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A CONFORMATIONAL STUDY OF ELAIOPHYLIN BY X-RAY CRYSTALLOGRAPHY AND DIFFERENCE ^{1}H NMR METHODS; OBSERVATION OF A SELECTIVE SIGN REVERSAL OF THE NUCLEAR OVERHAUSER EFFECT

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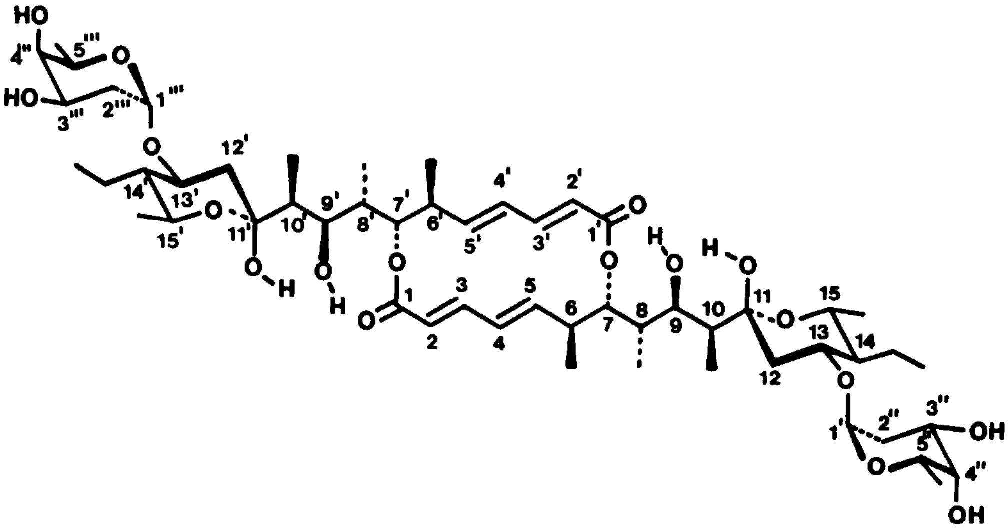
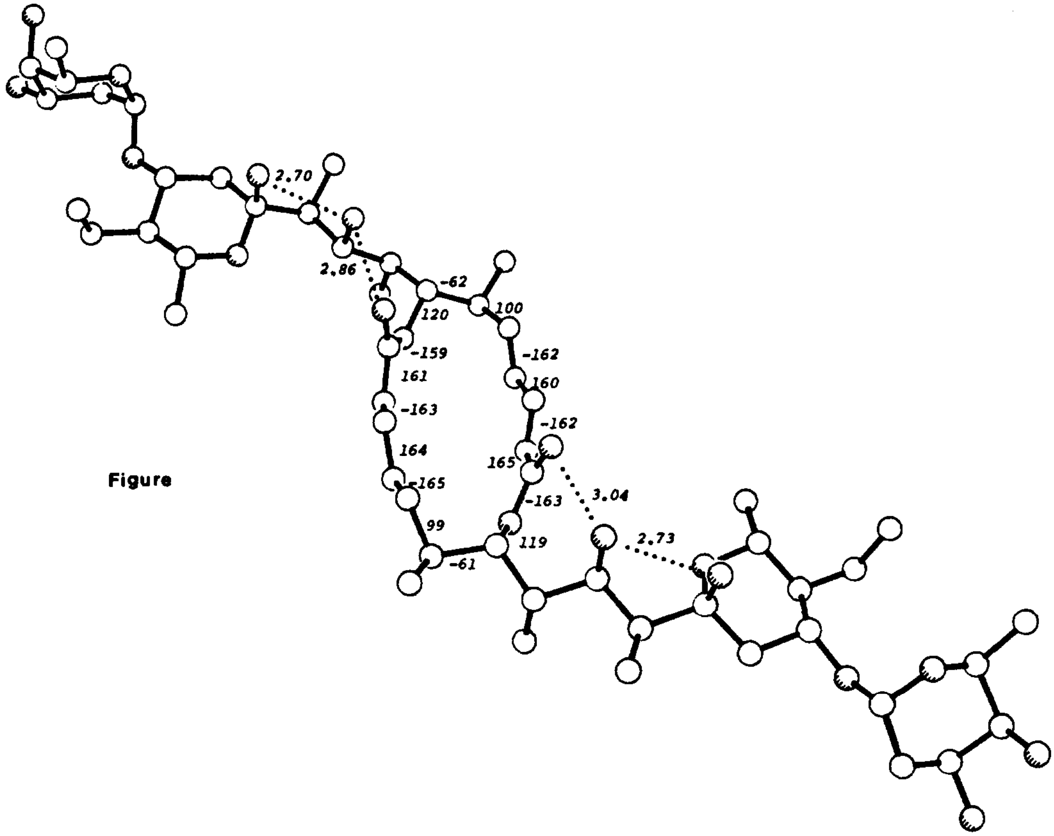
SUMMARY: During the structure determination of elaiophylin by X-ray crystallography and n.O.e. difference spectroscopy, the crystal and solution conformations were established and a selective sign reversal of the n.O.e. observed; this is rationalised in terms of the molecule's internal motions.

