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ACYLGLUCOSYL STEROLS FROM *MOMORDICA CHARANTIA**

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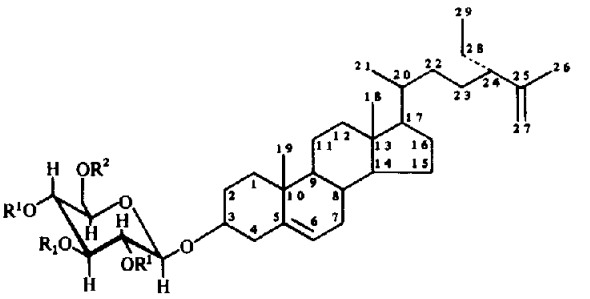
Key Word Index—*Momordica charantia*; Cucurbitaceae; balsam pear; bitter gourd, acylglucosyl sterol; 3-*O*-[6′-*O*-palmitoyl-β-D-glucosyl]-stigmasta-5,25(27)-diene, 3-*O*-[6′-*O*-stearyl-β-D-glucosyl]-stigmasta-5,25(27)-diene

Abstract—A mixture of acylglucosylsterols was isolated from the green fruits of *Momordica charantia* (balsam pear or bitter gourd) and the structure elucidated by high field ^{1}H NMR, ^{13}C NMR, FTIR and mass spectrometry and chemical modification studies followed by spectral and chromatographic analysis The major acylglucosyl sterol was 3-*O*-[6′-*O*-palmitoyl-β-D-glucosyl]-stigmasta-5,25(27)-diene while the minor component was 3-*O*-[6′-*O*-stearyl-β-D-glucosyl]-stigmasta-5,25(27)-diene. The isolation and structure elucidation of these acylglucosyl sterols are reported for the first time.

INTRODUCTION

A number of studies have been made on the chemical constituents of *Momordica charantia* and other plants of the family of Cucurbitaceae. In 1965, Sucrow [1] reported the isolation of charantin, a 1:1 mixture of the glucosyl derivatives of sitosterol and the then new compound stigmasta-5,25-diene-3β-ol. Subsequently, Okabe and co-workers [2–6], isolated and characterized a number of triterpene glycosides from the fruits and seeds. Ulubelen [7] studied the steroid and hydrocarbon constituents of the leaves while Ishikawa studied the fatty acid and sterols [8], the steam volatile constituents [9], and the triterpene alcohols [10] of the seed oil. Akihisa [11, 12] reported the sterol compositions of the seeds and mature plant materials from 32 species of 12 genera of the family Cucurbitaceae, among which was *Momordica charantia*. The results showed the predominance of Δ^7-sterols and the presence of saturated and Δ^5- and Δ^8-sterols. In most cases, however, sufficient amounts of the Δ^5- and Δ^8-sterols were not isolated to allow extensive characterization.



1a R^1 = H, R^2 = palmitate
1b R^1 = H, R^2 = stearate
2a R^1 = Me—C(=O)—, R^2 = palmitate
2b R^1 = Me—C(=O)—, R^2 = stearate

RESULTS AND DISCUSSION

An intractable mixture of acylglucosyl sterols (**1a**, **1b**) was isolated from the ethanol extract of the green fruits of *Momordica charantia* and purified by repeated and sequential column chromatography followed by preparative HPLC

The ^{1}H NMR signals of the acetylated mixture (**2a**, **2b**) which were attributed to the sterol moiety were consistent with published data for stigmasta-5,25(27)-diene-3β-ol [1, 13, 14]. The side chain signals appeared at δ0.92 (3H, *d*, *J* = 6.5 Hz, H-21), 0.82 (3H, *t*, *J* = 7 Hz, H-29), 1.54 (3H, *br s*, H-26), and 4.73 (1H, *m*, *J* = 1 Hz, H-27)/4.56 (1H, *br d*, *J* = 2 Hz, H-27). The olefinic signals at δ4.73 and 4.56 indicated the presence of an exo-methylene group (H-27). The angular methyl groups appeared at δ0.68 (3H, *s*, H-18) and 1.01 (3H, *s*, H-19). An olefinic signal appeared at δ5.36 (1H, *br d*, *J* = 5 Hz, H-6) and a multiplet at δ3.54 (1H, *m*, *J* = 5 Hz, H-3), characteristic of Δ^5-3β-hydroxy sterols.

