STRUCTURE ELUCIDATION OF ANGIOTENSIN CONVERTING ENZYME INHIBITOR L-681,176 FROM *STREPTOMYCES* SP. MA 5143a

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(Received for publication December 17, 1983)

L-681,176 (**1**, $C_{12}H_{23}N_5O_7$) is an inhibitor of angiotensin converting enzyme produced by *Streptomyces* sp. MA 5143a. The structure of L-681,176 has been determined by NMR and mass spectral analysis.

In the preceding paper,[1] the fermentation, isolation and characterization of the angiotensin converting enzyme inhibitor, L-681,176, has been reported. In the present paper, the structure determination of L-681,176 is described.

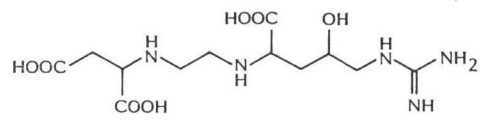
Structure of L-681,176

L-681,176 (**1**) is a water soluble compound which exhibits no UV absorption in the region 220~350 nm. The molecular weight of **1** was found to be 349 by field desorption mass spectrometry, which afforded ions at *m/z* 350 $(M+H)^+$ and 332 $(M-H_2O+H)^+$. Useful mass spectra of **1** were not obtained by electron impact. Treatment of **1** with bis-trimethylsilyltrifluoroacetamide in pyridine at 90°C for 3 hours afforded the hexa-trimethylsilyl (hexa-TMS) derivative **2**, which by high-resolution mass measurement (HR-MS) indicated the molecular formula $C_{12}H_{23}N_5O_7$ for **1** (Calcd for $C_{12}H_{23}N_5O_7+Si_6C_{18}H_{48}$, 781.3969; Found 781.3980).

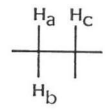
The ^1H NMR spectrum of **1** is listed in Table 1. Decoupling studies gave the partial subunits **a** and **b** and disclosed the almost singlet appearing, four-proton multiplet at 3.50 (H_j, H_k, H_l, H_m). Partial subunit **a** is reminiscent of an aspartate residue which was confirmed by a pH titration in the range 1.0~11.6 followed by NMR. H_a and H_b are implicated in titration of both carboxyls, but the two *pKa*'s are not clearly separated. Many of the resonances were affected by more than one ionizable group but those with primary shifts were observed as follows: *pKa* 1.75, H_c and H_h; 6.5, H_h and two of the δ 3.50 reso-

Table 1. ^1H NMR spectrum of **1** in D_2O (25°C, pH 5.32).

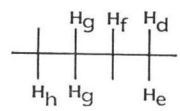
Chemical shift (ppm)	Proton integration	Multiplicity (*J* in Hz)	Assignment
2.73	1	dd (9, 18)	H_a
2.88	1	dd (18, 4)	H_b
3.91	1	dd (9, 4)	H_c
3.23	1	dd (14.5, 7.5)	H_d
3.35	1	dd (14.5, 3.5)	H_e
4.00	1	m	H_f
2.10	2	t (5.5, 5.5)	H_g
3.90	1	t (5.5)	H_h
3.50	4	m	H_j, H_k, H_l, H_m



1

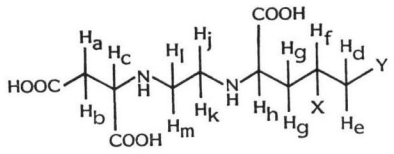


a



b

nances (H_j, H_k); and 10.2, H_e and the other two δ 3.50 resonances (H_l, H_m). Because of severe overlap problems, the titration behavior of the four protons at δ 3.50 could not be accurately followed but they appear to titrate as indicated. Only slight shifts of the four protons are observed on titration with acid, but the addition of base leads to upfield shifts and appears to titrate one of the four protons faster than the rest. Decoupling at various alkaline pH readings demonstrated conclusively that all four protons are coupled and never at any stage formed a simpler spin-spin pattern than ABCX which defied analysis. There are few permutations that allow four protons to be coupled, and an ethylenediamine unit seemed most probable. This assignment was corroborated by the observed *pKa*'s of 6.5 and 10.2 which are typical of the two-step protonation of the nitrogens of such a moiety.[2]



