Acnistins, a New Class of Steroidal Lactones from *Acnistus Ramiflorum* Miers; *X*-Ray Structure of Acnistin E

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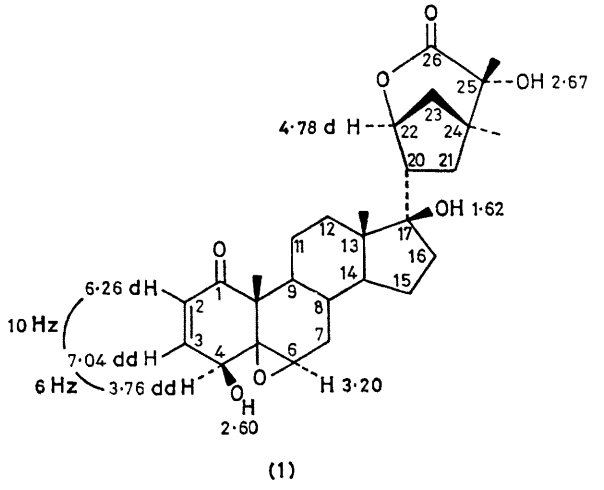
Summary The structures of two new C_{28} steroidal lactones have been established; an *X*-ray diffraction analysis of acnistin E shows that it has a novel side chain with the C-21 methyl group fused to the lactone ring.

SEVERAL steroidal lactones have been isolated from the genera Acnistus,[1] Dunalia,[2] Physalis,[3] and Withania,[4] which belong to the Solanaceae family. Structures showing epoxidation in the side-chain[5] as well as 13,14-seco-steroids[6] have been described. These compounds are believed to represent different stages of the biosynthetic pathway leading to the physalins.[7] The compounds isolated from *Acnistus ramiflorum* Miers illustrate a new biogenetic pathway involving activation of the C-21 methyl group to afford a five-carbon ring in the lateral chain.

Column chromatography of a methanolic extract obtained from the leaves yielded mixtures of up to six different products labelled A to F. The most abundant product, acnistin E (**1**) (Figure 1) was separated from the



(1)

FIGURE 1. Structure of acnistin E (**1**) showing some relevant ^{1}H n.m.r. signals (δ values, 220 MHz, $CDCl_3$ soln.).

others by taking advantage of its solubility in hot benzene. A high-resolution mass spectrum indicated a structural formula $C_{28}H_{38}O_7$ and its spectroscopic properties λ_{max} 214 nm (log ε 3·83), ν_{max} 1675 and 1730 cm^{-1} suggested an αβ-unsaturated ketone and a lactone carbonyl. The ^{1}H n.m.r. spectrum showed four tertiary methyls at δ 0·85, 1·21, 1·40, and 1·50. Analysis of the downfield signals indicated that the substance contained the same A- and B-ring functionalities as withaferine A.[8] Since the quantities available precluded an elaborate chemical degradation to establish the nature of the side-chain, the structure was elucidated by an *X*-ray diffraction study. The A/B rings are *cis*-fused with the C-10 methyl and the epoxide oxygen lying on the β-face. The B/C and C/D rings are *trans*-fused with the C-13 methyl lying on the β-face and the C-17 side-chain exhibiting α-orientation. The C-21 methyl group is fused to C-24 of the normal withanolide-type lactone ring. The six-membered lactone ring exhibits a very distorted chair conformation while the five-membered ring is in an envelope conformation with C-23 being the flap. The A ring can be described as 1,3-diplanar, the B ring 1,2-diplanar, and the C ring has almost a normal chair conformation.

Crystal data: orthorhombic, space group $P2_12_12_1$, a = 13·352(4), b = 16·034(7), c = 13·316(7) Å, U = 2851(2) Å^3.

All *X*-ray data were collected on a Syntex $P2_1$ diffractometer using Cu-K_α radiation and a graphite monochromator. The structure was solved by direct methods and refined by least-squares to an interim R factor of 0·113 for 2656 reflexions. The crystallographically derived structure of acnistin E is shown in Figure 2.†

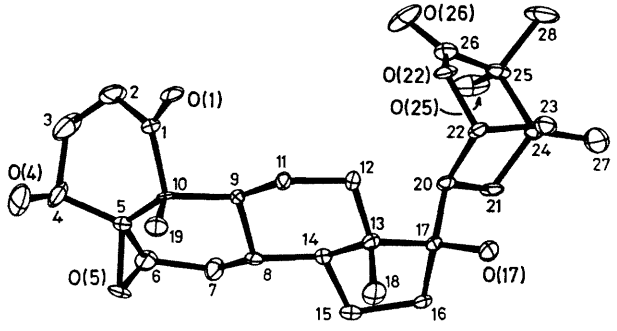


FIGURE 2. The molecular structure of acnistin E.

Acnistin A, m.p. 285—287 °C, M^+ 470 ($C_{28}H_{38}O_6$), a minor product isolated by means of repeated preparative t.l.c., exhibits two bands in the carbonyl region of the i.r. spectrum at 1655 (αβ-unsaturated ketone) and 1730 cm^{-1} (lactone) The ^{1}H n.m.r. (Me_2SO) spectrum shows the 2- and 3-proton at δ 7·04 (dd, J=10 and 3 Hz) and δ 5·96 (dd, J=10 and 2 Hz), a proton on a carbon carrying an epoxide at δ 3·18, and four tertiary methyls at δ 1·29, 1·13, 1·06, and 0·78. The structure of this compound is similar to that of acnistin E except for the absence of the β-hydroxy group at C-4. This was shown by heating at 50 °C the acnistin E methyl toluene-*p*-sulphonate, adsorbed on alumina, for 6 h. The alumina was extracted with $CHCl_3$–MeOH, and the product, purified by preparative t.l.c., was identical to acnistin A (i.r., ^{1}H n.m.r. and mixed m.p.).

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† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

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