The identity of the sterol moiety was confirmed by the EI mass spectrum of the free sterol obtained from the methanolysis of the acylglucosyl sterol mixture (**1a**, **1b**). The spectrum showed $[M]^+$ at *m/z* 412, corresponding to $C_{29}H_{48}O$, together with fragment ions at *m/z* 397 $[M-Me]^+$, 394 $[M-H_2O]^+$, 271 $[M-C_{10}H_{19}$ (side chain)$-2H]^+$, indicating that it was a C_{29}-sterol with two double bonds, one in the C_{10}-side chain and the other

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in the skeleton [15]. The fragment ions at *m/z* 213, 231 and 273 are typical of the Δ^5-3β-hydroxysterol nucleus [16]. The ions at *m/z* 328 and 314 are diagnostic of C-25 unsaturated side chains of sterols [17]. The occurrence of these two ions has been explained by the migration of the C-25 double bond prior to fragmentation, an electron impact-induced rearrangement of the Δ^{25}- to its Δ^{24}-isomer followed by a McLafferty rearrangement via a six- or seven-membered cyclic transition state This mass fragmentation pattern is consistent with the structure of stigmasta-5,25(27)-diene-3β-ol [14, 15].

Final confirmation of the identity of the sterol moiety was provided by the ^{13}C NMR spectrum of the arylglucosyl sterol mixture (**1a**, **1b**) (Table 1). Comparison with spectral data published in the literature of related sterols indicated that the chemical shifts of the signals due to the ring system carbons (C-1 through C-19) agreed well with sitosterol [13], confirming the presence of a Δ^5-nucleus. The chemical shifts of the signals due to the side chain (C-20 to C-29) were consistent with a 25(27) double bond [18].

The very good match of the signals of the sterol side chain in terms of ^{1}H and ^{13}C NMR chemical shifts and coupling constants with data published for C-24-alkyl sterols with confirmed 24β-configuration suggested that the stigmasta-5,25(27)-diene moiety also possessed the 24β-ethyl configuration, consistent with previous observations for $\Delta^{25(27)}$-sterols [11, 14, 19–21]

The very intense broad ^{1}H NMR singlet at δ1.26 (ascribed to a long methylene chain) and the triplet at 2 36 (2H, *t*, J=6 5 Hz, $-CH_2-C=O$), the ^{13}C NMR signal at 174.6 (ascribed to $-C=O$) and the intense signal at δ29 (many $-CH_2$'s) and the IR peak at 1734 cm^{-1} (ester linkage) were indicative of the presence of a long chain fatty acid. The GC-MS of the fatty acid methyl esters obtained from the saponification reaction (followed by

Table 1 ^{13}C NMR chemical shifts

C	**1a, 1b**	Sitosterol [13] Δ^5-3β-OH	Sitosterol [13] Δ^5-3β-OAc	Stigmastadiene-3β-OAc [18] $\Delta^{7,25(27)}$	Stigmastadiene-3β-OAc [18] $\Delta^{8\ 25(27)}$
1	37 3	37 3	37 0	36 8	34 8
2	29 3	31 8	27 8	27.5	27 2
3	74.0	71 8	74.0	73 4	78 8
4	38 9	42 3	38 1	33 8	36 0
5	140 3	140 8	139 1	40 1	47 1
6	122 2	121 7	122 6	29 5	20 7
7	32 0	32.0	31.9	117 3	28 1
8	31 9	32 0	31.9	139 5	133 3
9	50 2	50.2	50 0	49 3	134 8
10	36 7	36 6	36.6	34 2	36 2
11	21 1	21 3	21 0	21 4	21 8
12	39 8	39 8	39 7	39 5	25 4
13	42 3	42 3	42 3	43 3	44 5
14	56.8	56 9	56 7	55 0	49 8
15	24 3	24 4	24 3	23 0	31 0
16	28 2	28 9	28.2	27 9	30 7
17	56 1	56 1	56.0	56 0	50 4
18	11 8	12.2	11 9	12 1	15 7
19.	19 4	19 4	19.3	13 0	18 8
20	36 3	40 4	36 3	36 0	36 2
21	18 7	21 1	18 8	18 8	18.6
22	33 7	33 8	33 9	33 6	33 9
23	29 2	29.4	26 4	29 5	29 7
24	49 5	51 3	46 1	49 5	49 5
25	147 5	32 0	29.0	147 4	147 6
26	17 8	21.3	19 0	17 7	17.8
27	111 4	19.0	19 0	111 4	111 4
28	26 5	25 5	23 0	26 5	26 6
29	11 9	12 1	12 3	11 8	12 0
1′	101 3				
2′	73 9				
3′	76 3				
4′	70 5				
5′	76 3				
6′	63.4				
1″	174 6				
2″	32 0				
3″–14″	29 7/29.3				
15″	22 7				
16″	14 1				

