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A steryl ester from *Lepidium sativum*

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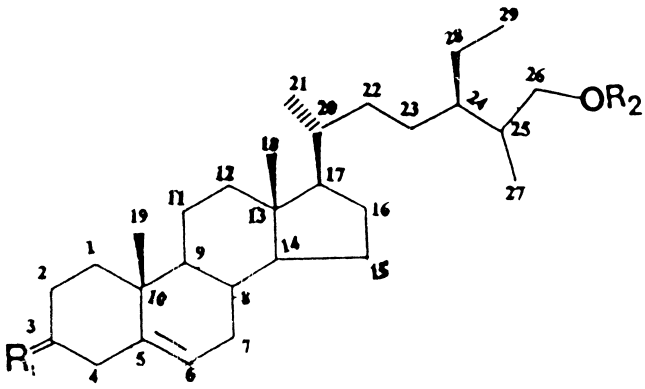
Abstract

A new steryl ester isolated from the aerial parts of *Lepidium sativum*, has been identified as stigmast-5-en-3β,27-diol 27-benzoate on the basis of spectral data analyses and chemical reactions.

Keywords: Lepidium sativum; Brassicaceae; Steryl ester; Stigmast-5-en-3β,27-diol 27-benzoate

1. Introduction

Lepidium sativum L. commonly called 'Garden cress' is a polymorphic species. Despite its great medicinal value, *L. sativum* has not received the attention it deserves. So far there are few reports on systematic investigation on its biochemistry. Pharmacological studies reveal its cardiovascular and diuretic properties (Vohra & Khan, 1977; Chandra & Babbar, 1987). Despite its potential therapeutic value no serious attempt has been made to work out its phytochemistry. This paper deals with the isolation and characterization of a new steryl ester from the aboveground parts of the plant



2. Results and discussion

Compound **1**, a steryl ester, was obtained as colorless flakes. It responded positively to the Liebermann–Burchard test indicating the sterol nature of the molecule. The IR spectrum of **1** showed the presence of a hydroxyl group (3413 cm^{-1}) and ester linkage (1734 cm^{-1}). Its mass spectrum exhibited the diagnostically important ion peaks at *m/z* 429 $[M–C_6H_5CO]^+$, 413 $[M–C_6H_5COO]^+$, 399 [413-Me]$^+$, 396 [413-H_2O]$^+$, 381 [396-Me]$^+$, 273 [M-side chain, SC, $C_{17}H_{25}O_2]^+$, 255 [273-H_2O]$^+$, 231 [273-ring D fission]$^+$, 213 [255-ring D fission]$^+$, 122 $[C_6H_5–COOH]^+$ and 105 $[C_6H_5CO]^+$ suggesting that the molecule contained a saturated side chain esterified with benzoic acid, an olefinic linkage and a hydroxyl group in the stigmastane carbon framework (Clark-Lewis & Dainis, 1967; Reichstein, Kaufman, Stocklin, & Reichstein, 1967; Gupta, Lal, & Shukla, 1981). The ion peaks appearing at *m/z* 83 $[C_{2,3}–C_{5,10}–C_{7,8}$ fission, ion a]$^+$, 69 [ion a–CH_2]$^+$, 72 $[C_{1,10}–C_{4,5}$ fission, ion b]$^+$, 54 [ion b–H_2O]$^+$, 138 $[C_{7,8}–C_{9,10}$ fission, ion c]$^+$, 120 [ion c–H_2O]$^+$, 124 [ion b–H_2O]$^+$, disclosed the presence of the hydroxyl group in ring A, which was placed at C-3 on the basis of biogenetic grounds and the existence of a vinylic linkage in ring B at C-5 (Budzikiewicz, Djerassi, & William, 1981). The saturated nature of rings C and D was inferred from ion peaks generated at *m/z* 177 $[C_{9,11}–C_{13,14}$ fission, ion d], 192 $[C_{12,13}–C_{8,14}$ fission, ion e]$^+$, 178 [ion e–CH_2]$^+$, 164 [178-CH_2]$^+$, 174 [ion e–H_2O]$^+$, 160 [174-CH_2]$^+$, 146 [160-CH_2]$^+$, 159 [177-H_2O]$^+$ and 144 [159-Me]$^+$ (Budzikiewicz et al., 1964; Dawidar & Fayez, 1969; Das, Venkateswarlu, Srinivas, & Rama Rao, 1992).

