Acanthamolide, a Melampolide Amide from *Acanthospermum glabratum* (Compositae)

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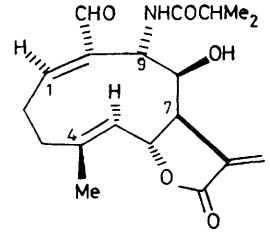
Summary The structure of acanthamolide (I), a novel melampolide sesquiterpene containing nitrogen, is deduced by interpretation of spectroscopic data.

ACANTHAMOLIDE (I) was obtained as colourless, rhomboid crystals, m.p. 249—251 °C (C_6H_6–MeOH), from a basic extract of *Acanthospermum glabratum* (DC.) Wild (Compositae). The i.r. spectrum (KBr) indicated the presence of hydroxy (3520 cm^{-1}) and secondary amide (3385 cm^{-1}), groups, an $\alpha\beta$-unsaturated δ-lactone (1770 cm^{-1}), an $\alpha\beta$-unsaturated ketone or aldehyde (1670 cm^{-1}), and an amide group (1640 cm^{-1}). The ^1H n.m.r. spectrum (δ, CD_3OD) confirmed the presence of an α-methylene lactone, with doublets (J 3 Hz) at 5·588 and 6·221 for 13a-H and 13b-H, respectively.[1] In addition, an α-substituted $\alpha\beta$-unsaturated aldehyde, with the aldehyde proton at 9·351 and a complex β-proton at 6·602 was indicated. Two other signals were readily interpretable: a vinylic methyl group at 1·991 and two three-proton doublets (J 6·8 Hz) at 1·005 and 1·089, indicative of an isopropyl residue. A molecular ion at *m/e* 347 indicated the formula $C_{19}H_{25}NO_5$ (347·1734; $C_{19}H_{25}NO_5$ requires 347·1733). Acanthamolide readily formed a monoacetate derivative, confirming the presence of a hydroxy group. The oxygen and nitrogen functions were therefore an aldehyde, an $\alpha\beta$-unsaturated γ-lactone, an isobutyryl amide, and a hydroxy group.

The chemical shift of the aldehyde group was characteristic of a *cis* geometry,[2] and from biogenetic considerations a germacradienolide of the melampolide (*cis* 1,10-double bond) type was suggested.



(I)

The stereochemistry of 7-H in all well characterized sesquiterpene lactones is α.[1] The magnitude of the coupling of 7-H in acanthamolide with the C-13 protons indicated a *trans*-fused lactone,† in which 6-H is β. By analogy with the chemical shifts and multiplicity of 5-H, 6-H, and 15-H in other melampolide derivatives,[3,4] the stereochemistry of the C-4, C-5 double bond was deduced to be *trans*, with 5-H in the α-configuration.

† Melampolide derivatives also obey Samek's rule, see ref. 3.

The C-1 proton (δ 6·602) appeared as a broad doublet of doublets (*J* 10·1 and 6·9 Hz) indicating that C-2 was unsubstituted. Two vicinally coupled doublets of doublets, at 4·407 (*J* 8·6 and 1·86 Hz) and 5·136 (*J* 8·7 and 2·2 Hz), could be assigned to the 8-H and 9-H, and because the aldehyde proton appeared as a doublet (*J* 1·86 Hz), the signal at 4·407 could be assigned to 9-H. Examination of Dreiding models indicated that 9-H and 14-H could only be placed in a W relationship when 9-H is β.‡

The C-9 proton also exhibited substantial coupling (*J* 8·6 Hz) with 8-H indicating a large dihedral angle between 8-H and 9-H and an α-orientation for 8-H. Confirmatory evidence for this stereochemical assignment was derived from the small (*J* 2·2 Hz) coupling of 8-H and 7-H which is only possible if 7-H and 8-H are *cis* and α.

The location of the hydroxy and isobutyramide groups was determined by examination of the monoacetate derivative of acanthamolide. A new doublet of doublets was observed at δ 6·09 and the peak originally at 5·136 was absent. The hydroxy group was therefore located at C-8 and the isobutyramide at C-9.

Substantiating evidence came from a rationalization of two fragment ions at *m/e* 167 ($C_9H_{13}NO_2$) and *m/e* 97 (C_5H_7NO). The former ion appeared to be derived by allylic cleavage at the 2,3- and 8,9-bonds, with subsequent loss of the isobutyrate residue and concomitant proton transfer to afford $[MeCH{=}(CHO){-}CH{=}NH]^+$, *m/e* 97.

Acanthamolide (NSC-280482) therefore has the structure (I). Marginal cytotoxic activity was observed in the 9KB system in cell culture (ED_{50} 2·2 μg ml^{-1}).[5]

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‡ In this configuration there is also maximum π-orbital overlap of the αβ-unsaturated carbonyl system.

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