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ABUTASTERONE, AN ECDYSONE FROM *ABUTA VELUTINA**

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Key Word Index—*Abuta velutina*; Menispermaceae; abutasterone; ecdysone; NMR and mass spectra.

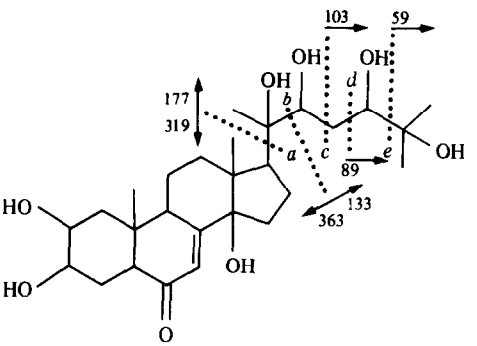
Abstract—The ecdysone abutasterone has been isolated from the Amazonian plant *Abuta velutina* and its structure elucidated by spectral means.

In continuation of a study of alkaloid constituents of plants of the Menispermaceae family an investigation of the menispermaceous vine *Abuta velutina* Gleason, indigenous to the Amazon region, was undertaken. The present communication reports the isolation and characterization of a non-alkaloidal ingredient of the plant.

Extraction of the leafless stem of the vine with methanol and chromatography of the extract on Si gel led to a crystalline ($C_{27}H_{44}O_8 \cdot H_2O$) compound (0.3% of dry stem wt), mp 257–259°. Its IR spectrum (KBr) revealed a broad hydroxy absorption band (3380 cm^{-1}) and strong conjugated carbonyl absorption (1660 and 1640 cm^{-1}), characteristic of an α,β-unsaturated keto unit, and its UV spectrum (MeOH) exhibited an absorption maximum at 242 nm (ε 13 800), characteristic of a 3-alkyl-2-cyclohexenone or its acyclic equivalent.

The mass spectrum indicated the compound, herewith named abutasterone, to possess an ecdysone-like structure. In analogy with the behavior of many ecdysones [1] the compound lacked a molecular ion peak at m/z 496, but showed a $[M-(H_2O)_2]^+$ peak at m/z 460 as the highest mass number in the spectrum. The tendency toward the loss of one or two water units was revealed by all important mass fragments. Two fragmentation patterns revealed the presence of an ecdysone ring system with a tetrahydroxy side chain attachment: (a) fragmentation mode *a* in formula **1** yielding a peak at m/z 319 representing the ring system, accompanied by water loss peaks at m/z 301 and 283, and a side chain peak of m/z 177, accompanied by m/z 159 and 141 peaks; and (b) fragmentation path *b* affording a peak at m/z 363 for the ring system together with peaks at m/z 345 and 327 for water loss, and a side chain m/z 133 peak, along with m/z 115 and 97 peaks [2, 3].

While the m/z 177 and 133 side chain fragments had shown the presence of a 20-hydroxy group, the following three side chain fragments (see paths *c–e* in formula **1**) [m/z 103 (accompanied by m/z 85 and 67 peaks), m/z 89 (alongside the base peak at m/z 71 and a 53 peak) and m/z 59 (with a m/z 41 peak)], indicated hydroxy groups at positions C-22, C-24 and C-25.



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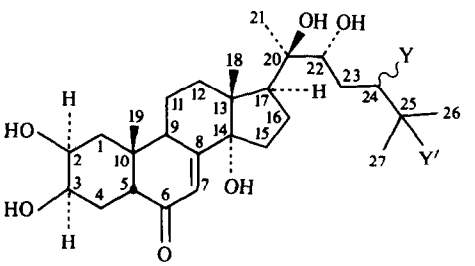

2a Y = Y′ = H; 5β–OH; Δ^{9-11}
2b Y = H, Y′ = OH
2c Y = α–OH, Y′ = H
2d Y = Y′ = OH

*Part LXXIX in the series "Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances". For Part LXXVIII see Reis Luz, A. I., da Rocha, A. I., Porter, B. and Wenkert, E. (1983) *Phytochemistry* **22**, 2301.