In the course of isolation of metabolites from *Streptomyces spp*[†], a crystalline product was obtained (from methanol solution) and shown, by a comparison of spectral data, to be identical to elaiophylin[1], the structure of which has recently been reported[2]. Here we discuss the X-ray crystallography and further important nmr spectroscopic properties of this molecule.

Elaiophylin approximates closely to non-crystallographic $\underline{C}_2$ symmetry[‡]. The torsion angles (Figure) show this symmetry to be particularly marked in the 16-membered bis-lactone ring where all 'equivalent' angles are the same to within 4°. Additionally, these angles highlight the non-planarity of both the chains of trans-olefinic bonds. There are rotations of between 15° and 18° about these bonds.



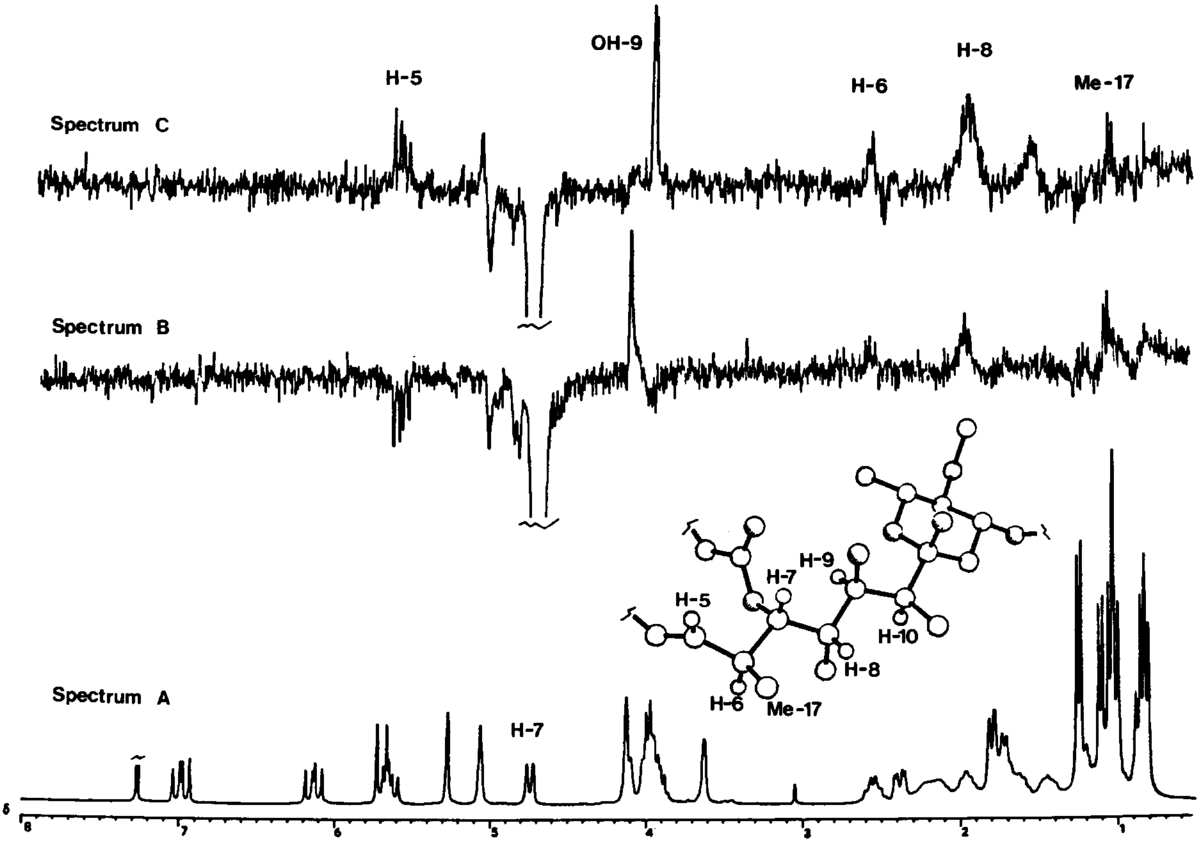


Figure

The structure is dominated by a pattern of inter- and intramolecular hydrogen bonds. Both screw and translationally related molecules are linked by hydrogen bonds formed between trapped methanol molecules and the sugar units at each end of the molecule. Despite its not being possible to locate unambiguously all the side-chain OH hydrogen atoms, it is clear from the relative orientations of these groups and the ring carbonyls (Figure) and from their interatomic distances that a pattern of hydrogen bonds exists linking these side-chain OH's and the ring carbonyl oxygens.

