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Supplementary Material Available: ^{17}O NMR spectra for the Fe(II)·BLM-mediated formation of $H_2{}^{17}O$ from $^{17}O_2$ (2 pages). Ordering information is given on any current masthead page.

Stereostructure of Pimaricin

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Pimaricin, isolated in 1957 from *Streptomyces natalensis*,[1] is the first polyene macrolide whose correct covalent structure **1** was established[2] after a 10-year period of numerous revisions.[3,4] Its comparison with the very similar tetraenic antibiotic tetrin A[5] in which the *S* configuration at C_{25} was established[6] led to a confusing stereochemical situation for pimaricin for which the same *S* configuration at C_{25} was given.[7] Even more disconcerting was the description of the absolute configurations of other asymmetric centers which were by no means proven.[8] Pimaricin represents a prototype molecule of the glycosylated polyene macrolides,[9] important for antifungal therapy and promising for other properties including antiviral activity, stimulation of the immune response, and action in synergy with other antifungal drugs or antitumor compounds.[10] A long-standing lack of stereostructural information has been the major obstacle for interpreting structure–activity relationships. We now report the complete stereostructure of pimaricin,[11] whose convenient solution arose from our recent study on nystatin A_1.[12]

The basis of our approach includes (a) a combined use of phase-sensitive DQF-COSY,[13] NOESY,[14] and/or ROESY[15] 2D proton NMR experiments[16] for assessing relative configurational features, (b) a search for unambiguous proton–proton through-space contacts with the sugar D-mycosamine taken as an internal chiral probe to attain the absolute configuration, and (c) execution of minimal chemical modifications of the natural substance to identify configurations left unknown after the above procedures.

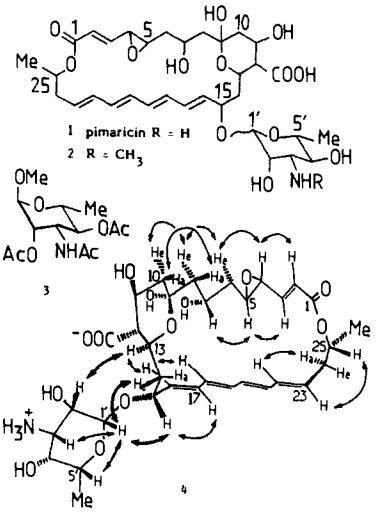

Figure 1. The double-headed arrows in structure **4** connect the pairs of hydrogens that are correlated by NOE (ROE) interactions. Only selected NOE (ROE) connectivities are shown; contacts between scalar coupled protons are not quoted. Ha(e) at C_6, C_8, C_{14}, and C_{24} refer to the pseudoaxial (a) or pseudoequatorial (e) orientation of these protons relative to the average plane of the macrocycle.

The D series of the mycosamine sugar[3a] was first confirmed by standard deglycosidation of pimaricin (HCl, MeOH, reflux, 2 h) and acetylation to di-*O*-acetate **3**, mp 139 °C, $[\alpha]_D$ +30°, identical with the compound obtained from nystatin A_1.[17] Analysis of phase-sensitive DQF-COSY experiments (10 mM MeOH-d_4 or DMSO-d_6 solutions) furnished the complete ^1H-^1H coupling pattern of pimaricin **1** and its *N*-acetyl derivative **2** (see supplementary material). This information combined with pertinent NOE contacts (see structure **4**, Figure 1) made the following structural assignments possible: (a) the chair conformation of the C_9–C_{13} segment with substituents at C_{11}, C_{12}, and C_{13} equatorials ($J_{10a,11}$ = 11.0, $J_{10e,11}$ = 4.8, $J_{11,12}$ = 10.5, and $J_{12,13}$ = 10.5 Hz) confirming previous observations;[18,19] (b) the axial orientation of OH_9, the first direct observation for structurally related polyene macrolides in solution ($J_{OH9-H10a}$ = 0.5–1 Hz in DMSO-d_6); (c) the diastereotopicity of the H_8 protons (see structure **4** for NOE contacts), hence the configuration at C_7 relative to the C_9–C_{13} tetrahydropyran; and (d) an accurate local geometry of the C_{13}–C_{16} segment by the *J* connectivities ($J_{13,14a}$ = 8.4, $J_{13,14e}$ = 1.0, $J_{14a,15}$ = 2.0, $J_{14e,15}$ = 3.5, and $J_{15,16}$ = 8.3 Hz) and observations of the NOE contacts, especially for the proton pairs H_{13}–H_{16} and H_{15}–H_{17}, defined the diastereotopicity of the H_{14} protons and thus the configuration at C_{15} relative to the one at C_{13}.