The ^{1}H NMR spectrum of **1** displayed three downfield

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multiplets at δ 7.94 (2H) assignable to H-2′, H-6′, 7.53 (2H) ascribable to H-3′, H-5′ and 7.48 (1H), accountable to H-4′ of the benzene nucleus. A one-proton doublet at δ 5.34 with coupling constant of $J=3.91$ Hz was associated with the H-6 vinylic proton. Two doublets at δ 4.22 ($J=7.08$ Hz) and 4.08 ($J=7.08$ Hz), integrated for one proton each were attributed to H-27a and H-27b of the C-27 oxygen substituted methylene group. A one-proton multiplet at δ 3.66 with $W_2^1=16.0$ Hz was due to the C-3 carbinol proton. The C-19 and C-18 tertiary methyls resonated as broad singlets at δ 1.00 and 0.67, respectively. The C-21 and C-26 secondary methyl functionalities and C-29 primary methyl group, indicating an ethyl group in the side chain located at C-24 on biogenetic grounds, appeared as doublets correspondingly at δ 0.94 ($J=6.0$ Hz), 0.87 ($J=6.0$ Hz) and 0.83 ($J=6.0$ Hz). The presence of all the methyls in the range of δ 1.00–0.67 indicated that these groups were located on saturated carbons (Alam, Chopra, Ali, Niwa, & Sakae, 1994; Sharma, Ali, & Singh, 1996). The H_3-29 resonance of 24*R*-configuration (δ 0.83) was more upshielded as compared to the 24*S*-resonance (δ 0.86) (Rubinstein, Goad, Clague, & Mulheirm, 1976). The assignments of the chemical shifts were made by comparison with δ values of the corresponding proton in the structurally similar sterols such as sitosterol (Greaca, Manaco, and Prevetera, 1990; Gupta, Ali, Alam, Niwa, & Sakae, 1992). The ^{13}C NMR spectrum suggested a sitosterol-type side chain and revealed the presence of one carbinol carbon (δ 71.83), one esterified carbon attached to a secondary oxygen-substituted carbon, an olefinic linkage and a benzene nucleus. The ^{13}C NMR values were compared with sitosterol (Greaca et al., 1990), lawsaritol (Gupta et al., 1992) and pleuchinol (Alam et al., 1994). The assignments of -CH, -CH_2, -CH_3 and olefinic carbon resonance were made in the ^{13}C-DEPT experiments and the assignments of proton signals were carried out using ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra. The signals of C-3, C-6, C-18, C-19, C-21, C-26, C-27 and C-29 in the ^{13}C NMR spectrum could be easily correlated with the corresponding protons in the ^{1}H NMR spectrum. The coupling interaction was illustrated by the ^{1}H–^{1}H correlated spectroscopy (COSY 45°C) which showed the connectivity of 3/3 to the protons at C-2 and C-4 and the connectivity of 6-H with 7-H_2. Acetylation of **1** yielded a monoacetyl product (**1a**) thus demonstrating the presence of one acetylable alcoholic group in the molecule. Jones oxidation of **1** produced a keto derivative (**1b**) which responded positively to the Zimmermann test (Barton & De Mayo, 1954), for 3-keto steroids. Alkaline hydrolysis of **1** released benzoic acid (**1d**) and a dihydroxy steroid (**1c**).

On the basis of the spectral data and chemical reactions, the structure of **1** was characterized as stigmast-5-en-3β,27-diol 27-benzoate. This is a new member of the sterol class and the first sterol isolated from *L. sativum*.

3. Experimental

3.1. Plant material

The aerial parts of *L. sativum* were collected from the Herbal Garden of Jamia Hamdard and identified by Dr. M. P. Sharma (Taxanomist), Department of Botany. A voucher specimen is preserved in the Herbarium of the Department of Botany, Faculty of Science, Jamia Hamdard New Delhi-110 062.

3.2. General

Mps were determined on a perfit melting point apparatus and are uncorr. The UV spectrum was recorded on Beckmann DU-64, IR spectrum on Shimadzu 8201 PC FTIR spectrophotometer, ^{1}H NMR on Bruker WM-400 (400 MHz FT) instrument, ^{13}C NMR spectrum on 100 MHz and mass spectrum on Jeol D-30 spectrometer.

