3.67 (1H, *m*), 2.73 (1H, *q*, $J = 7.0$, Hz), 2.25 (2H, *m*), 2.08 (3H, *s*), 2.04 (3H, *s*), 2.02 (3H, *s*), 2.00 (3H, *s*), 1.49 (3H, *d*, $J = 6.8$ Hz), 0.98 (3H, *s*), 0.91 (6H, *d*,

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **3b** in $CDCl_3$–CD_3OD solution*

C	^{13}C	^{1}H	HMBC (J_{C-H} = 10 Hz)
1	37.0 *t*	1.75 *m*	—
2	29.3 *t*	1.78 *m*, 1.48 *m*	2.29 (H-4eq)
3	78.8 *d*	3.48 *m*	4.30 (H-1′), 2.29 (H-4eq), 1.78 (H-2)
4	38.4 *t*	2.29 *dd* (12.9, 2.6), 2.17 *dd* (12.9, 8.6)	5.27 (H-6)
5	140.0 *s*	—	2.29 (H-4eq), 2.17 (H-4ax), 0.91 (H-19)
6	121.8 *d*	5.27 *brd* (4.8)	2.29 (H-4eq), 2.17 (H-4ax)
7	31.6 *t*	1.80 *m*, 1.40 *m*	5.27 (H-6)
8	31.7 *d*	1.30 *m*	...
9	49.9 *d*	0.84 *m*	1.90–1.05 (H-12), 0.91 (H-19)
10	36.4 *s*	—	5.27 (H-6), 2.29 (H-4eq), 1.78 (H-2)
11	20.7 *t*	1.40 *m*	—
12	39.4 *t*	1.90 *m*, 1.05 *m*	0.59 (H-18)
13	42.0 *s*	—	1.05 (H-12 and H-17), 0.59 (H-18)
14	56.4 *d*	0.89 *m*	1.90 (H-12), 1.00 (H-15), 0.59 (H-18)
15	23.9 *t*	1.40 *m*, 1.10 *m*	...
16	27.9 *t*	1.16 *m*	...
17	55.7 *d*	1.05 *m*	0.59 (H-18)
18	11.4 *q*	0.59 *s*	1.05 (H-12 and H-17)
19	18.9 *q*	0.91 *s*	—
20	35.8 *d*	1.30 *m*	1.16 (H-16), 1.05 (H-17), 0.85 (H-21)
21	18.9 *q*	0.85 *d* (6.4)	—
22	35.7 *t*	1.38 *m*	0.85 (H-21)
23	27.5 *t*	1.65 *m*	2.73 (H-25)
24	145.5 *s*	—	2.73 (H-25), 0.87 (H-26 and H-27)
25	28.3 *d*	2.73 *q* (7.0)	0.87 (H-26 and H-27)
26	20.5 *q*	0.87 *d* (7.0)	—
27	20.6 *q*	0.87 *d* (7.0)	...
24^1	116.2*d*	5.00 *q* (6.8)	2.73 (H-25), 1.49 (H-29)
24^2	12.3 *q*	1.49 *d* (6.8)	5.00 (H-28)
1′	100.8 *d*	4.30 *d* (7.8)	3.48 (H-3)
2′	73.2 *d*	3.16 *m*	3.34 (H-3′ and/or H-4′)
3′	69.9 *d*	3.34 *m*	3.73–3.65 (H-6′), 3.48 (H-1′), 3.20 (H-5′)
4′	76.2 *d*	3.34 *m*	3.15 (H-2′)
5′	75.5 *d*	3.27 *m*	3.73–3.65 (H-6′)
6′	61.5 *t*	3.73 *dd* (12.0, 2.9), 3.65 *dd* (12.0, 4.5)	—

* Chemical shifts are referred to TMS. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses.

J = 7.0 Hz), 0.87 (3H, *d*, J = 6.4 Hz), 0.64 (3H, *s*). ^{13}C NMR (125 MHz, $CDCl_3$: δ 170.5 (*s*), 170.3 (*s*), 169.4 (2*s*), 145.8 (*s*), 140.3 (*s*), 122.2 (*d*), 116.4 (*d*), 99.6 (*d*), 80.1 (*d*), 72.9 (*d*), 71.7 (*d*), 71.5 (*d*), 68.5 (*d*), 62.1 (*t*), 56.7 (*d*), 55.9 (*d*), 50.1 (*d*), 42.2 (*s*), 39.6 (*t*), 38.9 (*t*), 37.2 (*t*), 36.6 (*s*), 36.1 (*d*), 35.9 (*t*), 31.9 (*d*), 31.8 (*t*), 29.4 (*t*), 28.5 (*d*), 28.1 (*t*), 27.8 (*t*), 24.2 (*t*), 20.7 (*t*, C-11), 21.1 (*q*), 21.0 (*q*), 20.9 (*q*), 20.8 (*q*), 20.7 (*q*), 20.6 (*q*), 19.2 (*q*), 18.8 (*q*), 12.6 (*q*), 11.8 (*q*).

Acid hydrolysis of compound **3b**. Compound **3b** (15 mg) was heated in 2N HCl (0.5 ml) at reflux for 30 min. The reaction mixt. was extracted with EtOAc and the solvent evapd 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 (6 mg).

Acknowledgements—This research was supported by the Progetto Straegico 'Tecnologie Chimiche Innovative' C.N.R. Rome. We thank Dr M. C. Manca for the sugar analyses. Mass spectra and NMR spectra were provided by 'Servizio di Spettrometria di Massa del CNR-Napoli' and "Servizio NMR, ICMIB-CNR", respectively. The assistance of the staff of both services is gratefully acknowledged.

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