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PIMARICIN—VIII

STRUCTURAL AND CONFIGURATIONAL STUDIES BY ELECTRON IMPACT AND FIELD DESORPTION MASS SPECTROMETRY, ^{13}C (25.2 MHz) AND ^{1}H (270 MHz)-NMR SPECTROSCOPY

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Abstract—Attempts to obtain a molecular ion from the polyene macrolide antibiotic pimaricin by EI and FD mass spectrometry were unsuccessful. The loss of carbon dioxide and a varying number of water molecules from the molecular ion made a molecular-weight determination impossible. ^{13}C-NMR spectroscopy of N-acetylpimaricin, its dodecahydroderivative, and of HH-2, a hydrogenation-hydrogenolysis product of N-acetylpimaricin, confirmed that the antibiotic has structure **3** containing a hemi-ketalic ring and lacking an OH group at C-8. The value of the anomeric coupling constant, $J_{C\text{-}1\text{-}H\text{-}1}$, indicates that the mycosamine moiety is β-glycosidically bound to the aglycone.

The structure and configuration of the antibiotic have been studied by analysis of the 270 MHz ^{1}H-NMR spectra of N-acetylpimaricin and HH-2. By comparison of the two spectra and by extensive decoupling experiments, all signals in the spectrum of N-acetylpimaricin have been assigned to the protons in structure **3**. From chemical-shift, coupling-constant, and integral values, it was deduced that the mycosamine ring is pyranoid with a chair conformation, the hemi-ketal is 6-membered and occupies a chair conformation with the substituents in equatorial positions, the epoxy protons as well as the olefinic protons are *trans* to each other, and the antibiotic is diastereomerically pure.

The first structure for the polyene macrolide antibiotic pimaricin was proposed by Patrick *et al.* in 1958.[1] After extensive reinvestigations, which included the isolation of the molecular framework as a saturated hydrocarbon, we presented structure **1** (see Fig. 1) for pimaricin in 1964.[2] Two years later this structure was revised by Rickards *et al.*[3] who proposed structure **2** in which the C-8 OH group is absent (see Fig. 1).

Rickard's structure revision was based on molecular weight determinations by electron impact (EI) mass spectrometry of persilylated N-acetylpimaricin (N-Ac-PM) and some of its derivatives and also on the results of periodate titrations of N-acetyldodecahydropimaricin (N-Ac-12HPM) in basic aqueous solution. Recently, Desiderio *et al.*[4] confirmed Rickard's measurements by the use of high-resolution mass spectrometry.

Since there are experimental difficulties in ascertaining complete silylation in a molecule of this complexity, the reactivity of epoxides towards silylating agents has been little studied, and since some of the results of our earlier reported periodate oxidations[2] seemed difficult to explain, we decided to perform some further structural investigations on pimaricin.

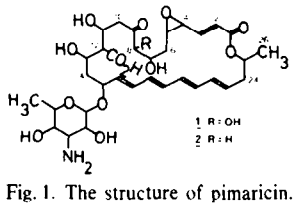

Fig. 1. The structure of pimaricin.

We have recently reported on the behaviour of simple epoxides towards silylating agents,[5] and in the present communication we present additional studies on the periodate oxidation, the EI and field desorption (FD) mass spectra, and ^{13}C-NMR studies of pimaricin and some of its derivatives. The results of our studies confirm that the aglycone of pimaricin does not contain an OH group at C-8 and are thus in accord with structure **2** proposed by Rickards.

The Lederle group reported, without specifying solvent or pH, that N-Ac-PM consumes 2 moles of periodate,[1] and we similarly claimed that N-Ac-PM, and also N-Ac-12HPM, consumed 2 moles of sodium metaperiodate in aqueous solution.[2] We have now verified Rickard's observation that N-Ac-PM in basic aqueous solution and the dodecahydro derivative in aqueous methanol do not consume any periodate. In aqueous methanol and in slightly acidic aqueous medium, however, N-Ac-PM consumes *ca.* 6 moles of periodate as measured by the arsenite method[6] and by UV spectroscopy.[7] The periodate probably reacts with the polyene system since during the periodate treatment, the tetraene UV-absorption of N-Ac-PM changed into an absorption characteristic of a triene.

The EI mass spectra of pimaricin and its N-acetyl derivative do not, as could be expected, display any molecular ion corresponding to formula **1** ($M^+ = 681$) or formula **2** ($M^+ = 665$). In the spectra of both compounds

Table 1. Relative intensities and assignments of the peaks in the mass region above $m/e = 404$ in the EI and FD mass spectra of pimaricin and N-acetyl-pimaricin

Pimaricin M^+=665				N-acetyl-pimaricin M^+=707		
EI	FD		m/e		EI	FD
			627	M - (CO_2 + $2H_2O$)	2	25
			609	M - (CO_2 + $3H_2O$)	25	15
4	100	M - (CO_2 + $2H_2O$) + H	586			
2	50	M - (CO_2 + $3H_2O$) + H	568			
-	25	M - (mycosamine)	502	M - (N-Ac mycosamine)	-	5
10	20	M - (mycosamine + CO_2 + H_2O)	440	M - (N-Ac mycosamine + CO_2 + H_2O)	5	20
100	40	M - (mycosamine + CO_2 + $2H_2O$)	422	M - (N-Ac mycosamine + CO_2 + $2H_2O$)	50	100
25	2	M - (mycosamine + CO_2 + $3H_2O$)	404	M - (N-Ac mycosamine + CO_2 + $3H_2O$)	100	25

The exact mass of the peaks at 609 and 422 mu were determined to correspond to $C_{34}H_{43}NO_9$ and $C_{26}H_{30}O_5$ respectively.

five peaks are found in the region above $m/e = 404$, the ones at highest mass corresponding to the loss of carbon dioxide and two (from **2**) or three (from **1**) molecules of water from the molecular ion. If we assume that the molecular weight of pimaricin is 665 and that of N-Ac-PM is 707, the fragments listed in Table 1 can be assigned to structures in which carbon dioxide, water, and mycosamine have been expelled from the molecular ion.

