Absolute Configurations of Macrolide Antibiotics of the Bafilomycin and Leucanicidin Groups

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Antibiotics of the bafilomycin[1] and leucanicidin groups[2] isolated from various microorganisms of the order *Actinomycetales* have been known for some years to possess a broad spectrum of biological activity against bacteria, fungi, yeasts, insects, protozoa, cestodes, and free-living nematodes. More recently they have been shown to have potent nematocidal activity against the free-living stages of intestinal parasitic nematodes of major importance in the animal health industry.[3] Furthermore the bafilomycins have been suggested as possible therapeutic agents in the treatment of peptic ulceration, by virtue of their inhibition of cell vacuolisation induced by the bacterium *Helicobacter pylori*[4~6].

The molecular structures of both the bafilomycins and the leucanicidins are characterised by the presence of a similar 16-membered macrolide nucleus to which various side chains are attached at the lactone terminus C15 (Fig. 1), leading to the generic term bafilolides for these antibiotics[3]. The only bafilolide for which the complete stereochemistry has been rigorously secured is bafilomycin A_1. X-ray crystallography of its 21-(2′,2′,2′-trichloroethylcarbonate) derivative established the absolute configuration depicted in structure **1**[7], confirming that predicted from ^{1}H NMR data and computer modeling in comparison with the known related antibiotic elaiophylin[8]. Both crystalline and solution conformations of bafilomycin A_1 are stabilised by a hydrogen-bonding network involving C19-OH, C17-OH and C1=O, which fixes the configuration of the potentially epimerisable C19-hemiacetal hydroxyl group[7,9~11].

Other bafilomycins and the leucanicidins (Fig. 1) have been assumed to correspond in absolute configuration at their respective stereogenic centres to bafilomycin A_1 (**1**)[3,12]. Evidence for such correspondence, however, is incomplete for both the bafilomycins and the leucanicidins. In view of the increasing biological significance of these antibiotics and the consequent need for accurate stereochemical information, we present here evidence which firmly establishes the correspondence of the absolute configurations of bafilomycins A_1, B_1, C_1 and D, and of leucanicidin. We also consider the stereochemical status of the related antibiotics bafilomycin A_1 21-*O*-(α-L-rhamnopyranoside), 7-*O*-isobutyrylleucanicidin, and L-681,110B_1.