To attain the absolute configuration of this structural segment, the NOE map furnished three crucial data: the previously suggested[18a] β-configuration of the anomeric linkage at O_{15} of the aglycon ($H_{1'}$–$H_{3'}$ and $H_{1'}$–$H_{5'}$ NOE contacts), the proximity of the anomeric proton $H_{1'}$ to *both* H_{14e} and H_{15} of the aglycon ($H_{1'}$–H_{14e} and $H_{1'}$–H_{15} NOE contacts), and the sufficiently close location of $H_{2'}$ to H_{13} of the aglycon to observe a NOE connectivity. Only an *R* configuration at C_{15} can conform to the last

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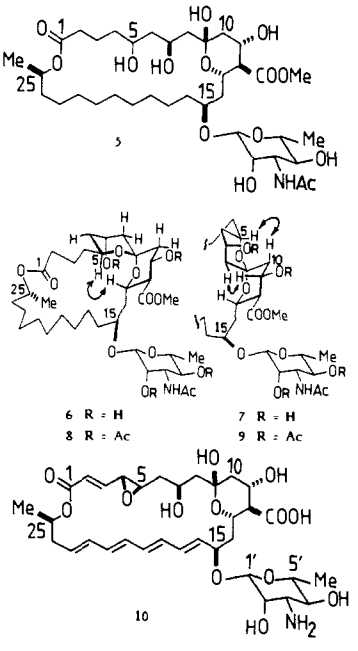

Figure 2.

three NOE distance constraints, a situation already observed in the stereostructural study of nystatin A_1.[12c] Consequently, the 7*R*, 9*S*, 11*S*, 12*R*, 13*S*, and 15*R* configurations were assigned for the pimaricin aglycon.

A precise definition of the local geometry of the C_{22}–C_{25} portion was easily derived from combined data taken from the DQF-COSY ($J_{23,24a}$ = 8.8, $J_{23,24e}$ = 2.5, $J_{24a,25}$ = 11.0, and $J_{24e,25}$ = 5.6 Hz) and NOESY experiments (H_{22}–H_{24a} and H_{23}–H_{25} NOE contacts, structure **4**). The *all-E* extended C_{16}–C_{23} tetraene (H_{17}–H_{18}, H_{19}–H_{20}, and H_{21}–H_{22} all antiperiplanar by pairs) behaves indeed as a long-range sensor which defines the orientation of H_{23} relative to H_{16} and hence to the C_9–C_{15} chiral segment. This observation combined with the C_{22}–C_{25} relative geometry described above defined the diastereotopicity of the H_{24} protons and, therefore, the *R* configuration at C_{25}.