The EI mass spectra do not allow a molecular-weight determination of pimaricin since the fragments listed can be related to structure **1** just as well as they can be related to structure **2**, the difference corresponding to the possibility of losing one additional molecule of water.

FD mass spectrometry has previously been successfully used to obtain molecular ions of non-volatile and thermally unstable natural products, including macrolide antibiotics.[8] The FD mass spectra of pimaricin and of N-Ac-PM, recorded at low temperature (see Experimental) did not display any ion that would represent the molecular weight. Instead, the spectra showed the same pattern of peaks above $m/e = 404$ as observed in the EI mass spectra. The assignments, which are the same as in the EI spectra, and relative intensities are summarized in Table 1.

It was recently reported[9] that ionization in the presence of Li-salts gives rise to abundant molecular ions of polar compounds which fail to show molecular ions in EI and FD spectrometers. By this method also the highest observable peak in the mass spectrum of N-Ac-PM corresponds to the loss of carbon dioxide and two molecules of water from the molecular ion.†

The absence of a molecular ion in the FD spectra of pimaricin and N-Ac-PM is somewhat surprising since macrolide antibiotics and carbohydrates of similar molecular weights give molecular ions of considerable intensity.[8] We therefore assume that the molecules contain an arrangement which is set up for a facile elimination of carbon dioxide and two molecules of water.

The ^{13}C-NMR and the 270 MHz ^{1}H-NMR spectra (*vide infra*) of these compounds show that the C-9 CO group and the C-13 OH group form a pyranoid hemiketal with chair conformation in which the C-12 carboxyl group is equatorial. The configuration at C-9 cannot be deduced from the NMR-spectra. If we assume, however, that the OH group occupies the axial position, leaving the long alkyl chain equatorial, a bicyclic lactone can form from which one molecule of water and carbon dioxide can be eliminated as indicated in Fig. 2. This leads to a pyrone with the molecular weight observed in the FD and EI spectra.‡

The next part of this communication will discuss the ^{13}C-NMR spectra of N-Ac-PM, N-Ac-12HPM, and of HH-2.[10] The last compound is a hydrogenation-hydrogenolysis product of N-Ac-PM whose structure has been proven independently.[10] It contains N-acetylmycosamine glycosidically bound to the macrocyclic lactone

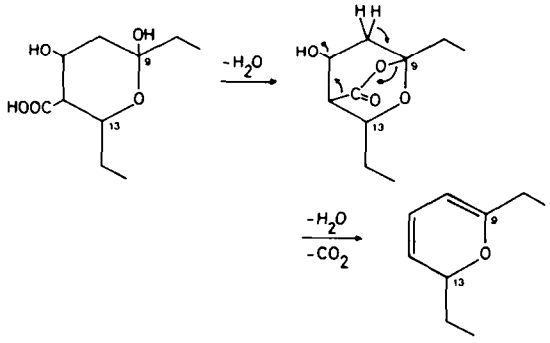

Fig. 2. Suggested mechanism for the loss of 2 molecules of water and 1 molecule of carbon dioxide from the molecular ion of pimaricin and N-acetylpimaricin.

†We are indebted to Dr. H. J. Veith, Technical University, Darmstadt, for the performance of the measurements.

‡After completion of our investigations, Dornberger *et al.*[16] reported a detailed analysis of the EI and negative ion (EA = 'Elektronenanlagerung') spectra of several macrolides, including pimaricin and its N-acetyl derivative. They observe no molecular ions but the same loss of carbon dioxide and 2 moles of water from them as we do. However, they present a different interpretation to explain the eliminations.

which is stripped of all other oxygen functions except the C-9 CO group. There is no reason to believe that mycosamine or the remaining aglycone part of the molecule has changed ring size or configuration during the degradation procedure.

The ^{13}C chemical-shift values for a certain type of atom vary very little in different molecules. The chemical shifts for the mycosamine C atoms and C-15, C-25 and C-26 in the aglycone of HH-2 (Fig. 3) have therefore been used to simplify the interpretation of the spectra of N-Ac-PM (Fig. 4) and N-Ac-12HPM (Fig. 5). The spectra were recorded using pyridine-d_5 as the solvent, the only solvent found in which every[11] carbon signal in the spectra of N-Ac-PM and N-Ac-12HPM was resolved at 25.2 MHz.

Schaffner *et al.*[12] have shown by X-ray crystallography that the structure of N-iodoacetyl amphotericin B contains a 6-membered hemiketalic ring instead of the C-17 hydroxy—C-13 keto system originally proposed.[13] Pimaricin, which contains the same arrangement (C-13–C-9) has been proposed to have the analogous structure **3**[14] (see Fig. 4). This conclusion was based on the fact that N-Ac-PM, its dodecahydro derivative and the products formed by sodium-borohydride reduction of these compounds exhibit almost identical ORD-curves. Dispersion curves do not give any information as to whether both of the two possible epimers have formed, nor do they tell about the position of the equilibrium between the open and the cyclic forms. Both these points should be conveniently clarified by ^{13}C-NMR spectroscopy.

