Structure of Calonysterone, an Unusually Modified Phytoecdysone

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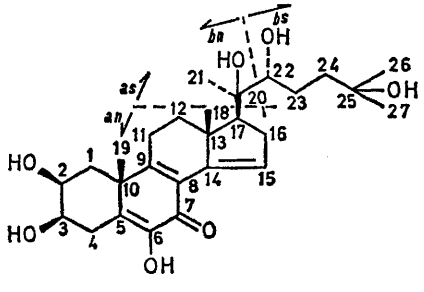
Summary The structure of 2β,3β,6,20*R*,22*R*,25-hexahydroxycholesta-5,8(9),14-trien-7-one (I) has been assigned to calonysterone, a phytoecdysone isolated from kaladana (*Ipomoea sp.*), by ^{13}C and ^{1}H n.m.r. spectroscopy.

SEEDS of a plant of the Ipomoea family, contain, in addition to previously known ecdysone, crustecdysone, and makisterone A, three new phytoecdysones, muristerone A,[1] kaladasterone,[2] and calonysterone. Here, we report the determination of the structure of calonysterone (I).

Calonysterone, $C_{27}H_{40}O_7$ (M^+ at *m/e* 476·2774) unlike more than 40 phytoecdysones characterized so far[3] is highly unsaturated and contains an α-diketone grouping; m.p. 234—235° (MeOH); $[\alpha]_D^{20}$ + 76·8 (MeOH); ν_{max} 3380, 3350 (OH), 1638, 1619, and 845 cm^{-1} (C=C, C=O conj.); λ_{max} (MeOH) 222 (ε 20,700), 244 (ε 13,500sh), and 294 nm (ε 7850) due to the presence of a differently conjugated system than is usual in phytoecdysones.[3]



(I)

The ^{1}H n.m.r. spectrum in $(CD_3)_2SO$ (Me_4Si as internal ref.) exhibits the presence of five methyl groups at δ 1·16 (3H, 21-H), 1·05 and 1·07 (3H and 3H, 26-H and 27-H), 1·00 (3H, 18-H), and 1·41 (CH, 19-H), three secondary OH groups at 4·32, 4·54, and 4·88, and three tertiary OH groups at 3·64, 4·04, and 7·95 and a vinylic proton at 6·78. The last mentioned signal is shifted even more downfield (7·41) in C_5D_5N. The methyl proton signals for methyls 26-C and 27-C are identical, and the signal for methyl 21-C is very close to the signals of corresponding methyls in the ^{1}H n.m.r. spectrum of crustecdysone (*cf.* ref. 3 and values above) and this suggested that the side chains of calonysterone and crustecdysone might be identical. This presumption was confirmed by finding fragments at *m/e* 161, 143, 125, 99, and 81 in the mass spectrum‡ of (I) resulting from *bs* and *as* splittings [(*cf.* formula (I)[4]] and by comparison of ^{13}C n.m.r. spectra of calonysterone and crustecdysone§[5,6] in $(CD_3)_2SO$: 25-C 68·6 (68·6), 20-C 74·9 (75·6), 22-C 76·4 (76·1), 26-C and 27-C 29·8 (29·8), and 23-C 20·0 (20·0).

Calonysterone forms a 2,3,6,22-tetra-acetate (II) and a 2,3;20,22-diacetonide (III). Their properties were in accordance with the structures proposed.

The tetracyclic portion of the calonysterone molecule contains two additional unsaturations (in comparison with usual phytoecdysones) as can also be seen from prominent *an* and *bn* fragments[4] present in the mass spectrum. Moreover, all phytoecdysones possessing the O=C-6–C-7 H=C-8 system show a similar pattern in the ^{13}C n.m.r. spectrum[5,6] which is not repeated in the case of calonysterone exhibiting signals for seven sp^2 carbons . From the sp^3 part of the spectrum (in comparison with that of crustecdysone) easily identifiable 14-C and 5-C signals disappeared which are fairly typical values of phytoecdysone chemistry. Even the 2-C and 3-C signals are shifted downfield.

On hydrogenation over Pd–C in MeOH, calonysterone takes up two equivalents of hydrogen yielding the 8ξ, 9ξ, 14ξ-15-tetrahydrocalonysterone (IV); M^+ at *m/e* 480; m.p.

‡ Correct exact masses of all fragments of (I) and (IV) quoted were determined.

§ Parenthesized n.m.r. values are those of crustecdysone.

257—258° (MeOH); $[\alpha]_D^{20}$ + 10° (MeOH); i.r. (KBr) 3900—3200 (OH), 1660, 1620 cm^{-1} (C = O, C = C, conj.); λ_{max} (MeOH) 285 nm (ϵ 9435), this in 5% NaOH in MeOH shifts to 340 nm (ϵ 7570) indicating the presence of an α-diketone grouping. The ^1H n.m.r. spectrum in $(CD_3)_2SO$ shows the CH_3-protons at the same resonances as in crustecdysone,[3] the low-field signal exchangeable with D_2O at 7·09 ascribed to the enolized keto group, in addition to five remaining OH groups, and no vinylic proton. In addition to the above evidence for the presence of the α-diketone in (I) and (IV), signals in the ^{13}C n.m.r. spectrum of calonysterone (I) agreed well with this conclusion. Moreover, (III) on treatment with CH_2N_2 in $CHCl_3$ solution gives a 2,3;20,22-diacetonide-6-methyl ether whose ^1H n.m.r. spectrum ($CDCl_3$) exhibits a $=C-OCH_3$ signal at 3·73 (s, 3H). All the data presented so far can be accommodated only by (I) for the structure of calonysterone. It explains the low-field resonance of the vinylic proton due to the deshielding cone of the carbonyl group, the upfield resonance of 2-H caused by the effect of the shielding zone of the C-5=C-6 double bond, and the mass spectral behaviour of (I) and its derivatives. Whereas crustecdysone and other common ecdysones show no significant *an* fragments [*cf.* (I)], calonysterone and its diacetonide show abundant *an* fragments at *m/e* 315 and 355 followed by the loss of a proton to give fragments at *m/e* 314 and 354. The retention of the positive charge on the tetracyclic part and the successive loss of the proton can be well explained by the presence of the C-14=C-15 double bond and its stabilizing effect. Calonysterone also exhibits an early tendency in its mass spectrum to split off a methyl group at variance with other ecdysones. Driving force for such a splitting—in fact easier than most dehydrations—can be seen in the aromatization of the ring B.

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