Evidence concerning the conformation in $CDCl_3$ solution was provided by extensive nmr experiments. The virtual absence of coupling from H-7 to H-8 and from H-9 to H-10 suggests that the torsion angles (H-7,C-7,C-8,H-8) and (H-9,C-9,C-10,H-10) are unfavourable, the coupling constants probably being further reduced by antiperiplanar oxygen substituents. The large coupling constants from H-6 to H-7 and from H-8 to H-9 show that both these pairs of protons adopt an antiperiplanar arrangement.

Difference decoupling of OH-11 showed a substantial W-coupling ($J = 1.5$ Hz) to the H-12 axial proton indicating that the OH-11 hydrogen atom completes a six-membered ring chair arrangement by its hydrogen bonding to O-9. Saturation transfer during this experiment provided immediate identification of all the remaining exchangeable protons and revealed the chemical shifts of OH-3" and OH-4" (δ 2.05-2.35). In combination these data strongly support



Normal (A) and n.O.e. difference spectra (B and C) of elaiophylin, recorded at 250 MHz in undegassed $CDCl_3$. Spectra B and C are vertically expanded approx. 100 fold relative to A. Sample concentration was approx. 5 mM, and accumulation required 10 h at each temperature. The entire normal spectrum was assigned by extensive decoupling experiments.

the retention of the crystallographic conformation for this portion of the molecule in solution.

More direct evidence for the intramolecular hydrogen bonding was obtained from the n.O.e. results. The relatively large enhancement of OH-9 resulting from pre-irradiation of H-7 (spectrum B) shows that these protons are spatially close as is required if OH-9 is to hydrogen bond to the C-1 carbonyl group. A totally unexpected feature of the n.O.e. difference spectrum B (recorded at 23°C) is the negative sign of the enhancement of proton H-5. We consider this effect to be a consequence of a longer correlation time for the relaxation vector connecting H-7 and H-5, compared with those of other relaxation vectors involving H-7. Such a difference must presumably arise through the existence of internal motions. In the present case this implies that segmental motion of the side-chain is more rapid than overall molecular tumbling so that the relaxation vector H-5 to H-7 is the only one in this particular experiment to adopt the overall correlation time of the molecule. This interpretation is consistent with the known dependence of maximum observable n.O.e. (η_{max}) on molecular tumbling[3] provided that the overall correlation time of the molecule is just below the value that would give zero n.O.e.

Alternative explanations and artefacts were excluded by consideration of the following. (a) The geometry of the molecule and the absence of suitable strong positive n.O.e.'s precludes the enhancement of H-5 being a 3-spin effect[4]. (b) H-5 is selectively enhanced from within a region of spectral overlap. (c) Identical results were obtained using the O-3'', O-4'', O-3''', O-4''' tetraacetate of elaiophylin. Final confirmation was given by the results obtained at 50°C (Spectrum C). Thus, at the higher temperature the generally decreased correlation times result in a change of sign in the enhancement of H-5.

Very approximate calculations suggest that a change of somewhat less than two-fold in correlation is involved in these effects. Nonetheless, such a two-fold increase would be sufficient to change η_{max} from +18% to -16% [3]. We suggest that in cases where sensitivity is not a severe problem, observation of similar sign reversals of the n.O.e., possibly coupled with manipulation of η_{max} through its dependence on field strength, temperature and viscosity[3], might provide a probe for internal molecular motions.

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† Extraction details are available on request.

‡ Elaiophylin crystallises from methanol incorporating two molecules of solvent to each of the parent molecules. Crystals, $C_{54}H_{88}O_{18}\cdot 2(CH_3OH)$, are monoclinic, space group $P2_1$, a = 9.935(2), b = 10.106(2), c = 31.069(6) Å, β = 92.71(2)°, U = 3116 Å^3, Z = 2, D_c = 1.16 gcm^{-3}. Of the 4472 independent reflections ($\theta \leq 58^{o}$) measured on a diffractometer using Cu-K_α radiation 441 had $F_o < 3\sigma(F_o)$ and were classed as unobserved. The structure was solved by direct methods and refined anisotropically to give R = 0.056 . The crystals slowly decompose on standing by loss of solvent. Coordinates of this work have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Cambridge, CB2 1EW, U.K.

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