A new stigmastane derivative from roots of *Malva parviflora*

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The roots of *Malva parviflora* yield a new stigmastane derivative along with 3-methyl-triacontane and 5α-stigmast-9(11)-en-3β-ol. The structure of the sterol has been elucidated as 5α-stigmast-9(11)-en-3-one by spectral analysis and chemical reactions.

*Malva parviflora* is used as a pot-herb, a rich source of calcium, iron and carotene. In Mexico, the plant is cooked like spinach. Infusion of the leaves is taken as a nervine tonic, decoction is used as a taenicide and for profuse menstruation. Seeds are demulcent, used to treat cough and ulcers in the bladder. In La Reunion the plant is used as an emollient. The leaf is applied as a hot poultice to wounds and swellings. A lotion is prepared from the plant for bruised limbs. In Kumaoon, the root is used for cleaning hair and washing woollen clothes. However, the plant is considered poisonous to livestock.

Results and Discussion

Column chromatography of ethanolic extract of the roots of *Malva parviflora* afforded known compounds 1 and 3, which were identified as 3-methyl-triacontane, earlier isolated from *Cotoneaster microphylla*, and 5α-stigmast-9(11)-en-3β-ol, by comparing melting points, UV, IR, NMR and MS. In addition to it, the structure of a new isolated compound 2 has been elucidated as follows:

**Compound 2:** named malvasterone, was obtained as a colourless crystalline powder from chloroform-methanol (99:1) eluants. A purple colour with vanillin-H₂SO₄ and positive Liebermann-Burchard test indicated the steroidal nature of the compound. The compound responded positively to Zimmermann test for 3-keto steroids. Its IR spectrum demonstrated the presence of carbonyl group (1715 cm⁻¹).

It had a molecular ion peak at m/z 412 corresponding to the molecular formula C₂₉H₄₈O. It indicated six degrees of double bond equivalents; four of them were completely adjusted in the four rings of the steroidal framework, one in carbonyl group and the remaining one to an olefinic linkage. The ¹H NMR spectrum of 2 exhibited the presence of one-proton multiplet at δ 5.06 assignable to H-11, two two-protons broad singlets at δ 2.20 and 2.10 attributable to C-2 and C-4 methylene groups adjacent to the carbonyl group, three broad singlets at δ 1.06, 0.86 and 0.70, integrating for three protons each, ascribable to C-19, C-21 and C-18 methyl functionalities and a nine-protons broad singlet at δ 0.83 associated with C-26, C-27 and C-29 methyl groups. The absence of any signal in between δ 3.00-4.50 ruled out the location of any carbinol protons.

The EI mass spectrum of 2 showed diagnostically important ion fragments at m/z 397 [M-Me]⁺, 369 [M-C₄H₈]⁺, 354 [369-Me]⁺, 339 [354-Me]⁺, 284 [397-C₃H₇]⁺, 256 [397-SC, 141]⁺, 241 [256-Me]⁺, 213 [256-ring D fusion]⁺, 95 [256-C₁₁₋₁₃-C₈₋₁₄ fission]⁺ and 81 [256-C₁₂₋₁₃-C₈₋₁₄ fission]⁺, which suggested the stigmastane skeleton of the sterol nucleus. The ion peaks at m/z 201 [M-side chain-ring A]⁺, 159[201-ring DJ]⁺ and 81 [C₃₋₅-C₉₋₁₀ and C₈₋₁₄-C₁₂₋₁₃ fission, CH₂CH=C-C=CH₂=CH₂=CH₃]⁺ suggested that it was not a Δ⁴-sterol. The ion fragments at m/z 70 [ion a]⁺, 138 [ion b]⁺, 124 [ion b-C₃H₇]⁺, 110 [174-CH₂]⁺, 83 [ion d]⁺, 69 [ion d-CH₃]⁺ and 55 [69-CH₃]⁺ indicated the saturated nature of side chain and rings A and B and the presence of the carbonyl group in ring A which was placed at C-3 on the basis of biogenetic grounds.
Sodium borohydride reduction of 2 yielded 5α-stigmast-9(11)-en-3β-ol 3 which had been reported from the roots of *Costus speciosus*.

These data led to establish the structure of malvasterone 2 as 5α-stigmast-9(11)-en-3-one. This sterol is being reported for the first time from *M. parviflora*.