The CO region (*ca.* 160–220 ppm)[15] in the spectra of N-Ac-PM and N-Ac-12HPM each contains only three signals, while the N-acetyl derivatives of structures **1** and **2** require four. The C-9 CO carbon atom in HH-2 absorbs at 210.3 ppm, but no signal close to this value can be detected in the spectra of N-Ac-PM and N-Ac-12HPM. We assign the peaks at 176.2 ppm and 176.5 ppm respectively in these spectra to the carboxyl carbon atom since this signal is absent in the spectrum of HH-2. The CO carbon atom of the N-acetyl group corresponds to the signals at 171.4 (N-Ac-PM), 171.5 (N-Ac-12HPM), and 171.0 ppm (HH-2). The lactone CO carbon atom resonates at 173.2 (N-Ac-12HPM) and 173.0 ppm (HH-2) in a saturated surrounding, but at 165.2 ppm as part of the α,β-unsaturated system in N-Ac-PM. The absence of a C-9 CO resonance in the spectra of N-Ac-PM and N-Ac-12HPM strongly indicates the presence of a masked CO group and the absence of a free CO group.

Acetalic and ketalic C atoms usually resonate around 100 ppm.[15] The single frequency off-resonance decoupled (SFORD) spectra of N-Ac-PM and N-Ac-12HPM display singlets at 98.4 ppm and doublets at 98.1 and 98.7 ppm respectively. The doublets are assigned to C-1′ in the pyranoid mycosamine moiety and the singlets to the quarternary C-9 in the 6-membered hemi-ketal in structure **3**.

In addition, the presence of only one set of acetalic-hemiketalic resonances in each compound strongly indicates that only one of the two possible C-9 epimers has formed in the ring closure reaction. The same observation also indicates that in the reductive opening of the epoxide to form N-Ac-12HPM the OH group is placed on C-4. A C-5 OH group would most likely result in a mixture of the C-5—C-9 and the C-13—C-9 hemiketals.

For some aldohexoses ^{13}C-chemical shift values have been successfully used to distinguish α- and β-configurations.[17] In mannose and rhamnose the chemical shift differences between the two anomeric C atoms are unfortunately too small to be useful, particularly in cases where only one form is available. For the mycosamine moiety, which has a mannopyranose configuration,[18] the C-1′ chemical shift values are 100.2, 98.1 and 98.7 ppm respectively in the three recorded spectra. These values are close to the ones reported for methyl mannopyranoside. However, Bock *et al.*[19] have reported a very clear correlation between the orientation of the anomeric proton and $J_{C\text{-}1—H\text{-}1}$, the anomeric coupling constant, which is 169–171 Hz for equatorial protons and 158–162 Hz for axial ones. The anomeric coupling constant in N-Ac-PM was determined to 156 Hz which indicates that the glycosidic configuration of the mycosamine moiety is β.

We now want to turn to the number of OH groups in pimaricin. Carbon atoms carrying one single-bonded O atom generally absorb between 50 and 80 ppm.[15] In the pimaricin case, C-12, carrying the carboxyl function and flanked by two oxygen-carrying C atoms, and C-3′ in the mycosamine moiety also belong to this category. Structure **1** contains nine C atoms of this type in the macrocyclic ring while structure **3** contains eight. In addition, the mycosamine moiety contributes four more atoms of the same type. Therefore, structure **1** requires a total of thirteen resonance signals in the above-mentioned region and structure **3** twelve. By the same reasoning, the numbers are twelve and eleven for the dodecahydro structures.

The spectrum of N-Ac-PM displays twelve resonance lines between 54.5 and 75.6 ppm in accord with structure **5**. The signals between 54.5 and 59.3 ppm, which all become doublets in the SFORD spectrum, form a well-separated subgroup. For these we propose the following assignments. The peak at 56.3 ppm is present in the spectrum of HH-2 also and is therefore assigned to C-3′ in mycosamine, which carries the N atom. The signal at 59.3 ppm is assigned to C-12, partly because of its presence in the spectrum of N-Ac-12HPM and partly because the value of the reduced coupling constant in the SFORD spectrum indicates that the proton attached to it resonates at a relatively high field. The two remaining signals, at 59.1 and 54.5 ppm, correspond to the two epoxy C atoms. This is in accord with reported chemical-shift values[20] and also with the fact that the signals are absent in the spectrum of N-Ac-12HPM.

The chemical shifts of the epoxy C atoms in 3,4-epoxycyclopentene, -hexene and -octene have been reported to be different with the α-C atoms resonating at higher field than the corresponding β-C atoms.[20] We therefore assign the signal at 54.5 ppm to C-4 and the signal at 59.1 ppm to C-5.

The three lines around 67 ppm are also present in the spectrum of N-Ac-12HPM, but not in the spectrum of HH-2. Consequently, these lines are assigned to the three saturated C atoms which carry O atoms in the two former compounds and which are methylene C atoms in HH-2, namely C-7, C-11 and C-13.

Dorman and Roberts[21] have reported ^{13}C-NMR shift values around 73 ppm for C-2, C-4 and C-5 in the β-form of rhamnopyranose, which is the oxygen analog of mycosamine. We therefore tentatively assign the peaks at 74.5, 72.5 and 70.9 ppm to these three C atoms in mycosamine. The three peaks are also present in the spectra of N-Ac-12HPM and of HH-2 within a range of 0.5 ppm.

