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A REVISED STRUCTURE FOR TAGETITOXIN

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Summary: New MS and NMR data for tagetitoxin required the structure to be revised to one consisting of two fused 6-membered heterocyclic rings.

Tagetitoxin is a phytotoxin produced in liquid cultures of the plant pathogenic bacterium Pseudomonas syringae pv. tagetis[1]. The toxin has been isolated and characterized[1], and a structure for it proposed[2]. However, stereochemical aspects of the proposed structure have remained tentative, since our inability to recover suitable crystals has precluded X-ray analysis. We have therefore undertaken more refined NMR and MS measurements which encourage us to propose a revised structure for tagetitoxin as a substituted 9-oxa-3-thiabicyclo[3.3.1]nonane.

Results from the present work (Table 1) were consistent with, and substantiated all of the previous NMR data recorded for tagetitoxin[2]. FAB mass spectrometry gave $(M+H)^+$ = 417.0361 ($C_{11}H_{18}N_2O_{11}PS$ requires 417.0369) indicating that tagetitoxin has a molecular formula $C_{11}H_{17}N_2O_{11}PS$ (c.f. $C_{11}H_{18}NO_{13}PS$ deduced previously[2]). The functional group containing the additional nitrogen was deduced from NMR data to be an amide, since the ^{13}C NMR definitively revealed only one C-N bond, and since there was no phosphoramide N

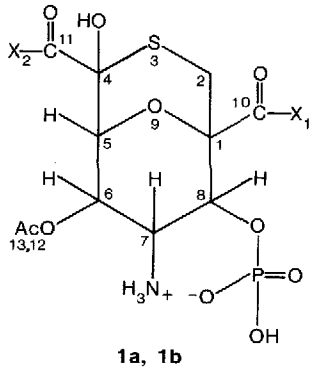
Table 1. ^1H- and ^{13}C- NMR data** for tagetitoxin (structure 1)

Carbon no	^1H δ ppm	^{13}C δ ppm	Multiplicity
1		71.7	Cq
2	2.92, 3.32	33.4	CH_2
4		85.7	Cq
5	4.38	73.0	CH
6	5.16	79.8	CH
7	3.46	43.9	CH
8	4.73	77.1	CH
10		174.5	Cq
11		171.2	Cq
12		174.1	Cq
13		23.2	CH_3

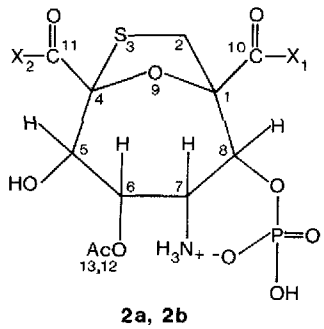
** spectra were referenced relative to external 4,4-dimethyl-4-silapentane sodium sulphonate

as evidenced by the ^{31}P chemical shift. The oxygen-containing functional groups of tagetitoxin are one acetyl, one phosphate, one carboxylic acid, one carboxamide, and two oxygens present in either hydroxyl or ether groups. A fully saturated non-cyclic compound with these substituents would have 21 H, and since from the MS tagetitoxin has 17 H and from the NMR no carbon-carbon multiple bonds it must therefore have 2 rings.

A linear series of four C-H protons, with the substituents shown in the figure and as in the original proposed structure, was substantiated from ^{1}H chemical shifts and coupling patterns, and also from ^{13}C chemical shifts. This partial structure is flanked at each extremity by quaternary carbons, and must therefore constitute part of one of the two rings of tagetitoxin. These data, in conjunction with considerations of carbon chemical shifts (Table 1), allow only two possible bicyclic ring structures (1 and 2) for tagetitoxin.



