STRUCTURE ELUCIDATION OF FICELLOMYCIN

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The structure of ficellomycin, an antibiotic previously discovered by ARGOUDELIS *et al.*, is elucidated as valyl-2-[4-guanidyl-1-azabicyclo[3.1.0]hexan-2-yl]glycine (**1**) by NMR, MS, and derivatization studies. The 1-azabicyclo[3.1.0]hexane moiety in **1** represents an unusual ring system making ficellomycin a unique natural product compound.

In a previous report,[1] ARGOUDELIS *et al.* described the production, isolation, and chemical characterization of ficellomycin. We now wish to report the structure of ficellomycin.

Experimental

TLC Procedures

TLC's were run on silica gel using ethanol - 3 M ammonium hydroxide (3 : 2) as the solvent system. The antibiotics were detected by bioautography on an agar medium seeded with *Staphylococcus aureus* agar tray or spraying with ninhydrin reagent.

NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer operated at 300 MHz for ^{1}H and 75 MHz for ^{13}C nuclei. D_2O was used as the solvent. ^{1}H-^{1}H correlation spectrum (COSY)[2] was obtained from a $128 \times 2,048$ data matrix, which resulted after zero filling in the F1 dimension in a $2K \times 2K$ data matrix. The spectral width was 1,400 Hz. Sixteen scans were recorded for each t_1 value with a delay time of 2 seconds between scans. ^{1}H-^{13}C COSY[3] was obtained from a $128 \times 4,096$ data matrix. The spectral widths were 13,500 Hz and 700 Hz in the F2 and F1 dimensions, respectively. For each t_1 value, 384 scans were recorded, and the delay time between scans was 1.5 seconds. The MLEV decoupling method was used with the decoupler positioned in the center of the ^{1}H spectrum. Long-range C-H correlation (COLOC)[4] spectrum was obtained with the same data size as the ^{1}H-^{13}C correlation experiment. The number of scans of each experiment was 1,024. Composite decoupling was used during acquisition.

Titration of Ficellomycin

The pH of a D_2O solution of ficellomycin was adjusted with dropwise addition of 2 N DCl directly into the NMR tube containing the sample. The chemical shift value was referenced to internal dioxane (67.4 ppm).

Methyl Ester Derivatization

Ficellomycin, 100 mg, was dissolved in 10 ml of 0.8 N methanolic HCl and refluxed for 2.5 hours. The solution was added to a mixture of acetone and diethyl ether (200 ml/50 ml) to precipitate the HCl salt. Fast atom bombardment (FAB)-MS gave a peak (M+H) at 327 amu confirming the formation of the methyl ester. Ester carbonyl absorbance was also observed in a Fourier transformation (FT)-IR spectrum of the methyl ester derivative of ficellomycin.

Results and Discussion

The physico-chemical properties of **1** were described in the previous report.[1] It was established

Table 1. ^{1}H and ^{13}C NMR data of ficellomycin.

Number	^{13}C chemical shift (ppm)	Multiplicity	^{1}H chemical shift (ppm) and coupling constant (Hz)
1	177.15	s	
2	57.43	d	4.32 (d, J=7)
3	52.62	d	3.61 (dd, J=11.5, 7)
4	27.98	t	β 2.13 (dd, J=13.4, 7.8)
			α 1.37 (dd, J=13.4, 11.5)
5	52.65	d	4.35 (ddd, J=9.8, 7.8, 5.3)
6	39.67	d	2.62 (dd, J=5.3, 3.5)
7	22.34	t	β 1.93 (dd, J=3.5, 2.1)
			α 1.61 (dd, J=5.5, 2.1)
8	157.54	s	
1′	176.65	s	
2′	61.39	d	3.15 (d, J=6.1)
3′	32.85	d	1.87 (m)
4′	18.04	d	0.84 (d, J=7)
5′	19.67	d	0.88 (d, J=7)

that **1** contained a valine unit which was linked to the remaining part of **1** by a peptide linkage. It was also found that there was a primary guanidino functional group in the molecule as evidenced by the positive Sakaguchi test, the strongly basic character of the antibiotic, its behavior in acidic or basic hydrolysis, and the 157.5 ppm peak in the ^{13}C NMR spectrum. The molecular formula was established in the previous study as $C_{13}H_{24}N_6O_3$ by the high resolution (HR)-MS method.

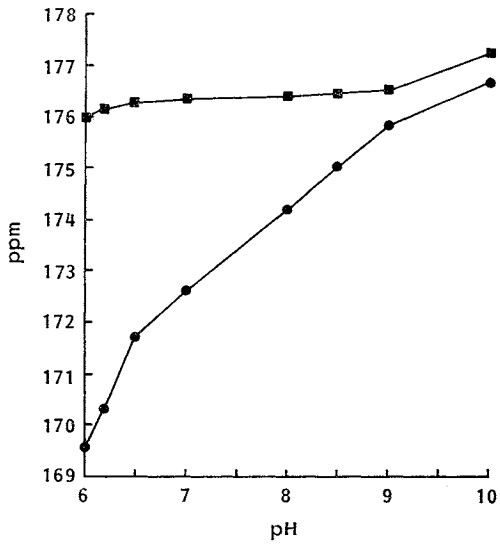
The ^{1}H and ^{13}C NMR results are listed in Table 1. The multiplicities of the ^{13}C NMR signals were determined by a distortionless enhancement by polalization transfer (DEPT) experiment. The correlation between protons and carbons was determined by a two-dimensional (2D) experiment.