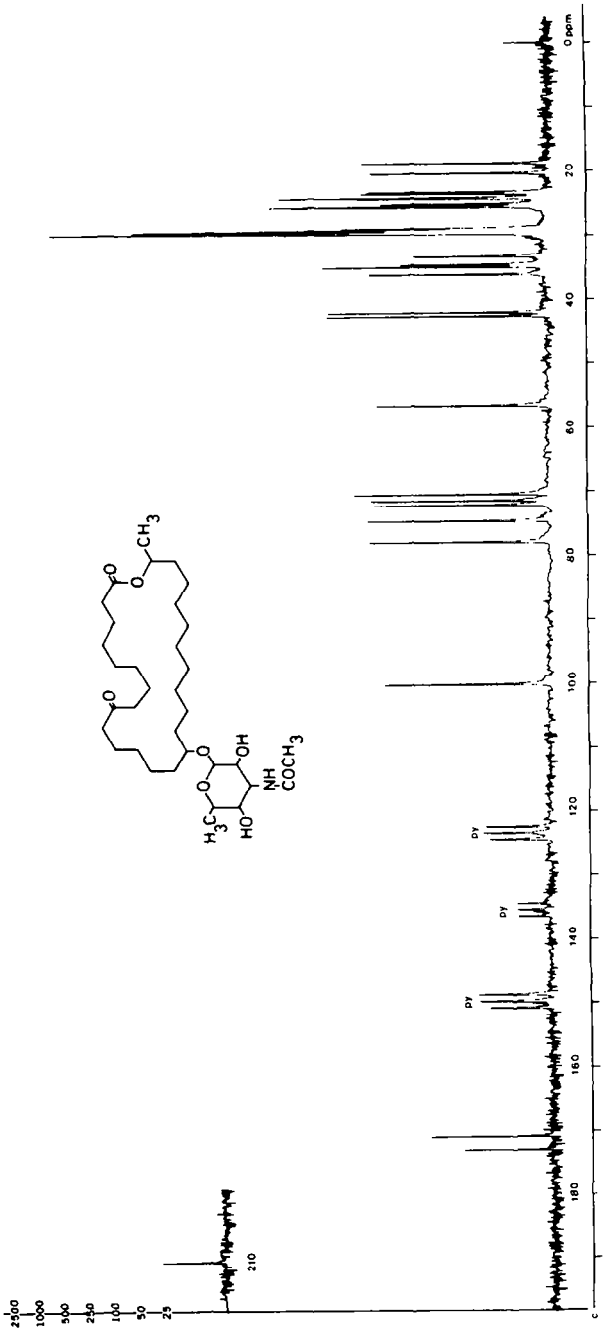

Fig. 3. Noise-decoupled ^{13}C-NMR spectrum of HH-2 (pyridine-d_5).

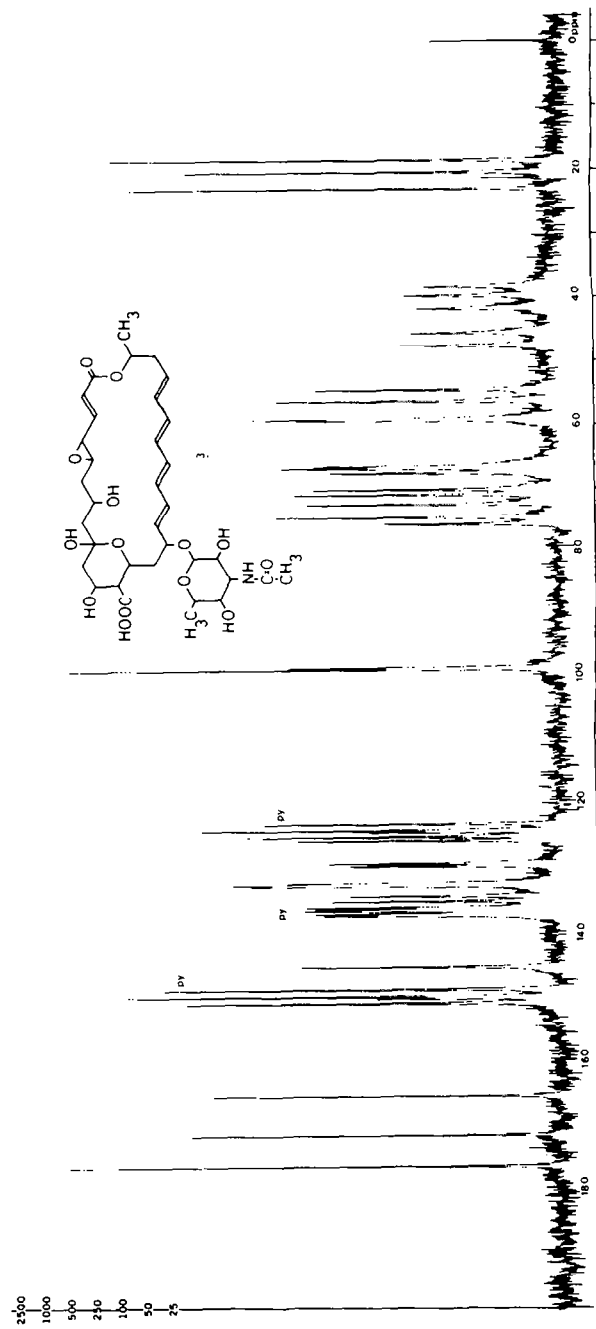

Fig. 4. Noise-decoupled ^{13}C-NMR spectrum of N-acetylpimaricin (pyridine-d_5).

