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STRUCTURE OF THE DITERPENE PORTION OF A NOVEL BASE FROM THE SPONGE *AGELAS MAURITIANA*

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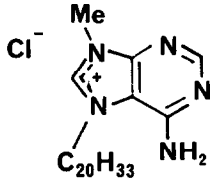
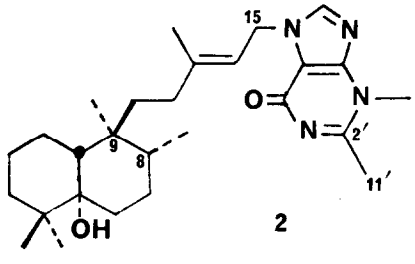
Summary. The structure of purino-diterpene 2, derived from an unknown antimicrobial metabolite of *Agelas mauritiana*, was determined by X-ray analysis.

In 1975, Cullen and Devlin[1] reported the isolation and partial characterization of agelasine (1), a novel quarternary 9-methyladenine derivative of a diterpene, from the Caribbean sponge *Agelas dispar*. Despite the unusual character of agelasine (1), the complete structure of agelasine (1) has not been subsequently reported nor have there been reports of similar compounds from marine sponges. We have examined several samples of *Agelas dispar* but could not detect agelasine (1) in the crude extracts.[2] In this paper, we report the structural determination of an unusual purino-diterpene 2, an artifact derived from an unstable base that is the major antimicrobial metabolite of *Agelas mauritiana*.



1



2

The crude methanolic extract of the sponge *Agelas mauritiana* from Enewetak showed significant antimicrobial activity and was partially characterized by ^{1}H NMR spectroscopy. Chromatography of the extract on Sephadex LH-20 gave fractions with diminished antimicrobial activity against *Bacillus subtilis* and *Vibrio anguillarum* and with more complex ^{1}H NMR spectra. The active fractions all contained at least three purine derivatives of a diterpene that could only be separated after acetylation of the mixture with acetic anhydride in pyridine. The least polar acetylation product[3] gave a crystal, m.p. 95-98°C from benzene, that was submitted for single crystal X-ray analysis.

Only one crystal with dimensions .5x.2x.1 mm was available for diffraction analysis. Preliminary X-ray photographs showed orthorhombic symmetry and accurate lattice constants, determined by a least-squares fit of fifteen 2θ-values, were **a** = 7.510(2), **b** = 17.172(5) and **c** = 25.050(7)Å. Systematic extinctions, the presence of chirality and an estimated density were uniquely accommodated by space group $P2_12_12_1$ with one molecule of $C_{27}H_{42}N_4O_2$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer controlled four-circle diffractometer using graphite monochromated CuK$\bar{\alpha}$ radiation (1.54178Å) and a variable speed, 1° ω-scan. Of the 2549 unique reflections, only 1527 (60%) were judged observed after correction for Lorentz, polarization and background effects. A phasing model was developed with the MULTAN series of programs with six special and one general reflection as the starting variable phases.[4] The best phasing model showed all of the non-hydrogen atoms. Block diagonal least-squares refinements with anisotropic non-hydrogen atoms and isotropic hydrogens at calculated positions have converged to a standard crystallographic residual of 0.0725 for the observed reflections. Additional crystallographic details are available.[5]

A computer generated perspective drawing of the final X-ray model of the purine derivative **2** is shown opposite. The X-ray analysis defined only the relative configuration so the enantiomer shown is an arbitrary choice. Both the cyclohexane rings in the *trans*-decalin are in the chair conformation and the substituted purine ring is planar. The alkyl substituent at C-9 and the methyl at C-8 are equatorial. The double bond at C-13 has E stereochemistry. In general all bond distances and angles agree well with accepted values.

In an attempt to deduce the structure of the original sponge metabolite we have compared the ^{1}H NMR spectrum of the purino-diterpene **2** with those of the original methanol extract and of fractions representing each stage of the isolation procedure. These spectra provide evidence for formation of the purino-diterpene **2** from an N-acetyl precursor with C-2' and C-11' arising from the acetyl group and concomitant loss of a small nitrogen-containing fragment. Without knowing the identity of the fragment lost, we cannot identify the original sponge metabolite.