methylation) of the acylglucosyl sterol confirmed the presence of palmitate and stearate at a ratio of 2.3:1.

The ^{1}H NMR signals of **2a**, **2b** attributed to the sugar moiety indicated the presence of a β-D-glucose [22]. The anomeric proton appeared as a sharp doublet at δ4.58 (1H, *d*, J=8 Hz, H-1′), indicative of a β-D-glucosidic linkage. The non-equivalent protons H-6′ appeared as doublets of doublets at δ4.22 (1H, *dd*, $J_{6'A6'B}$=12 Hz, $J_{6'A5'}$=5 Hz, H-6′$_A$) and 4.12 (1H, *dd*, $J_{6'B6'A}$=12 Hz, $J_{6'B5'}$=2.5 Hz, H-6′$_B$). The H-5′ signal appeared at δ3.70 (1H, *m*, $J_{5'6'B}$=2.5 Hz, H-5′) while H-2′, H-3′, and H-4′ gave rise to triplets at δ4.95 (1H, *t*, $J_{2'3'}$=8.5 Hz, H-2′), 5.21 (1H, *t*, $J_{3'4'}$=9.5 Hz, H-3′), and 5.05 (1H, *t*, $J_{4'5'}$=9.5 Hz, H-4′), respectively.

Comparison of the ^{13}C NMR signals of **1a**, **1b** attributed to the glucosyl moiety with published data on aldopyranoses [23, 24] also confirmed the identity of the β-D-glucose. Further comparison with data on methyl glycopyranoses indicated that the fatty acids were ester-linked to the hydroxyl group at the C-6′ of glucose.

Similar comparison of the ^{1}H NMR glucosidic signals of **1a**, **1b** and the glucosylsterol obtained from the saponification reaction of **1a**, **1b** showed that only the signals due to the H-6′ were significantly shifted upfield upon saponification. On the other hand, the ^{1}H NMR spectrum of the acetylated glucosylsterol indicated that only one molecule of fatty acid was ester linked per glucosylsterol.

The sole presence of D-glucose as the sugar unit was confirmed by the gas liquid chromatogram of the trimethylsilylated glucose obtained from trimethylsilylation of the sugar from the methanolysis reaction of **1a**, **1b**.

In the light of these data, the identity of the major acylglucosylsterol, **1a**, isolated from *Momordica charantia* was assigned as 3-*O*-[6′-*O*-palmitoyl-β-D-glucosyl]-stigmasta 5,25(27)-diene. The minor component, **1b**, was 3-*O*-[6′-*O*-stearyl-β-D-glucosyl]-stigmasta-5,25(27)-diene. This is the first report of the isolation and structure elucidation of these acylglucosylsterols.

Similar acylglucosylsterols have been previously isolated from other sources: from snake [22] and chicken [25] epidermis, where the fatty acid components were palmitic, stearic, and oleic and the sterol component was cholesterol in snake epidermis and cholestanol/cholesterol in chicken epidermis; from plant materials like *Cucumis sativus* [26], where the sterols were sitosterol, stigmasterol and stigmastanol; from millet seeds [27] and from wheat flour [28], where the major sterols were sitosterol and campesterol.