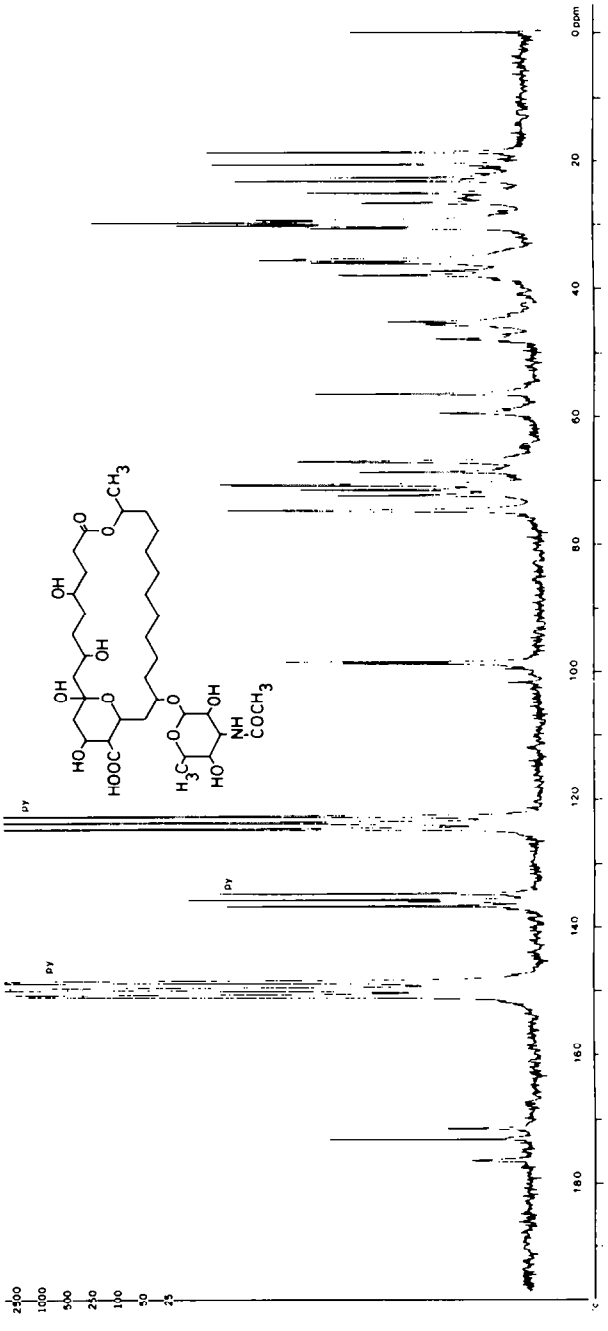

Fig. 5. Noise-decoupled ^{13}C-NMR spectrum of N-acetyldodecahydropimaricin (pyridine-d_5).

The signal at 75.6 ppm, which is shifted upfield to 74.7 ppm in the spectrum of the dodecahydro derivative, is assigned to C-15 which is α to the tetraene system in N-Ac-PM. The remaining signal at 70.1 ppm, which has approximately the same chemical-shift values in the two other spectra, corresponds to C-25 in the aglycone.

The assignments of these twelve lines between 54.5 and 75.6 ppm are supported by their presence in the spectrum of N-Ac-12HPM also. The corresponding signals are found within 0.5 ppm from the values reported for N-Ac-PM except for the signals from the epoxy C atoms which are absent. A new signal has appeared at 70.6 ppm representing C-4, now carrying an OH group, while the signal from the methylene C atom C-5 is found in the methylene region. The assignments to the oxygen-carrying C atoms of the signals in the spectra of N-Ac-PM and N-Ac-12HPM all support structure **3**.

The same confirmation of structure **3** can be obtained from the number of methylene signals in the spectrum of N-Ac-PM. Structure **1** requires four while structures **2** and **3** require five.

Between 38.5 and 47.5 ppm five peaks, which all become triplets in the SFORD spectrum, are present. These are safely assigned to the methylene carbons C-6, C-8, C-10, C-14 and C-24 and therefore structure **3** is the correct structure.

In the olefinic region of the spectrum ten lines appear between 145.1 and 125.2 ppm. The outer lines are assigned to the β- and the α-carbon atoms respectively in the unsaturated lactone system, while the eight inner lines correspond to the C atoms in the tetraene system.

The high field region displays three signals corresponding to the three Me groups in the molecule. By using known chemical-shift rules,[15] the peaks at 18.5, 20.4 and 23.1 ppm are assigned to C-6' in mycosamine, C-26 in the aglycone, and the CH_3-group in the acetyl group in mycosamine, respectively.

This completes the assignments of most of the 35 lines in the spectrum of N-Ac-PM and the corresponding signals in the spectra of N-Ac-12HPM and HH-2. This information is summarized in Table 2. Our chemical-shift values and assignments are in full accord with those reported for various other macrolide antibiotics.[22] In conclusion, the ^{13}C-NMR investigation has proved that the aglycone part of N-acetylpimaricin exists completely in the hemi-ketalic form **3**, that only one diastereoisomer is present, and that the mycosamine ring is probably β-glycosidically bound to the aglycone.

The remaining part of the present communication describes ^{1}H-NMR studies of N-Ac-PM and HH-2 at 270 MHz which enable us to assign all signals in the spectrum of **3** even though the ones which belong to the tetraene protons and to the methylene protons at C-6, C-8, C-14 and C-24 are not completely separated. The identifications have been made possible by extensive decoupling experiments and also by comparison between the spectra of the two compounds. The decoupling experiments discussed here are indicated in the spectra

Table 2. Assignments of the peaks in the ^{13}C-NMR spectra of HH-2, N-acetylpimaricin, and N-acetyldodecahydropimaricin