1a, 1b



2a, 2b

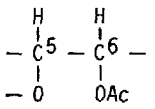
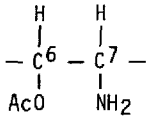
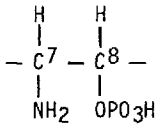
a $X_1 = OH$, $X_2 = NH_2$

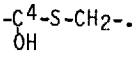
b $X_1 = NH_2$, $X_2 = OH$

These ring systems and substitution patterns were supported by NOE experiments. A strong NOE was observed between the geminal protons on C-2 and an NOE was also found between the vicinal protons on C-5 and C-6. A definitive NOE between one of the C-2 protons (at 2.92 ppm) and the C-7 proton (at 3.46 ppm) indicated that the molecule is relatively rigid and that there is a definite spatial proximity between these two protons which are attached to carbons far removed in the bonding framework. Such a proximity is conformationally possible in the ring structures of **1** and **2**, but would be unlikely to occur in the monocyclic ring structure proposed by Mitchell & Hart[2]. This NOE effect therefore substantiates the deduction from the mass spectral analysis, that the molecule is bicyclic, rather than the MS result being an artefact of an intramolecular dehydration.

The coupling constants between the protons of the four-carbon series (Table 2) allow the dihedral angles to be deduced. These angles are best accommodated by structure **1** where the protons on C-6 and C-7 are in a true diaxial interrelationship. In contrast, the 5-membered ring in structure **2** places a constraint on the 7-membered ring that does not allow a true diaxial relationship between the C-6 and C-7 protons to be attained. On this basis we favour the structure **1**, although the data do not entirely eliminate **2**.

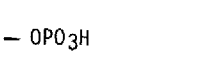
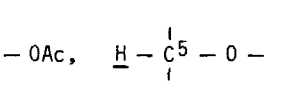
Table 2. Coupling in ^1H NMR and dihedral angles

Part-structure	Coupling constant H_z	Estimated dihedral angle
	3.6	50°
	12.4	180°
	6.0	35°

Structural information relating to the nature of the oxathiane ring and its substituents was obtained from ^{13}C-^1H long range shift correlation data (COLOC pulse sequence). In the aliphatic region of the ^{13}C NMR there was a very strong correlation between the quaternary carbon at 85.7 ppm and both protons of the methylene, which is consistent with the part structure  .

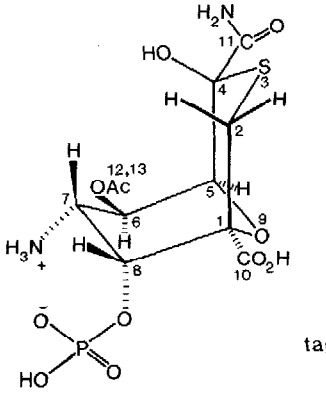
In the carbonyl region of the ^{13}C NMR spectrum, correlations found are listed in Table 3.

Table 3. Long range ^{13}C-^1H shift correlations (using a COLOC pulse sequence)

^{13}C-Carbonyl at δ ppm	Long range correlation to proton	Assignment of carbonyl C
174.5		C-10
174.1		acetyl carbonyl
171.2		C-11

The carbon at 174.1 ppm is assigned to the acetate carbonyl, which is consistent with this being the strongest of the three carbonyl signals. The carbon at 174.5 ppm correlates with the proton on C-8, and this provides evidence that the quaternary C-1 (adjacent to C-8) bears a carboxylic acid substituent, or its amide. Similarly, the carbon at 171.2 ppm correlates with the proton on C-5, and this provides evidence that the quaternary C-4 bears a carboxylic acid substituent, or its amide. The placement of the amide at C-10 or C-11 is not definitive from these data. However, we favour its location at C-11 on the basis of the smaller chemical shift of C-11. All of the ^{13}C COLOC data provide supportive evidence for the oxathiane ring-structure in tagetitoxin and the nature of its substituents.

The collective data allows the full assignment of the ^{13}C NMR spectrum (Table 1). The structure that best fits the collective data, **1a**, is proposed for tagetitoxin, although the placement of the amide at C-11 is equivocal. Thus tagetitoxin consists of two fused 6-membered rings, one a tetrahydropyran in the chair conformation, the other a 1,4-oxathiane in the boat conformation. The structure is depicted by the 3-dimensional presentation below where, with the exception of C-4, all of the relative stereochemistry of the molecule is defined by the data. Because of the boat conformation of the oxathiane ring, the favoured stereochemistry at C-4 is with the hydroxyl and amide substituents axial and equatorial respectively.



tagetitoxin

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1. Mitchell, R.E., Durbin, R.D. Physiol. Plant Pathol. 1981, 18, 157-168.
2. Mitchell, R.E., Hart, P.A. Phytochem. 1983, 22, 1425-1428.

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