14-Deoxymuristerone, a Compound exhibiting Exceptional Moulting Hormonal Activity

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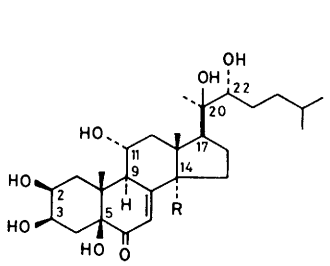
The product resulting from treatment of muristerone (**1**) with Me_3SiCl–NaI exhibits extraordinary activity when assayed on *Drosophila* Kc-H cells; spectroscopic studies define this compound as 14-deoxymuristerone (**2**).

The phytoecdysteroid muristerone (**1**)[1] is a potent insect moulting hormone.[2] Treatment of (**1**) with trimethylsilyl chloride (TMS-Cl)–NaI[3] in an attempt to obtain iodinated derivatives gave rise instead to a previously unreported ecdysteroid analogue, 14-deoxymuristerone (**2**).

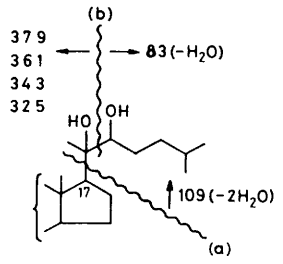
The biological activity of (**2**) was assayed by the morphological response of *Drosophila* Kc-H cells in culture.[2] In this system various ecdysteroids have been shown to be active approximately in proportion to their reported affinities for the ecdysteroid receptor.[2,4] Relative to 20-hydroxyecdysone, the activities of (**1**) and (**2**) were approximately 10 and 80, respectively. Comparably high activities were observed when hormonal activity was assayed by acetylcholinesterase or ecdysteroid-inducing polypeptide induction.[5,6] Thus (**2**) is, at least for *Drosophila* cells, the most potent ecdysteroid known.

The synthesis of (**2**) was as follows: (**1**) (Sigma, 9 mg) and NaI (43 mg) were dissolved with stirring in anhydrous MeCN† (22 ml) under Ar, and TMS-Cl (27 μl) was added slowly over a period of 5 min. The reaction was allowed to continue at room temp. for 3.5 h and was subsequently quenched with 10% *w/v* sodium thiosulphate–0.1 M sodium phosphate buffer (2.2 ml, pH 7.0). Phosphate buffer (80 ml) was added and the solution partitioned with $CHCl_3$ (2 × 15 ml) and EtOAc (2 × 25 ml). The combined organic extracts were washed with buffer, dried over anhydrous Na_2SO_4, evaporated, and the residual oil partially purified by silica t.l.c. (*v/v/v*, 14.8:1.9:0.1, $CHCl_3$:EtOH:H_2O). Pure (**2**) (2 mg, 25%) was ultimately acquired by repeated h.p.l.c.‡ (Rainin C_8 ultrasphere, 1:1, MeOH:H_2O).

14-Deoxymuristerone displayed the following spectral characteristics: negative ion desorption chemical ionisation-mass spectrum (CH_4 reactant gas) $C_{27}H_{44}O_7$, *m/z* 479 (*M* − 1); λ_{max} (MeOH) 242 nm (ε 8500); ν_{max} (NaCl) 1671 cm^{-1};



(1) R = OH muristerone
(2) R = H
(4) R = OH and 5β-H ajugasterone C



(3)

δ($CDCl_3$, 250 MHz) 0.810 and 0.998 (s, 18-H_3 and 19-H_3), 0.914 and 0.928 (d, *J* 6.2 Hz, 26-H_3 and 27-H_3), 1.25 (s, 21-H_3), 3.38 (br. d, *J* 10 Hz, 22-H), 4.00 (br. m, $W_{1/2}$ 11 Hz, 3-H and 11-H), 4.24 (ddd, *J* 11, 9.2, 5 Hz, 2-H), and 5.83 (br. s, 7-H).