Carbon atom	HH-2	N-Ac-PM	N-Ac-12HPM
Aglycone			
1	173.0	165.2	173.2
2		125.2	
3		145.1	
4		54.5	70.6
5		59.1	
6		38.3-47.5	
7		66.5-67.5	67.1-68.7
8		38.3-47.5	
9	210.3	98.4	98.4
10		38.3-47.5	
11		66.5-67.5	67.1-68.7
12		59.3	59.4
13		66.5-67.5	67.1-68.7
14		38.3-47.5	
15	77.9	75.6	74.7
16-23		128.9-137.0	
24		38.3-47.5	
25	70.5	70.1	70.7
26	20.3	20.4	20.6
27		176.2	176.5
Mycosamine			
1	100.2	98.1	98.7
2,4,5	71.4-75.7	70.9-74.5	71.4-74.6
3	56.5	56.3	56.5
6	18.7	18.5	18.7
C=O	171.0	171.4	171.5
CH_3	23.2	23.1	23.1

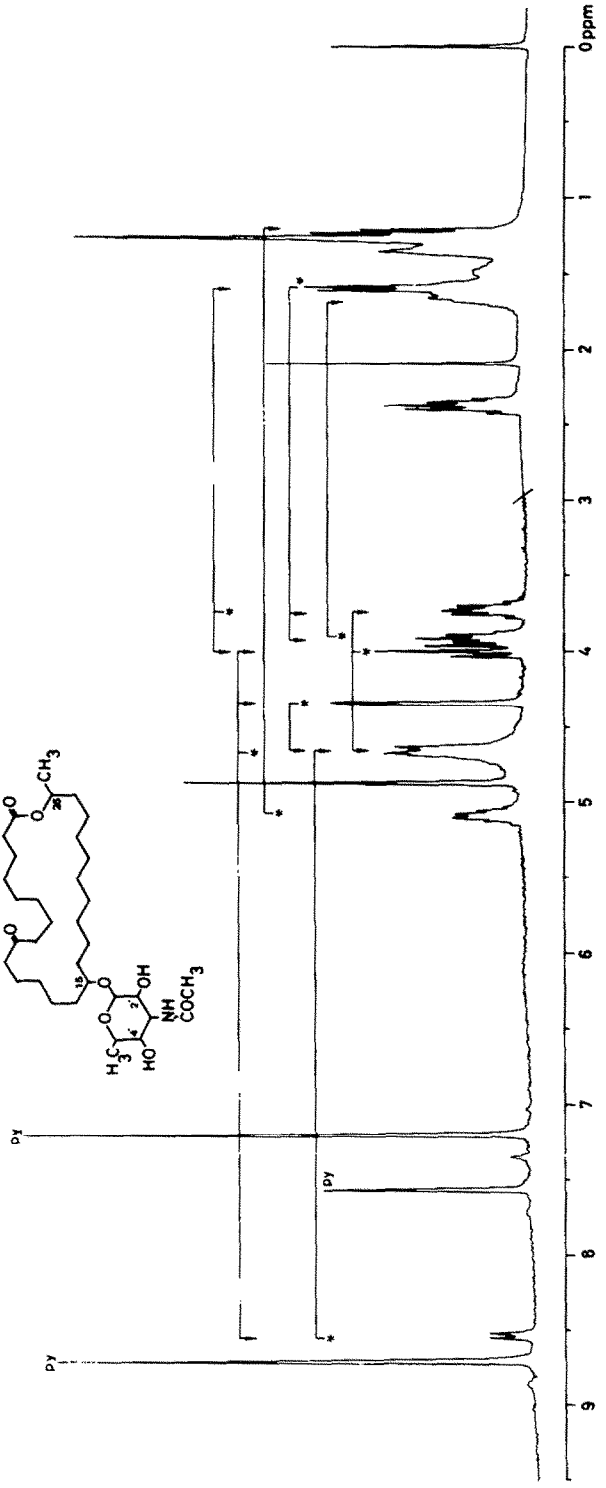

Fig. 6. ^{1}H-NMR spectrum of HH-2 (pyridine-d_5).

(Figs. 6 and 8) with an asterisk at the point of irradiation and arrows at the signals affected. Additional decoupling experiments to confirm the assignments, also indicated in the spectra, are all in accord with the reported results. From coupling-constant, chemical-shift and integral values, it can be deduced that: (a) the mycosamine ring is pyranoid, occupies a chair-like conformation, and is epimerically pure (b) the hemiketal is 6-membered, occupies a chair-like conformation with the protons at C-11, C-12 and C-13 all in axial positions, and that only one C-9 epimer is present (c) the olefinic protons H-2 and H-3 and the epoxy protons H-4 and H-5 both are *trans* to each other (d) C-8 carries two protons, and no OH group, in accord with structure **3**, and (e) the antibiotic is diastereomerically pure.

As in the ^{13}C-NMR investigations, the choice of solvent was of crucial importance. Of all solvents tested, pyridine-d_5, which is known to 'be useful to better approximate first-order spectra',[23] was the only one in which most of the signals in the 270 MHz FT-NMR spectrum of N-Ac-PM were sharp and separated. In dimethyl sulfoxide-d_6 the signals were broad and the absorptions from many different protons overlapped. In pyridine-d_5 the chemical-shift differences for the protons in N-Ac-PM that coupled were sufficiently large in most cases to allow the spin–spin coupling information to be analyzed by first-order technique.

In order to decrease the decoupling work and to simplify the assignments of the mycosamine protons in the spectrum of N-Ac-PM, we first, as in the ^{13}C-NMR studies, determined chemical shifts and coupling constants for the relevant protons in HH-2. The spectrum of HH-2 was also recorded using pyridine-d_5 as the solvent (Fig. 6). The key to the analysis of the signals from the mycosamine spin system was the observation that on addition of D_2O the three-proton signal at 4.70 ppm immediately became a one-proton signal and the doublet at 8.55 ppm (J = 8.1 Hz) slowly decreased without changing into a singlet. The last signal can therefore be assigned to the amide proton and the exchangeable part of the former to the two OH protons. After complete exchange of the amide proton, the one-proton signal that remained at 4.70 ppm displayed a doublet of doublets (J = 3.3 and 9.6 Hz). Irradiation of this signal before addition of D_2O caused changes in three other one-proton signals in the spectrum.