Due to the quasi-planar arrangement of the epoxide function, the spectroscopic study left the C_4–C_5 configurations undetermined. At this point, it was anticipated that the disjunction of the macrocycle structural rigidity allied with a regioselective epoxide opening would permit the formation of a bicycloketal, a conformationally biased skeletal framework ideally suited for structural investigation. Hydrogenolysis (H_2, Pd/C, MeOH, room temperature, 1.5 h) and methyl ester formation (CH_2N_2, MeOH) on *N*-acetylpimaricin (**2**) led to the single saturated polyol **5**, $[\alpha]_D$ −70°, with a hydroxyl group located at C_5[20,21] (Figure 2). Acid-catalyzed bicycloketalization (CSA cat., $CHCl_3$–MeOH, 9:1, room temperature) gave two easily separated isomers **6**, $[\alpha]_D$ −88°, and **7**, $[\alpha]_D$ − 105°, in a ratio of 2:1 (2-h reaction) transformed to a 40:1 ratio (72-h reaction, 85% yield). This chemical behavior strongly suggested a 5,7-*syn*-diol system.[22] Proton NMR analysis of tetra-*O*-acetates **8** (major isomer), $[\alpha]_D$ −71°, and **9** (minor isomer), $[\alpha]_D$ −47°, fully confirmed this prediction. The *J* coupling pattern observed (not shown) led to chair–chair bicycloketals with a characteristic splitting pattern for an equatorial proton at C_7 in both isomers. This conformational and configurational situation was fully validated by long-range space contacts derived from NOESY (ROESY) maps (H_5–H_{13} in **8** and H_5–H_{10e} and H_{8e}–H_{11} in **9**). The 5*R* configuration was then determined, and by extension the 4*S* configuration, thus defining the complete stereostructure of pimaricin as **10**.

Analyzing through-space contacts between a well-characterized carbohydrate and a chiral aglycon of unknown absolute configuration represents a simple and powerful method of general interest for three-dimensional assignments of naturally or artifically glycosylated structures. In polyene macrolides containing polyhydroxy ketonic structural segments, bicycloketalization of saturated macrocycles may provide a useful protocol for stereostructure determination.

Acknowledgment. We express our gratitude to Gist-Brocades (The Netherlands) for a generous supply of pimaricin.

Supplementary Material Available: Physical data for **3** and **5**–**7**, phase-sensitive DQF-COSY data for **1**, **2**, **5**, **8**, and **9**, and NOESY/ROESY data for **1**, **2**, **8**, and **9** (5 pages). Ordering information is given on any current masthead page.

(20) Information obtained by the phase-sensitive DQF-COSY spectrum of **5** in DMSO-d_6 (15 mM, 298 K): H_5 (3.60 ppm), OH_5 (4.54), 2 H_6 (1.39–1.49), H_7 (4.08), and OH_7 (4.92).

(21) The same degradation sequence was described to give an hydroxyl group at C_4[18a] in contradiction with the previous findings of Golding et al.[2] placing the OH group at C_5 in the allylic hydrogenolysis of the epoxide function of *N*-acetylpimaricin (**2**).

(22) A 5,7-*anti*-diol system would cyclize in two isomers equilibrating under mild acidic conditions to a ratio of approximately 1:1, as no differences in electronic effects (stabilizing) and steric effects (destabilizing) between the two isomers would be observed.[23]

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[7.7]Paracyclophanes from Blue-Green Algae

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[*m.n*]Paracyclophanes[1] were first described by Cram and Steinberg in 1951.[2] These carbocyclic compounds, known to date only through synthesis, have provided interesting vehicles for host–guest chemistry. We report here the isolation and identification of [7.7]paracyclophanes from two species of cytotoxic blue-green algae belonging to the Nostocaceae. This marks the first time that this class of macrocyclic compounds has been found in Nature.

In an evaluation of blue-green algae for antitumor activity, extracts of two species belonging to the Nostocaceae, viz., *Cylindrospermum licheniforme* Kutzing (ATCC 29204) and *Nostoc linckia* (Roth) Bornet (UTEX B1932), were found to exhibit moderate cytotoxicity against KB and LoVo tumor cell lines at <20 μg/mL.[3,4] Each freeze-dried cyanophyte[5] was extracted with 70% aqueous ethanol and the resulting extract subjected to normal-phase (silica gel) and/or reverse-phase (C-18) column chromatography, to give a mixture of cytotoxic [7.7]para-

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