Bioassay conducted on the acylglucosylsterols (**1a**, **1b**) using the micronucleus test, indicated high antimutagenic activity against a well-known mutagen, mitomycin C. At a dosage of 0.0125 mg/g mouse, it reduced by 80% the number of micronucleated polychromatic erythrocytes induced by mitomycin C.

EXPERIMENTAL

Analytical TLC. silica gel developed in 2 5% MeOH–EtOAc and spots visualized with vanillin-concd H_2SO_4 spray and heating, prep HPLC silica gel 20 μ column (60 cm × 8 mm i.d.), 40% THF–hexane as mobile phase for **1a**, **1b** and 20% EtOAc–hexane for the free sterol (flow rate, 4 ml/min), RI detector; GC of the fatty acid methyl esters: 1 8 m × 4.5 mm 10% polyethyleneglycol succinate on celite at 180°, GLC of the trimethylsilylated glucose: 25 m × 0.2 mm vitreous silica gel column at 160° for 40 min then 3°/min to 200°; high resolution EI-MS (70 eV)· solid sample probe, low resolution EI-MS (70 eV). injection probe; FTIR spectra: KBr; ^{1}H NMR, COSY-2D-^{1}H NMR, ^{13}C NMR, and ^{13}C DEPT NMR. determined at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in $CDCl_3$ for **1a**, **1b** and **2a**, **2b** and the free sterol and in DMSO-d_6 for the glucosyl sterol.

Isolation of acylglucosylsterols. Fresh green fruits of *Momordica charantia* were homogenized in distilled EtOH at room temp. The filtered extract was concd under red. pres at 40° and subsequently partitioned between H_2O, CH_2Cl_2, petrol, MeOH, and CCl_4 using the method of ref. [29]. The CCl_4 extract was subjected to repeated and sequential flash CC using vacuum elution (silica gel 60 for TLC) (Coll., personal communication) using hexane–EtOAc mixtures of varying polarities until TLC-pure fractions were obtained. The elution was monitored by analytical TLC on precoated silica gel The acylglucosyl sterols eluted with EtOAc–hexane (3 7) and gave an R_f of 0 5 on analytical TLC The pure fractions (by TLC) were finally subjected to prep HPLC to isolate the intractable mixture of acylglucosyl sterols **1a**, **1b** (R_t = 12 6 min).

Chemical modifications Acetylation was carried out in Ac_2O–pyridine at room temp Methanolysis was carried out in MeOH–HCl gas (1 M) at 60° for 18 hr under N_2 gas. The resulting free sugars and methyl glycosides were reacted with trimethylsilylimidazole in dry pyridine (50·50) and analysed by GLC. The free sterol was isolated by prep HPLC Saponification was carried out by treatment with $CHCl_3$–MeOH–10 M NaOH (2·7 1) for 1 hr at 60°. The resulting fatty acids were methylated with BF_3 in MeOH and analysed by GLC.

3-O-[6′-O-*palmitoyl* (*and stearoyl*)-β-D-*glucosyl*]-*Stigmasta*-5,25(27)-*diene* (**1a**, **1b**). Mp 122–123°; IR ν_{max}^{KBr} cm^{-1} 3424 (O-H), 3072 (=C–H), 2923 and 2852 (aliphatic C-H), 1738 (–C=O of ester), 1080–1030 (–C–O), and 888 (=CH_2); EIMS (sample probe, 70 eV) *m/z* (rel. int.)· 574 [M – fatty acid]$^+$ ($C_{35}H_{88}O_6$) (4), 412 [M – fatty acid – glucose]$^+$ ($C_{29}H_{48}O$) (16.5), 394 [$C_{29}H_{48}O$ – H_2O]$^+$ (100)