Firstly, the amide doublet collapsed into a singlet, secondly the doublet at 4.35 ppm (J = 3.3 Hz) became a singlet, and thirdly the apparent triplet at 4.00 ppm (J = 9.6 Hz) formed a doublet with the same coupling constant as for the triplet. The 4.70 ppm resonance is thus assigned to H-3′ and the last two signals to H-2′ and H-4′. Irradiation of the multiplet (diffuse octet) at 3.75 ppm changed the Me doublet at 1.60 ppm to a singlet, and again, the triplet at 4.00 ppm to a doublet (J = 9.6 Hz). The last decoupling experiment shows that the multiplet at 3.75 ppm represents H-5′ and allows us to assign H-4′ the chemical shift 4.00 ppm and therefore H-2′ to the 4.35 ppm signal. The multiplets at 5.10 and 3.90 ppm are assigned to H-25 and H-15 respectively, since irradiation at 5.10 ppm changed the Me doublet at 1.25 ppm (J = 6.3 Hz) to a singlet, and irradiation in the methylene-region around 1.60 ppm changed the 3.90 ppm multiplet into a singlet. The only remaining unassigned signal in this region is then the singlet ($J < 0.5$ Hz) at 4.85 ppm which thus belongs to H-1′, the anomeric proton. The chemical-shift values and coupling-constant

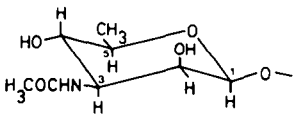

Fig. 7. Absolute configurations, chemical-shift (ppm), and coupling-constant data for the mycosamine spin system (pyridine-d_5).

H-1 = 4.85	$J_{1-2} < 0.5$ Hz
H-2 = 4.35	$J_{2-3} = 3.3$
H-3 = 4.70	$J_{3-4} = 9.6$
H-4 = 4.00	$J_{4-5} = 9.6$
H-5 = 3.75	$J_{5-6} = 6.2$
H-6 = 1.60	$J_{3-NH} = 8.1$
NH = 8.55	
CH_3CO = 2.10	

data for the mycosamine spin system are summarized in Fig. 7.

A Karplus type of treatment of the J-values demonstrates that mycosamine in HH-2, and consequently also in pimaricin, exists as a mannopyranose in chair conformation. Saltza *et al.*[24] proved that methyl mycosaminide obtained by acid methanolysis of the macrolide antibiotic nystatin possesses D-mannose configuration, and by X-ray crystallography Schaffner *et al.*[12] showed that the mycosamine moiety in amphotericin B has the same configuration and exists in a chair-like conformation.

Since only one set of signals can be detected in the spectrum of HH-2, only one ring size and one C-1′ epimer is present. In the α-form of methyl mycosaminide H-1′ absorbs at 4.60 ppm ($CDCl_3$) and in HH-2 the same proton signal appears at 4.55 ppm in the same solvent. The chemical shifts, however, are of limited value for configurational assignments in this case. Firstly, in mannopyranoses, axial and equatorial anomeric protons show very small chemical-shift differences,[25] and secondly, only the α-form of mycosamine has been characterized.[24] We therefore feel that the analysis of the $J_{^{13}C\text{-}1'—^{1}H\text{-}1'}$-value, which indicates β-configuration at the anomeric center, is a more reliable method. This configurational problem will be treated in greater detail in a subsequent communication.[26]

We now turn to the analysis of the N-Ac-PM-spectrum (Fig. 8). Based on a comparison of this and the spectrum of HH-2 and on some confirmatory decoupling experiments the amide proton, H-1′, H-3′, H-2′, H-4′, H-5′, CH_3CO and CH_3-C-5, all in the mycosamine moiety were assigned to the signals at 8.45, 5.10, 4.55, 4.50, 3.95, 3.65, 2.05 and 1.50 ppm respectively. These signals have been darkened in Fig. 8. In structure **3** thirty-seven protons now remain to be assigned.

We will analyze them in terms of three spin systems: (A) the protons on C-26—C-23 (B) the protons on C-16—C-10 and (C) the protons on C-8—C-2, (see Fig. 8). The systems B and C are separated by C-9, which carries no protons, and A and B are separated by the remaining olefinic protons whose pattern is too complicated to analyze at 270 MHz.

The easiest entrance to system A is via the Me doublet at 1.25 ppm (J = 6.6 Hz) which must belong to the C-26 protons since the other Me doublet has already been assigned to the mycosamine moiety. Irradiation of the doublet at 1.25 ppm changes the diffuse multiplet at 4.90 ppm into an imperfect doublet (J = 9 Hz). Irradiation of this resonance changes the above-mentioned Me doublet into a singlet and it also affects the methylene region around 2.2 ppm, which is not completely resolved