Table 1. ^{13}C NMR chemical shifts of the ecdysteroids **2a–2d***

Carbon No.	2a†	2b†	2c‡	2d
1	38.6	37.8	37.7	38.1
2	67.9	68.0	68.0	68.2§
3	69.5	68.0	68.0	68.1§
4	35.2	32.3	32.4	32.6
5	79.7	51.1	51.4	51.5
6	201.2	203.2	203.6	203.5
7	116.8	121.6	121.6	121.7
8	156.0	165.7	166.1	166.2
9	137.4	34.5	34.4	34.6
10	45.4	38.5	38.6	38.8
11	132.9	21.0	21.1	21.2
12	38.6	31.5	31.7	31.8
13	47.4	48.1	48.0	48.2
14	83.2	84.2	84.1	84.2
15	31.3	32.0	31.9	32.1
16	21.5	21.5	21.6	21.9
17	49.9	50.1	50.0	50.1
18	26.3	17.7	17.8	18.0
19	17.9	24.3	24.4	24.6
20	76.5	76.9	76.7	76.9
21	21.2	21.5	21.4	21.6
22	76.8	77.5	77.5§	78.3
23	30.2	27.2	35.6	33.1
24	37.0	42.4	76.7§	80.4
25	28.2	69.7	33.9	72.3
26	23.3	29.9	19.5	27.0
27	22.4	29.9	17.0	25.4

*In d_5-pyridine solutions; chemical shifts in [δ-values (ppm)] downfield from TMS; δ(TMS) = δ(d_5-pyridine) + 149.9 ppm.
†From ref. [4].
‡From ref. [5].
§Signals in any vertical column may be interchanged.

A ^{13}C NMR analysis of abutasterone and comparison of the chemical shift data with those of kaladasterone (**2a**) [4], ecdysterone (**2b**) [4] and pterosterone (**2c**) [5] proved the configuration of the tetracyclic ring system of the three substances to be identical and confirmed the hydroxy group attachment sites of the side chain (Table 1). Furthermore, inspection of the $\Delta\delta$-values of the side chain carbons for the **2a–2b** and **2c**-abutasterone pairs of ecdysones showed the four substances to possess the same C-20 and C-22 configuration, limiting abutasterone to structure **2d**.

EXPERIMENTAL

The mps were obtained on a Reichert micro hot-stage and are uncorr. The IR spectrum of a KBr pellet and UV spectrum in MeOH soln were recorded on Pye Unicam 3-200 and Kontron Uvikon 810 spectrophotometers, respectively. ^1H NMR spectra of a d_4-MeOH soln with TMS as int. standard (δ0) were taken on a 360 MHz NMR spectrometer with a highly modified Varian HR-220 console, an Oxford magnet and a Nicolet 1180-E computer system. ^{13}C NMR spectra were obtained on a Nicolet NT-200, wide-bore, broad-band spectrometer, operating with an Oxford magnet at 50.31 MHz in the Fourier transform mode. Low-resolution MS were recorded on a Finnigan 4021 GC/MS spectrometer.

Isolation of abutasterone (**2d**). An *Abuta velutina* Gleason plant (INPA-Manaus herbarium No. 75,777) was collected on the Manaus–Itacoatiara road. Its powdered, dried stem (6.20 kg) was extracted with 20 *l.* EtOH at room temp. over a 30-day period. Chromatography of 80 g of the extract (291 g) on 800 g Si gel and elution with C_6H_6–Me_2CO (9:1) gave a yellow oil, which was not investigated further, and 200 mg of a mixture of sitosterols, mp 151–154°, identified by GC/MS with an authentic specimen. Elution with $CHCl_3$ gave 14 mg of an unidentified material, while elution with MeOH yielded a solid, whose crystallization from aq. Me_2CO afforded 5.10 g (0.3% of stem dry wt) of colorless needles of abutasterone (**2d**), mp 257–259°; $[\alpha]_D^{20}$ 32° (MeOH; *c* 2.0). (Found: C, 62.74; H, 8.93. $C_{27}H_{44}O_8 \cdot H_2O$ requires: C, 63.04; H, 8.95%.) ^1H NMR (d_4-MeOH): δ0.89, 0.96, 1.14, 1.18, 1.22 (*s*, 3 each, Me_5), 3.17 (1H, *dt*, J = 8, 2 Hz, H-9), 3.54 (1H, *dd*, J = 9, 2 Hz, H-24), 3.68 (1H, *dd*, J = 9, 2 Hz, H-22), 3.85 (1H, *dt*, J = 11, 3 Hz, H-2), 3.97 (1H, *br s*, H-3), 5.83 (1H, *d*, J = 2 Hz, H-7); MS *m/z* (rel. int.): 460 (1), 442 (1), 424 (2), 363 (3), 345 (10), 327 (6), 319 (1), 301 (4), 283 (4), 177 (4), 159 (15), 141 (15), 133 (8), 123 (12), 115 (34), 103 (3), 97 (28), 89 (4), 85 (17), 71 (100), 67 (13), 59 (80), 53 (10), 41 (15).

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