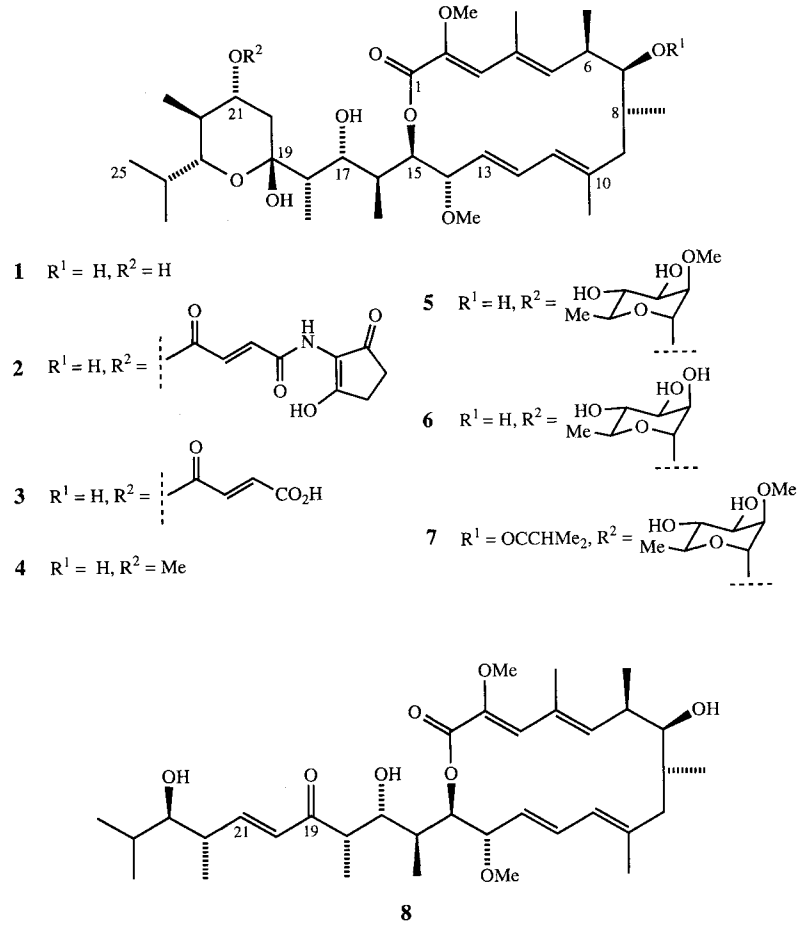
Results and Discussion

Absolute Configurations of Leucanicidin, 7-*O*-Isobutyrylleucanicidin and Bafilomycin A_1 21-*O*-(α-L-rhamnopyranoside)

The two-dimensional structure of the aglycone of leucanicidin (**5**) was defined primarily by NMR spectroscopy[13], and was confirmed[14] by comparison of its spectra and degradation products with those of bafilomycin A_1 (**1**)[12,15], which lacks the 21-(2′-*O*-methyl-L-rhamnoside) sugar moiety of leucanicidin. Since the C13~C25 segments of the two antibiotics afforded acyclic degradation products identical in all respects including optical rotation[14], these segments also correspond in absolute configuration, with the possible exception of the cyclic hemiacetal centre C19 which is potentially epimerisable and is destroyed in these reactions. ^{1}H NMR spectra of the intact antibiotics[10,13], however, show similar W-coupling between C19-OH and H20α, indicating that the configurational correspondence includes also C19. The remaining common stereogenic centres C6, C7 and C8 of the two antibiotics were shown to correspond at least in relative configuration, but no absolute conclusion could be reached[14]. Cleavage of the C5~C10 segment of each antibiotic afforded inseparable mixtures of two stereoisomers of 2,4-dimethylheptane-1,3,6-triol, but in different ratios thus precluding meaningful comparison of optical rotation values.

After considerable experimentation with the acid- and base-sensitive leucanicidin, we have shown that hydrolysis of the carbohydrate moiety by mild treatment with *p*-toluenesulfonic acid in aqueous acetonitrile, conditions which have been employed for the preparation of the aglycones of the macrolide antibiotics concanamycin A and elaiophylin[16], affords bafilomycin A_1 (**1**). In conjunction with the known absolute configuration and anomeric stereochemistry of the attached rhamnoside[13], this conversion confirms the previous structural and

Fig. 1. Structures of bafilomycins A_1 (**1**), B_1 (**2**), C_1 (**3**), D (**8**), bafilomycin A_1 21-*O*-(α-L-rhamnopyranoside) (**6**), leucanicidin (**5**), 7-*O*-isobutyrylleucanicidin (**7**), and antibiotic L-681,110B_1 (**4**).



stereochemical conclusions[13,14] and establishes the complete absolute configuration of leucanicidin as that depicted in structure **5**.

The ^{1}H NMR chemical shift and coupling constant data provided for 7-*O*-isobutyrylleucanicidin, a minor co-metabolite of leucanicidin in *Streptomyces halstedii*[17], are in complete accord (except for expected chemical shift differences in the vicinity of the additional ester substituent) with those of leucanicidin itself[13], and thus imply the corresponding absolute configuration **7**.

Streptomyces olivaceus produces leucanicidin (**5**) together with a related antibiotic, which was assigned the two-dimensional structure **6** and named bafilomycin A_1 21-*O*-(α-L-rhamnopyranoside) on substantial but incomplete evidence similar to that described above for leucanicidin[14]. With the subsequent establishment of the stereochemistry of bafilomycin A_1 itself as **1**[7], this rhamnoside if correctly assigned would have the stereochemistry **6**, and from the present work leucanicidin (**5**) would be its 2′-*O*-methyl ether. Detailed consideration of the reported ^{1}H and ^{13}C NMR data[13,14] for the rhamnoside and leucanicidin confirms their relative stereochemical correspondence, and in conjunction with their similar optical rotations confirms the absolute configuration **6** for this rhamnopyranoside. We attribute minor disagreements in the NMR data to recording errors, apart from the obligatory differences in the sugar resonances.

