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MICROBIAL TRANSFORMATION OF SARSASAPOGENIN BY *FUSARIUM LINI*

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Key Word Index—microbial transformation; sarsasapogenin; *Fusarium lini*; spasmolytic activity; 3-acetoxysarsasapogenin; 7α-hydroxysarsasapogenin.

Abstract—The fermentation of sarsasapogenin with *Fusarium lini* yielded two metabolites identified as 3β-acetoxysarsasapogenin and 7α-hydroxysarsasapogenin. The metabolites showed dose dependent spasmolytic activity in rat duodenum.

INTRODUCTION

Sarsasapogenin (**1**), a spirostan-type steroidal sapogenin has been isolated from *Yucca schidigeria* and *Trigonella foenum-graecum* [1, 2]. The spirostan-type steroidal saponins have been extensively investigated on account of their interesting bioactivities, as well as of their economic importance as starting material in the industrial synthesis of steroidal hormones. Cortisone has been synthesized by chemicoenzymatic transformation of sarsasapogenin whereby a degradative intermediate of sarsasapogenin was hydroxylated at the 11α-position by microbial transformation and was further oxidized by a chemical method to yield cortisone [3, 4]. Steroidal sapogenins are also precursors of natural defensive chemicals in plants against microbial and predator invasions, e.g. 12-ketoporrigenin and 2,3-secoporigenin [5–7]. In this investigation, sarsasapogenin (**1**) was subjected to fermentation with *Fusarium lini* for 12 days to afford two metabolites, 3β-acetoxysarsasapogenin (**2**) and 7α-hydroxysarsasapogenin (**3**).

RESULTS AND DISCUSSION

The fermentation of sarsasapogenin (**1**) with *Fusarium lini* yielded the metabolites identified as 3β-acetoxysarsasapogenin (**2**) and 7α-hydroxysarsasapogenin (**3**). The EIMS of **2** displayed the molecular ion at *m/z* 458 which was further confirmed by FDMS. The HREI MS showed the exact molecular mass at *m/z* 458.3431 which corresponded to the molecular formula $C_{29}H_{46}O_4$ (calcd 458.3395). The ^{1}H NMR spectrum of **2** displayed a diagnostic signal at δ 5.03 (*t*, *J* = 2.4 Hz) for H-3α and a singlet for acetoxymethyl at δ 2.03. The ^{13}C NMR spectra showed resonances for 29 carbons where signals at δ 170.7 (*OCO* CH_3) and 21.5 (OCO*CH*$_3$) indicated the *O*-acetylation of **1** to afford **2**. These assignments were further compared with those of the 3-*O*-acetyl compound obtained by chemical acetylation of **1** [8].

The second metabolite was identified as 7α-hydroxy sarsasapogenin (**3**). The HREI mass spectrum of **3** displayed the molecular ion at *m/z* 432.3242 which corresponded to the molecular formula $C_{27}H_{44}O_4$ (calcd 432.3239) The ^{1}H NMR of **3** displayed a downfield signal at δ 3.67 (*td*, *J* = 3.5, 11.5 Hz) suggesting the presence of an additional OH in the molecule. The position of this OH at C-7 was deduced from the multiplicity pattern of H-7 and the HMBC correlation of H-7 (δ 3.67) with C-6 (δ 38.4) and C-8 (δ 35.3) and COSY interactions of H-7 (δ 3.67) with H_2-6 (δ 1.36, 1.67) and H-8 (δ 1.52). The stereochemistry of the new hydroxyl group was deduced from the multiplicity pattern of H-7β, which indicated the α-stereochemistry of the newly introduced hydroxyl group at C-7. Preliminary results have shown that the three sapogenins have spasmolytic activity with **2** > **3** > **1**.

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EXPERIMENTAL

General

HREI MS spectra were recorded on a Jeol-JMS HX110 mass spectrometer. The IR spectra were recorded on a JASCO IRA-1 infrared spectrophotometer and UV spectra were recorded on a Pye Unicam PV 8700 UV/visible spectrophotometer. A polatronic D polarimeter was used for measuring the optical rotations. The m.p.'s were taken on a Büchi 535 melting point apparatus and are uncorr. The purity of the samples was checked on TLC (silica gel G-254 precoated plates) and flash chromatography on silica gel was used to obtain the pure metabolites. The mass spectra were recorded on a Varian MAT 112S mass spectrometer. All ^{1}H-NMR spectra were recorded on a Bruker AMX 500 spectrometer at 500 MHz in $CDCl_3$ solution, while the ^{13}C-NMR spectra were recorded on the same instrument at 125 MHz.

Incubation experiments

Media for *Fusarium lini* was prepared by mixing the glucose (40 g), glycerol (40 ml), peptone (20 g), yeast extract (20 g) KH_2PO_4 (20 g) and NaCl (20 g) in distilled water (4.0 l). *Fusarium lini* (NRRL 68751), which were maintained on Sabraud Dextrose Agar, was cultivated in liquid medium broth and incubated on a rotary shaker for 2 days at 30°. The substrate (sarsasapogenin) (500 mg) was dissolved in 20 ml of Tween 80 and heated to get a clear soln which was evenly distributed among 40 flasks with well grown fungal cultures. The fermentation was carried out for a further 12 days. The mycelia were filtered, washed with EtOAc (500 ml) and the broth thus obtained was extracted with EtOAc (12 l). The organic extract obtained was dried over Na_2SO_4 and the solvent was evaporated on a rotary evaporator to afford a crude brown gum (1.8 g) which was adsorbed on an equal quantity of silica gel and chromatographed. Elution with 15% EtOAc:85% pet. ether yielded a brown gum identified as 3β-acetoxysarsasapogenin **2** (15 mg). Elution with 50% EtOAc:50% pet. ether afforded 7α-hydroxysarsasapogenin **3** (12 mg).

7α-hydroxysarsasapogenin (3)

M.p. 205°; $[\alpha]_D^{27}$ 7° (*c* = 1, MeOH); [M$^+$] at *m/z* 432.3221 $C_{27}H_{44}O_4$ requires 432.3239; EIMS *m/z* (rel. int.): 432 (8), 360 (15), 318 (25), 289 (22), 271 (10), 245 (5), 139 (100), 115 (27), 69 (12). ^{1}H NMR (500 MHz): 0.74 (3H, *s*, H-18), 0.98 (3H, *s*, H-19), 0.99 (3H, *d*, *J* = 7.5 Hz, H-21), 1.06 (3H, *d*, *J* = 7.0 Hz, H-27), 3.28 (1H, *d*, *J* = 11.0 Hz, H-26a), 3.92 (1H, *dd*, *J* = 3.0,11.0 Hz, H-26e), 3.67 (1H, *td*, *J* = 3.5, 11.5 Hz, H-7β), 3.99 (1H, *brs*, H-3α), 4.38 (1H, *q*, *J* = 8.0 Hz, H-16); ^{13}C NMR (125 MHz): 29.7(C-1), 26.4 (C-2), 67.7 (C-3), 32.3 (C-4), 35.5 (C-5), 38.4 (C-6), 69.9 (C-7), 35.3 (C-8), 41.3 (C-9), 35.7 (C-10), 21.1 (C-11), 40.2 (C-12), 40.4 (C-13), 56.3 (C-14), 31.7 (C-15), 80.9 (C-16), 62.1 (C-17), 16.5 (C-18), 23.7 (C-19), 42.1 (C-20), 14.3 (C-21), 109.9 (C-22), 26.0 (C-23), 25.8 (C-24), 27.1 (C-25), 65.1 (C-26), 16.1 (C-27).

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