**Experimental Section**

**Extraction and Isolation.** The dried roots of *M. parviflora* (1.150 kg) was collected from Kullu Valley region (Himachal Pradesh). The plant was then extracted with ethyl alcohol in a soxhlet apparatus. The brown viscous extract from the roots of *Costus speciosus* (1.150 kg) was collected from Kullu Valley for their help in collecting the plant material from the site. They also thank Dr M P Sharma, Taxonomist, Deptt of Botany, Jamia Hamdard (Hamdard University), New Delhi for identifying the plant species and where a voucher specimen has been retained.

**Compound 1.** Fractions eluted with petroleum ether furnished colourless crystals of 1, crystallized from CHCl₃, mp 68-69°, yield, 0.032 g (0.0028%), Rₛ 0.88 (petroleum ether–C₆H₆; 3:1) [α]D⁺ 5.13° (C=0.038, CHCl₃), IR(KBr); 2910, 2840, 1445, 1375, 1245, 1055, 1000, 790 cm⁻¹; ¹H NMR (60 MHz, CDCl₃); δ 1.50 (1H, br s, H-3), 1.03 (54H, brs, CH₃-26, CH₃-27), 0.73 (6H, br s, Me-I, Me-30), 0.65 (3H, brs, Me-31); EIMS m/z (rel. int.): 436 [M]+ (C₃₂H₄₃O) (20.8).

**Compound 2.** Elution of the column with CHCl₃–MeOH (99:1) yielded colourless crystals of 2, crystallized from CHCl₃–MeOH (1:1), mp 102-3°, yield 0.086 g (0.0075%), Rₛ 0.80 (CHCl₃–MeOH, 95:5), [α]D⁺ 4.45° (C=0.068, CHCl₃), UV(MeOH): 215 nm (log ε 4.5); IR(KBr); 2950, 2885, 1715, 1465, 1415, 1385, 1265, 1170, 1090, 1015, 800, 720 cm⁻¹; ¹H NMR (60 MHz, CDCl₃); δ 5.06 (1H, m, CH-11), 2.20 (2H, br s, CH₂-2), 2.10 (2H, br s, CH₂-4), 1.06 (3H, brs, CH₃-19), 0.86 (3H, br s, CH₂-21), 0.83 (9H, br s, CH₃-26, CH₃-27), 0.70 (3H, br s, CH₃-18); EIMS m/z (rel. int.): 412 [M]+ (C₃₄H₄₅O) (14.8), 397 (8.2), 369 (8.2), 354 (7.2) 339 (12.1), 284 (6.8), 256 (16.1), 241 (5.0), 213 (9.1), 201 (5.6), 159 (10.1), 138 (8.8), 128 (25.5), 124 (25.6), 114 (12.1), 110 (17.1), 95 (30.0), 97 (37.2), 95 (30.0), 83 (51.8), 81 (51.4), 70 (37.8), 69 (73.6), 57 (100), 55 (99.8).

**Acetylation of 3.** Compound 3 (10 mg) was treated with Ac₂O (3 mL) and pyridine (1 mL) for 24 hr. Water (10 mL) was added and the reaction mixture was treated with CHCl₃ (3x5 mL). The CHCl₃-layer was washed with water (2x5 mL), dried over fused Na₂SO₄, and evaporated to get colourless residue of 5α-stigmast-9(11)-en-3β-ol 3, mp 131-32° (lit. 17 mp 132-33°); IR(KBr): 3430 cm⁻¹; Rₛ 0.72 (C₆H₆–Me₂CO, 95:5).

**Compound 3.** On elution with CHCl₃–MeOH (49:1) gave colourless crystals of 3, crystallized form CHCl₃–MeOH (1:1), yield 0.1g (0.0017%), Rₛ 0.84 (CHCl₃–MeOH, 95:5), mp 131-33° (lit. 18 mp 132-33°), [α]D+ 3.39° (C=0.056, CHCl₃); UV(MeOH): 214 nm (log ε 5.6); IR(KBr); 3400, 2905, 2840, 1630, 1420, 1350, 1215, 1035, 945, 765 cm⁻¹; ¹H NMR (60 MHz, CDCl₃); δ 5.03 (1H, m, CH), 3.36 (1H, br m, ω₀=18.0 Hz, H-3), 1.06 (3H, br s, CH₃-19), 0.96 (3H, d, J=60 Hz, CH₃-21), 0.83 (3H, br s, CH₃-29), 0.80 (6H, br s, CH₃-26, CH₃-27), 0.63 (3H, br s, CH₃-18); EIMS m/z (rel. int.): 414 [M]+ (C₃₄H₄₅O) (100).