Absolute Configurations of Bafilomycins B_1, C_1, D and Antibiotic L-681,110B_1

Bafilomycins B_1 (synonym setamycin), C_1 (synonym antibiotic L-681,110A_1) and D (synonym tubaymycin) have been shown to have the two-dimensional structures **2**, **3**, and **8**, respectively[1]. No chemical interconversion has been reported between bafilomycin A_1 (**1**), which has established stereochemistry, and these related bafilomycins, although bafilomycins B_1 and C_1 have been stated[12] without further detail to be "C21 substituted derivatives of bafilomycin A_1". The published NMR data

do not allow comparison of stereochemistry, since the necessary complete detail is lacking for bafilomycins B_1 and D while different solvents were used for the reported ^1H spectra of bafilomycins A and C_1. The co-production of various bafilomycins by the same microorganism (*e.g.*, A_1, B_1, C_1 and D by *Actinomyces* sp. A239[3], and A_1, B_1 and C_1 by *Streptomyces griseus* subspecies *sulfurus*[1]) suggests configurational correspondence, but does not necessarily ensure that it is complete. The formation of epimeric alcohols, for example, by a single organism is not uncommon (*e.g.*, the lactones brefeldin A and 7-*epi*-brefeldin A by *Curvularia lunata*[18]). KRETSCHMER and coworkers, however, have converted bafilomycins B_1 and C_1 into bafilomycin D by warming at pH 13.5 in dioxane-water, which effects ring opening of the hemiacetal to the δ-hydroxyketone followed by β-elimination of the fumarate ester to yield the δ-hydroxy-α,β-unsaturated ketone[19]. These three bafilomycins must therefore correspond in configuration at all stereogenic centres, with the possible exceptions of the C19 hemiacetal centre and the C21 ester-carrying centre in B_1 and C_1, both of which are destroyed in these conversions into D.

Treatment of leucanicidin (**5**) at room temperature with aqueous methanolic sodium hydroxide afforded an anhydroaglycone, identical in all respects including optical rotation to bafilomycin D. These conditions have previously been employed to form anhydroaglycones from the related 18-membered macrolide antibiotics concanamycin A[20], B and C[21], and again involve ring opening of the hemiacetals to δ-hydroxyketones followed now by β-elimination of the sugar moieties. Since we have established the total absolute configuration of leucanicidin as **5**, then the present conversion establishes that of bafilomycin D as depicted in structure **8**. Taken in conjunction with the work of KRETSCHMER and coworkers[19] (see above), it also establishes that bafilomycins B_1 and C_1 have the configurations **2** and **3**, except for the C19 and C21 centres already noted. The large $10\sim12$ Hz coupling constants between the adjacent protons H20α, H21, H22 and H23 of bafilomycin C_1[22], however, define their *trans*-diaxial vicinal relationships in the chair-shaped tetrahydropyran ring; hence the C21 configuration of this antibiotic relative to the known centres C22 and C23 is as shown in structure **3**. Although the corresponding proton spin system in bafilomycin B_1 is partially obscured by other resonances, the critical signal of H21 appears as a doublet of triplets with J 5.0 and 11.0 Hz at δ 5.09 ($CDCl_3$), establishing the C21 configuration relative to C22 as shown in structure **2**. Whilst the C19 centres in bafilomycins B_1 and C_1 are potentially epimerisable, ^1H and ^{13}C NMR spectra[3,22] indicate that both antibiotics exist as single epimers in organic solvents. These epimers are probably those depicted in structures **2** and **3**, stabilised by hydrogen-bonding networks between C19-OH, C17-OH and C1=O similar to that established for bafilomycin A_1[7,9~11].