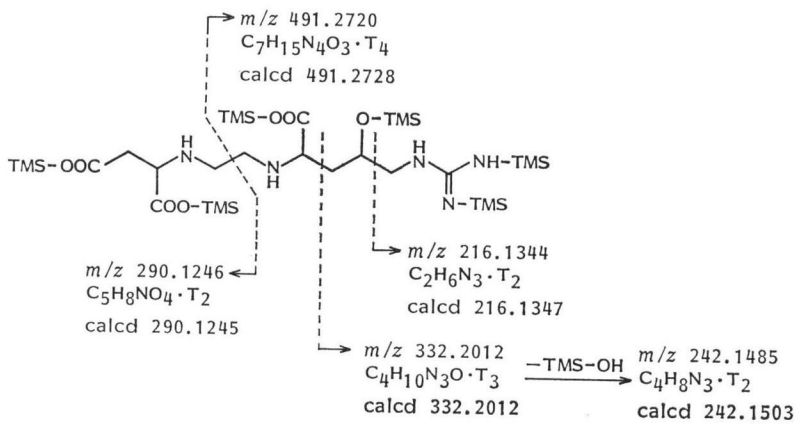
c

H_c and H_h are implicated in titration of a carboxyl group as well as the ethylenediamine nitrogens, thus suggesting the linkage of subunits **a** and **b** through the ethylenediamine unit as in **c**. Confirmation comes from the following observations: the *pKa* of 10.2 is reflected to a smaller extent in H_a, H_b, H_j and H_k, much smaller in H_h, and none in H_d, H_e, H_f and H_g; and the *pKa* of 6.5 is reflected to a smaller extent in H_g, H_l and H_m, much smaller in H_c and H_f, and none in H_a, H_b, H_d and H_e. The nature of X and Y in **c** was not immediately obvious from the NMR data and the HR-MS data for the trimethylsilyl derivative **2** afforded the answer.

The empirical formula was determined by HR-MS and the number of TMS groups was determined by comparison with the perdeutero-TMS derivative. The hexa-TMS derivative indicates the molecular formula $C_{12}H_{23}N_5O_7$ for **1** and partial structure **c** accounts for the elements $C_{11}H_{18}N_2O_6$ leaving CH_5N_3O for X plus Y. Neither group, X or Y, titrates in the pH range 1.0~11.6 and the chemical shift of H_f (δ 4.00) suggests that X=OH and Y=CH_4N_3. Several permutations are possible for Y, but the most likely is a guanidino group which usually has a *pKa* near 12.5 and was therefore not detected in the initial NMR titration. Upon reexamination, H_d and H_e were found to move upfield at pH 12.45. In addition, L-681,176 exhibited a positive SAKAGUCHI test.[3]

In the mass spectrum of **2** (Fig. 1), key fragment ions at *m/z* 332, 242 and 216 tend to define the

Fig. 1. Mass spectral fragmentation of **2** (the symbol·T stands for SiC_3H_9).



guanidino group *via* their high nitrogen: carbon ratios and suggest a hydroxyl group due to loss of TMS-OH (332 to 242). The ions at *m/z* 491 and 290 result from cleavage through the ethylenediamine unit, thus completing the sequence and allowing the assignment of structure **1**. Prolonged treatment of **1** with TMS reagent did afford the corresponding hepta-, octa- and nona-TMS derivatives as a mixture, thus corroborating the number of derivatizable functionalities in the molecule.

Structure **1** may be conceived biogenetically as the addition of aspartic acid and γ-hydroxyl arginine to pyruvate through their α-amino groups, accompanied by decarboxylation. In this regard they resemble the 'marasmine-type' amino acids[4~6] which, however, retain the carboxyl group.

Materials and Methods

General Methods

^{1}H NMR spectra were obtained on a Varian SC-300 instrument and high-resolution mass measurements were performed on a Varian MAT-731 mass spectrometer.

pH Titration

L-681,176 (3.6 mg) was dissolved in 100% D_2O and titrated *in situ* in a 5-mm NMR tube with 0.1 and 0.01 N NaOD and DCl using a Copenhagen Radiometer pH meter. ^{1}H NMR spectra were recorded at various pH's in the interval 1.02~11.61 and shifts of the various resonances plotted *versus* pH to determine *pKa*'s. No pH correction was applied to allow for titration in D_2O. The titration was completely reversible and no decomposition or racemization of **1** was noted in this pH range.

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