As depicted in (**3**), electron ionisation mass spectrum (23 eV) of compound (**2**) showed peaks at *m/z* 109 and 83 resulting from fissions (a) (17-C–20-C) and (b) (20-C–22-C),[7] respectively, thereby establishing that the 20,22-glycol was intact. Of diagnostic value were the series of peaks at *m/z* 361, 343, and 325 originating from the *m/z* 379 ion [direct fission (b)] *via* consecutive losses of HOH. In the ecdysteroids this indicates that only four hydroxy-groups are present on the nucleus, C-1 to C-17.[8,9] Thus, one nuclear OH present in (**1**) was absent in (**2**). This was substantiated by the presence of only one OH resonance [δ 3.80 (1H, br. s)] in (**2**)-20,22-acetonide-2,3,11-triacetate and two OH resonances [δ 4.17 and 4.56 (each 1H, br. s)] in (**2**)-20,22-acetonide-2,11-diacetate. The formation of these two derivatives and several others, including: (**2**)-2,3,20,22-diacetonide,§ (**2**)-2,3,20,22-diacetonide-11-acetate, and (**2**)-2,3,11,22-tetra-acetate¶ (possessing four *sec*-acetates) confirmed that the hydroxy-groups at C-2, 3, 11, 20, and 22 were intact.

The missing hydroxy-group in (**2**) was discerned to be the 14-OH in (**1**) on the basis of the following evidence: (i) ajugasterone C (**4**)[10] would have been generated if the 5β-OH had been lost but this was not the case, and (ii) the 7-H signal of (**2**)-20,22-acetonide-2,11-diacetate [δ 5.89 (dd, *J* 2.7, 2.7 Hz)] collapsed to a doublet (*J* 2.7 Hz) when either 9α-H [δ 2.99 (dd, *J* 2.7, 8.4 Hz)] or 14-H [δ 2.23 (dddq, *J* 10.1, 5.6, 27, *ca.* 0.5 Hz)] was irradiated. The observation of 14-H in this derivative as a dddq indicated that the C/D ring junction was *trans* as expected from the similarity between the methyl ^1H chemical shifts in (**1**) and (**2**).** The A/B ring junction was judged to be *cis* owing to the nuclear Overhauser effect (n.O.e.) observed for 2-H upon irradiation at 9α-H[7,10] of (**2**)-20,22-acetonide-2,11-diacetate (10%) and (**2**)-2,3,11,22-tetra-acetate (15%). Further evidence in favour of the stereochemistry of the steroidal nucleus as depicted in (**2**) was obtained by application of the additivity relationship in the amplitudes of exciton-split c.d. curves of ecdysteroid benzoates.[11]

Structure–function relationships among ecdysteroids have been reviewed recently.[12] In general, 14-deoxy-steroids are less active when assayed in whole animals than their 14α-OH

† Failure to adhere to strictly anhydrous conditions invariably led to a rapid conversion of (**1**) into a complex array of less polar products.

‡ Compound (**2**) co-chromatographed with two impurities; separation was accomplished by peak splitting and re-injection.

§ The 20,22-monoacetonide and 2,3,20,22-diacetonide of (**2**) were prepared in a combined 65% yield when (**1**)-2,3,20,22-diacetonide was treated as described above for (**1**).

¶ ^1H-n.m.r. and mass spectral data are consistent with these structures.

** (**1**) (CD_3OD) 18-H_3 δ 0.86, 19-H_3 δ 1.03; and (**2**) (CD_3OD) 18-H_3 δ 0.92, 19-H_3 δ 1.03; also by n.m.r. comparisons with C/D *cis*-14-deoxyecdysteroids which recently became available (manuscript in preparation).

counterparts.[13] In each of the cases cited in ref. 13 the 14α-OH compound is probably metabolized to a more active hormone *in vivo*. Evidently the 14α-OH is required for this process or for stability *in vivo*.[14] By contrast the Kc-H cell assays appear to measure the affinities of ecdysteroids for the hormone receptor and very little is known about the structural requirements for receptor binding. The present result indicates that in a different, and hormonally more active molecule, loss of the 14α-hydroxy-group may lead to a considerable enhancement of biological activity.

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