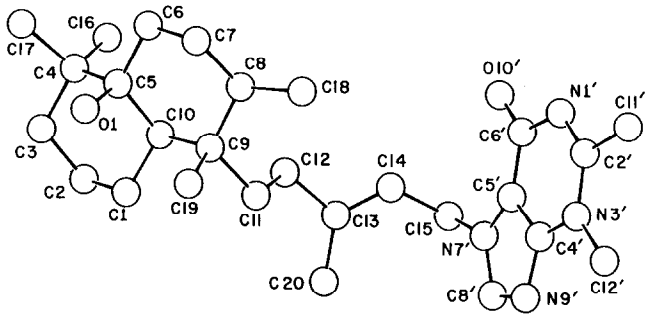


Figure. A computer generated perspective drawing of purino-diterpene 2. Hydrogens are omitted for clarity and no absolute configuration is implied.

The ^{1}H NMR spectrum of the purino-diterpene 2 contained signals at δ 0.79 (d, 3 H, J = 7 Hz), 0.82 (s, 3 H), 0.85 (s, 3 H), 0.97 (s, 3 H), 1.79 (br s, 3 H, C-20), 2.58 (s, 3 H), 3.83 (s, 3 H), 5.07 (d, 2 H, J = 7 Hz, C-15), 5.47 (t, 1 H, J = 7 Hz), 7.62 (s, 1 H), in good agreement with expected values. The ^{1}H NMR spectrum of the crude extract contained signals at 0.79 (d, 3 H, J = 7 Hz), 0.83 (s, 3 H), 0.85 (s, 3 H), 0.98 (s, 3 H), 1.85 (br s, 3 H, C-20), 5.43 (t, 1 H, J = 7 Hz), 5.53 (d, 2 H, J = 7 Hz, C-15), 8.01 (s, 1 H) and 8.31 (s, 1 H) with a number of sharp singlets in the N-Me region. The chemical shift values for the methyl signals are so nearly identical that a rearrangement of the diterpene portion of the molecule seems most unlikely. We therefore conclude that the diterpene portion of the molecule remained unchanged throughout. The observation that the chemical shift of the C-15 methylene proton signal is further downfield in the spectrum of the crude extract suggests a partial positive charge on the nitrogen attached to that position.[6] We can, however, reject the possibility of a 9-methyladenine derivative since these derivatives have a C-8 proton signal in the ^{1}H NMR spectrum at >10 ppm, and undergo hydrolysis to a formamide, neither of which were observed. The presence of two singlets at δ 8.01 and 8.31 tends to indicate a 3-methyladenine derivative with additional methyl groups at unspecified locations. We have been unable to obtain a fresh specimen of the sponge to complete this study.

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References and Notes

1. Cullen, E.; Devlin, J.P. *Can. J. Chem.*, **1975**, *53*, 1690.

2. R.J. Capon, unpublished data.

3. m.p. 95-98°C; UV (MeOH) 344 nm (ε 200), 267 nm (ε 3300), 221 nm (ε 9400); 360 MHz ^{1}H NMR ($CDCl_3$)--see text; HRMS, *m/z* 454.3315, $C_{27}H_{42}N_4O_2$ requires 454.3308.

4. All crystallographic calculations were done on a Prime 850 computer, operated by the Cornell Chemistry Computing Facility. Principal programs employed were REDUCE and UNIQUE, data reduction programs: Leonowicz, M.E., Cornell University, 1978. MULTAN 78, "A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data", direct methods programs and fast Fourier transformation routine (locally modified to perform all Fourier calculations including Patterson syntheses): Main, P.; Hull, S.E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M.M., University of York, England, 1978. NQEST, CYBER 173 version negative quartets figure of merit calculation: Weeks, C.M., Medical Foundation of Buffalo, Inc., August, 1976. BLS78A, anisotropic block-diagonal least-squares refinement: Hirotsu, K.; Arnold, E.; Cornell University, 1980. ORTEP, crystallographic illustration program: Johnson, C.K., Oak Ridge, TN, ORNL3794, June, 1965.

5. Crystallographic data has been deposited with Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, England. Any request should be accompanied by the full literature citation for this communication.

6. In a quarternary 9-methyladenine derivative, the ^{1}H NMR signal for C-15 occurs at δ 5.72 ($CDCl_3$).[2]

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