Antibiotic L-681,110B_1, a minor component of the sodium and potassium ion activated adenosinetriphosphatase inhibitor L-681,110, has been shown to have the two-dimensional structure **4** together with relative stereochemistry around the tetrahydropyran ring as depicted[22], and has been considered[1] to be 21-(*O*-methyl)-bafilomycin A_1. Comparison of its ^1H NMR chemical shift and coupling constant data[22] with that recorded for bafilomycin A_1 in the same solvent[10,15] shows excellent correspondence, except as expected for the chemical shifts of H20, H21 and H22 which are near the additional methyl ether group, and also for the assignments of C8-CH_3 and C24-CH_3 which should probably be reversed. Accordingly L-681,110B_1 is appropriately represented by the absolute configuration **4**.

Conclusion

The macrolide antibiotic leucanicidin has been converted into the related macrolides bafilomycin A_i (**1**) and bafilomycin D. In conjunction with the established absolute configuration of bafilomycin A_1, and the previous conversions of bafilomycins B_1 and C_1 into bafilomycin D, this establishes the complete absolute configurations of bafilomycin B_1 (**2**), C_1 (**3**), D (**8**), and leucanicidin (**5**) as depicted (Fig. 1). Detailed consideration of the available spectroscopic data for the related bafilomycin A_1 21-*O*-(α-L-rhamnopyranoside) (**6**), 7-*O*-isobutyrylleucanicidin (**7**) and antibiotic L-681,110B_1 (**4**) also settles their absolute configurations as shown. The stereochemistry of the carbon skeleton of these bafilolide antibiotics is specified by the descriptors 2*Z*, 4*E*, 10*E*, 12*E* and 6*R*, 7*S*, 8*R*, 14*S*, 15*R*, 16*S*, 17*R*, 18*S*, 19*R*, 21*R*, 23*R*, except for bafilomycin D which lacks tetrahedral centres at C19 and C21 but has a 20*E* olefinic bond. The chirality at C22 remains unchanged in all these metabolites, but its descriptor varies with the functionalisation of the C21-OH group.

Experimental

General

NMR spectra were recorded for acid-free $CDCl_3$ solutions on Varian Gemini-300 or VXR-500 instruments, with the solvent as internal reference. FAB-MS data were obtained on a VG ZAB2-SEQ spectrometer using 3-nitrobenzyl alcohol as the matrix. Leucanicidin was obtained as described previously[3].

Conversion of Leucanicidin (5) into Bafilomycin A_1 (1)

Leucanicidin (**5**) (18 mg) was treated with *p*-toluenesulfonic acid (22 mg) in MeCN - H_2O (4 : 1, 4 ml) at 25°C for 24 hours. The solution was cooled to 0°C, neutralised with saturated aqueous sodium bicarbonate, and extracted with EtOAc. Chromatograpy of the dried ($MgSO_4$), evaporated extracts on silica gel (CH_2Cl_2 - Me_2CO, 4 : 1) afforded bafilomycin A_1 (**1**) (1 mg, 5%), identified by comparison of ^1H and ^{13}C NMR spectra with literature data[10]; FAB-MS *m/z* 645 $(M+Na)^+$.

Conversion of Leucanicidin (5) into Bafilomycin D (8)

Leucanicidin (**5**) (10 mg) was treated with 0.03 N aqueous sodium hydroxide in MeOH (1 : 1, 1 ml) at room temperature, and the reaction monitored by TLC. After 1 hour the starting material had been consumed, and the mixture was neutralised with dil. hydrochloric acid and extracted with EtOAc. The extracts were dried ($MgSO_4$), concentrated, and subjected to flash chromatography on silica gel (CH_2Cl_2 - Me_2CO, 9 : 1) to yield bafilomycin D (**8**) (2.2 mg, 28%), identified by comparison of ^1H and ^{13}C NMR spectra with literature data[19]; $[\alpha]_D^{22}$ −246° (*c* 0.6, $CHCl_3$) {lit.[19] $[\alpha]_D^{25}$ −251° (*c* 0.6, $CHCl_3$)}.

Acknowledgments

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