3.3. Extraction and isolation

The dried and coarsely-powdered plant material (1 kg) was defatted with petrol and Soxhlet-extracted with MeOH. The concentrated brown colored mass (60 g) was fractionated into 0.3 M HCl soluble and insoluble portions. The HCl soluble portion was extracted with $CHCl_3$ (3×100 ml), basified with ammonia solution (pH 9) and reextracted with $CHCl_3$ (5×100 ml). The neutral portion was dried (Na_2SO_4) and concentrated under red. pres. to produce a dark brown viscous semisolid mass. It was adsorbed on alumina (60–120 mesh), dried in air and subjected to alumina CC. The column was eluted successively with petrol, $CHCl_3$ and MeOH in order of increasing polarity. Elution of the column with $CHCl_3$ (fraction 10–15) yielded colorless flakes of **1**, recrystallized from $CHCl_3$–MeOH (1:1), 105 mg (0.0093% yield), R_f 0.12, mp 140–141°C. UV_{max} nm: 205,240 ($\log\varepsilon$ 1.7, 1.0). IR_{max}^{KBr} cm^{-1} 3413, 2929, 2858, 1734, 1618, 1450, 1377, 1307, 1157, 1105, 1066, 1028, 999, 761, 729, 700. ^{1}H NMR ($CDCl_3$): δ 7.94 (2H, m, H-2′, H-6′) 7.53 (2H, m, H-3′, H-5′), 7.48 (1H, m, H-4), 5.34 (1H, d, $J=3.91$ Hz, H-6), 4.22 (1H, d, $J=7.08$ Hz, H_2-27a), 4.08 (1H, d, $J=7.08$ Hz, H_2-27b), 3.66 (1H, br, m, W_2^1 16.0 Hz, H-3a) 1.00 (3H, brs, H_3-19), 0.94 (3H, d, $J=6.0$ Hz, H_3-21), 0.87 (3H, d, $J=6.0$ Hz, $H_3=26$), 0.83 (3H, d, $J=6.0$ Hz, H_3-29), 0.67 (3H, brs, H_3-18). EIMS *m*/*z* (ret int.): 534 $[M]^+$ ($C_{36}H_{54}O_3$) (1.2), 429 (3.2), 413 (100), 400 (43.2), 399 (24.97), 396 (32.0), 381 (18.2), 317 (3.8), 353 (4.8), 329 (25.0), 315 (179), 303 (27.8), 301 (5.8), 273 (25.6), 261 (4.6), 255 (28.2), 231 (19.8), 229 (12.1), 213 (27.7), 205 (6.5), 199 (10.0), 19. 2(8.0), 177 (7.8), 174 (12.9), 164 (3.8), 163 (19.2), 160 (25.4), 159 (23.7), 146 (16.2), 144 (34.3), 138 (6.0), 131 (16.2), 124 (16.3), 122 (20.1), 120 (22.6), 105 (44.7), 95 (48.1), 83 (24.9), 72(23.1),69 (48.2), 54 (70.0). ^{13}C NMR ($CDCl_3$): δ 37.30 (C-1), 31.90 (C-2),

71.83 (C-3), 40.01 (C-4), 143.53 (C-5), 123.91 (C-6), 31.41 (C-7), 32.17 (C-8), 51.10 (C-9), 36.39 (C-10), 22.61 (C-11), 39.86 (C-12), 44.33 (C-13), 56.65 (C-14), 25.52 (C-15), 28.07 (C-16), 55.95 (C-17), 11.80 (C-18), 19.10 (C-19), 36.13 (C-20), 18.91 (C-21), 30.21 (C-22), 26.71 (C-23), 46.95 (C-24), 29.09 (C-25), 27.23 (C-26), 63.85 (C-27), 25.89 (C-28),14.01 (C-29), 129.71 (C-1′), 130 (C-2′), 128.21 (C-3′), 131.90 (C-4′), 129.0 (C-5′), 129.08 (C-6′), 178.02 (C-7′).

3.4. Acetylation of *1*

Compound **1** (10 mg) was heated with a mixture of Ac_2O (3 ml) and pyridine (1 ml) for 30 min and then left for 24 h. H_2O (10 ml) was added and the reaction mixture extracted with $CHCl_3$ (3×10 ml). The $CHCl_3$ layer was washed with water (3×5 ml), dried over Na_2SO_4 and evaporated to yield monoacetylated product (**1a**), mp 131–132°.

3.5. Jones oxidation of *1*

Compound **1** (5 mg) was dissolved in Me_2CO (10 ml) and cooled to 4°C. Freshly prepared Jones Reagent was added drop-wise till the persistence of a brown color. H_2O (20 ml) was added and the mixture was worked up as usual to produce the oxo-derivative (**1b**), mp-122–123°.

3.6. Hydrolysis of *1*

Compound **1** (15 mg) was heated with 0.1 ethanolic KOH (5 ml) for 3 h. H_2O (10 ml) was added and the reaction mixture extracted with $CHCl_3$ (3×10 ml). The organic phase after usual work up yielded sterol (**1c**), mp 135–136°. EIMS m/z (ret. int.) 430 $[M]^+$ ($C_{29}H_{50}O_2$) (11.3). The mother liquor after extraction with $CHCl_3$ was acidified (Congo red) and reextracted with $CHCl_3$ to isolate benzoic acid, mp 121–122°, Co-TLC comparable.

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