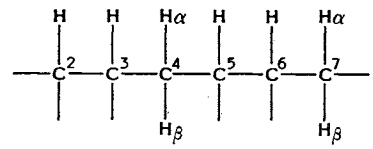
The NMR results in Table 1 are consistent with the molecular formula deduced from the HR-MS. Specifically, ^{13}C NMR exhibited the expected thirteen signals, five of them clearly due to a valine unit. ^{1}H NMR spectra displayed sixteen non-exchangeable protons, all of which can be correlated with protonated carbons by 2D C-H correlation experiments. Of the eight exchangeable protons, seven of these can be attributed to the guanidino group (4 exchangeable protons) and valine units (3 exchangeable protons), leaving one exchangeable proton unaccounted for. That this unaccounted exchangeable proton is due to the existence of a carboxylic acid group in the molecule is suggested by the following evidence: 1) One of the ^{13}C signals in the carbonyl region shifts upfield by $\sim$6 ppm on acid titration (Fig. 1); 2) the IR absorption in the carbonyl region changes markedly on addition of acid to the sample. A new band formed at 1728 cm^{-1}; and 3) methanolic HCl treatment of ficellomycin results in the decomposition of ficellomycin. However, the decomposed compound(s) give(s) an IR band at 1743 cm^{-1} and a three-proton signal at 3.7 ppm. All of the above evidence are consistent with the existence of a carboxylic acid functional group in the molecule.[5,6] It was established before,[1] that ficellomycin is inactivated at lower pH's (*vide infra*). The remaining portion of the molecule, therefore, should have C_6H_8N as the molecular formula. The 2D ^{1}H-^{1}H COSY clearly indicates that these eight protons belong to one spin network. It also identifies the other spin network as the valine protons. This eight proton spin network can be depicted as **I**.

The unusually small geminal coupling constant of 2.1 Hz between 7-H_α and 7-H_β is highly characteristic of an aziridine ring system.[7] The vicinal coupling constants between 6-H and 7-H are also

Fig. 1. Titration of ficellomycin chemical shift of carbonyls vs. pH.

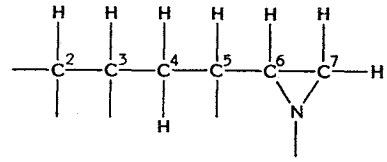
Functional group: ■ Amide, ● acid.



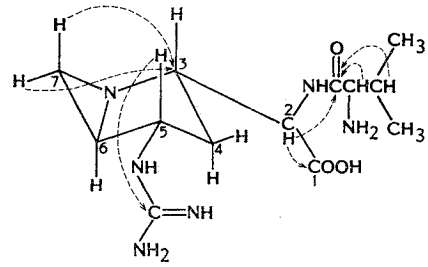


I

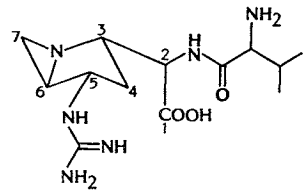
$J_{7\alpha,7\beta}$=2.1 Hz
$J_{6,7\alpha}$=5.3 Hz
$J_{6,7\beta}$=3.5 Hz
$J_{4\alpha,4\beta}$=13.4 Hz



II



III



1

consistent with the aziridine ring structure with the 7-H_α being *cis* to 6-H, while 7-H_β is *anti* to the N-R bond. The geminal coupling constant (13.4 Hz) between 4-H_α and 4-H_β indicates that they are part of a six-membered ring. The C_6H_8N fragment can be therefore constructed as **II**.

Evidence for the valine amido group attachment at C_2 comes from a 2D COSY study of a DMSO solution of **1**. The assignment of the amido proton is based on its slow exchange rate with D_2O. The fact that there are two functional groups to be linked to **II** and four valencies available suggests that **1** is a bicyclic compound. 2D long-range C-H correlation were utilized to place the two functional groups and close the ring, results are summarized on **III**.

The long-range correlation results suggest that the ring is cyclized between the aziridine nitrogen and C_3 carbon. Furthermore, the carbonyl group is attached to the main fragment at C_2, and the guanidino group is attached to the main fragment at C_5. The structure of ficellomycin, therefore, can be constructed as **1**.

The stereochemistry is assigned based on the coupling constants observed in the 500 MHz ^1H NMR spectrum. To our knowledge, **1** represents the first natural compound possessing a 1-azabicyclo-[3.1.0]hexane ring system. It has been speculated that this system may be generated *in situ* and plays a role in the antitumor activity observed in 593A.[8] In the case of ficellomycin, the aziridine ring-

system may be stabilized by the neighboring guanidino group. Nonetheless, the aziridine-ring system is probably responsible for the acid-labile property noted earlier. Indeed, treatment of **1** with acids would result in a bioinactive compound which is devoid of the aziridine moiety as suggested by the loss of characteristic ^{1}H geminal couplings and the downfield shift of the ^{13}C chemical shift values of C-6 and C-7. The isolation and identification of the bioinactive compound will be discussed in a later report.

The aziridine ring system is most likely responsible for the antimicrobial activities of this compound. The mode of action of ficellomycin could very well be the inhibition of DNA synthesis since the aziridine ring is often a very effective alkylating reagent.

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