Compounds **2a**, **2b**. ^{1}H NMR (400 MHz, $CDCl_3$). 5 36 (1H, *d*, J = 5 Hz, H-6), 4.73 (1H, *m*, J = 1 Hz, H-27), 4.65 (1H, *br d*, J = 2 Hz, H-27), 1.54 (3H, *br s*, H-26), 1.01 (3H, *s*, H-19), 0 92 (3H, *d*, J = 6 5 Hz, H-21), 0 82 (3H, *t*, J = 7 Hz, H-29), 0.68 (3H, *s*, H-18), 5 21 (1H, *t*, J = 9 5 Hz, H-3′), 5 05 (1H, *t*, J = 9 5 Hz, H-4′), 4 95 (1H, *t*, J = 8.5 Hz, H-2′), 4 58 (1H, *d*, J = 8 Hz, H-1′), 4 22 (1H, *dd*, J = 12/5 Hz, H-6′), 4.12 (1H, *dd*, J = 12/2 5 Hz, H-6′), 3.70 (1H, *m*, J = 4 5 Hz, H-5′), 2.36 (2H, *t*, J = 7.5 Hz, H-2″), 1 64 (2H, *m*, H-3″), 1.26 [very intense, *br s*, (–CH_2–)$_n$], and 0 89 (3H, *t*, J = 6 Hz, terminal Me), 2.05, 2.03, 2.01 (3H each, *s*, 3–Me–C=O)

Stigmasta-5,25(27)-*diene*-3β-*ol*. Mp 119 8–121°, EIMS (injection probe, 70 eV) *m/z* (rel. int.)· 412 [M]$^+$ ($C_{29}H_{48}O$) (18), 397 [M – Me]$^+$ (15), 394 [M – H_2O]$^+$ (21), 379 [M – Me – H_2O]$^+$ (25), 314 [M – C_7H_{14}]$^+$ (16), 299 [M – C_7H_{14}]$^+$ (21), 273 [M – C_9H_{13} – H_2O]$^+$ (18), 271 [M – $C_{10}H_{19}$ – 2H]$^+$ (39), 255 [M – $C_{10}H_{19}$ – H_2O]$^+$ (31), 231 [M – $C_{10}H_{19}$ – C_3H_6]$^+$ (20), 229 [M – $C_{10}H_{19}$ – C_3H_8]$^+$ (31), 213 [M – $C_{10}H_{19}$ – C_3H_6 – H_2O]$^+$ (58).

3-O-[β-D-*glucosyl*]-*Stigmasta*-5,25(27)-*diene*. Mp >250; ^{1}H NMR (400 Hz, d_6-DMSO): 5 32 (1H, *br d*, J = 4 Hz, H-6), 4 73 (1H, *br d*, J = 1 Hz, H-27)/4 63 (1H, *br d*, J = 2 5 Hz, H-27), 4 22 (1H, *d*, J = 8 Hz, H-1′), 3 64/3 4 (2H, *m*, 2H-6′), 3 00-3 20 (4H, *br m*, H-2′, H-3′, H-4′, and H-5′), 2.89 (1H, *m*, J = 5 Hz, H-3), 1 54 (3H, narrow *m*, J = 1 Hz, H-26), 0.96 (3H, *s*, H-19), 0.88 (3H, *d*, J = 6 5 Hz, H-21), 0.76 (3H, *t*, J = 7.5 Hz, H-29), and 0 65 (3H, *s*, H-18).

Acetylated glucosyl sterol ^{1}H NMR (400 MHz, $CDCl_3$) 5 36 (1H, *br d*, J = 5 Hz, H-6), 5 22 (1H, *t*, J = 9.5 Hz, H-3′), 5 08 (1H, *t*,

$J = 9.5$ Hz, H-4'), 4.97 (1H, *t*, $J = 9.5$ Hz, H-2'), 4.73 (1H, narrow *m*, $J = 1$ Hz, H-27)/4.65 (1H, *d*, $J = 8$ Hz, H-27), 4.60 (1H, *d*, $J = 8$ Hz, H-1'), 4.26 (1H, *dd*, $J = 12/4.5$ Hz, H-6')/4.12 (1H, *dd*, $J = 12/2.5$ Hz, H-6'), 2.08, 2.05, 2.03, 2.01 (12H, *s*, 4–Me–C=O), 1.58 (3H, narrow *m*, $J = 1$ Hz, H-26), 1.00 (3H, *s*, H-19), 0.91 (3H, *d*, $J = 6.5$ Hz, H-21), 0.81 (3H, *t*, $J = 7$ Hz, H-29), and 0.68 (3H, *s*, H-18)

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