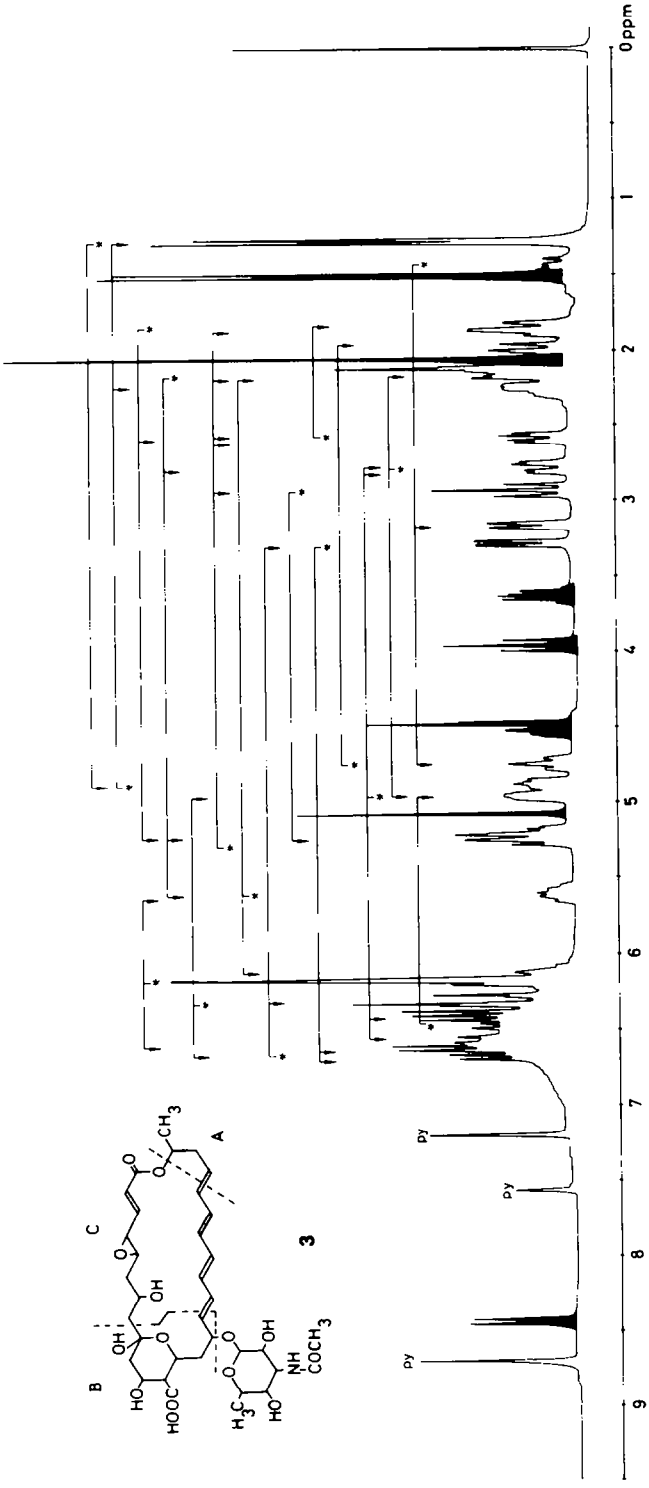

Fig. 8. ^{1}H-NMR spectrum of N-acetylpimaricin (pyridine-d_5). Darkened signals belong to the mycosamine spin system.

at 270 MHz. This means that H-25 absorbs at 4.90 ppm and that the two C-24 methylene protons both appear at 2.2 ppm. Finally, irradiation of the multiplet in the olefinic region at 5.60 ppm also causes deformation of the C-24 methylene signal at 2.2 ppm. In addition changes occur in the olefinic region at 6.2 ppm. The 5.60 ppm signal therefore belongs to H-23.

They key to spin system B is the observation that of the three unassigned signals at 4.75, 5.00 and 5.25 ppm, the region where protons attached to C atoms carrying singly bonded O atoms absorb, the last one represents two protons. The only two protons in structure **3** for which the environments seem to be sufficiently similar to allow identical chemical-shift values are H-11 and H-13. Irradiation at the two-proton signal at 5.25 ppm causes the following changes in the spectrum: (a) the triplet at 2.95 ppm (J = 10.3 Hz) collapses into a singlet (b) the doublet of doublets at 2.60 ppm (J = 12.1 and 4.7 Hz) loses the small coupling and forms a doublet (J = 12.1 Hz) (c) the broad triplet at 1.85 ppm collapses into a doublet (J = 12.1 Hz) and (d) the partially hidden signal at 2.2 ppm is affected.

The signal at 2.95 ppm can now be assigned to H-12 because (a) the chemical shift is that expected for a proton α to a CO function and (b) the change of the triplet shape into a singlet in the decoupling experiment requires two adjacent protons equivalent both in the chemical-shift and spin-coupling sense. This assignment is confirmed by irradiation at 2.95 ppm which affects only the two-proton signal at 5.25 ppm. The three other signals which were disturbed in the first decoupling experiment must then correspond to protons on C-10 and C-14.

Irradiation at 2.60 ppm again caused the triplet at 1.85 ppm to collapse into a doublet, now with J = 10 Hz, and distorted the two-proton signal at 5.25 ppm, but affected no other signals in the spectrum including those at 4.75, 4.90 and 5.00 ppm. Since C-9 carries no protons, we assign the signals at 2.60 and 1.85 ppm to the two protons at C-10. The coupling constant, 12.1 Hz, which was obtained in the first decoupling experiment, is in agreement with a geminal relationship between the two protons.

The signal at 2.2 ppm is assigned to one of the methylene protons at C-14 which was confirmed in the following way. Irradiation at 5.00 ppm changed the olefinic four-line signal at 6.4 ppm to an AB-doublet (J = 15 Hz) and caused collapse of the doublet of doublets at 2.80 ppm (J = 15.4 and 4.4 Hz) into one doublet (J = 15.4 Hz). The signal at 5.00 ppm is thus assigned to H-15 which couples with the olefinic H-16 and also with one of the protons at C-14 (J = 4.4 Hz), but shows no coupling with the other. Since irradiation at 2.2 ppm destroys the larger coupling in the signal at 2.80 ppm, this last signal is assigned to the other methylene proton at C-14. Also here the coupling constant, 15.4 Hz, indicates that the two protons resonating at 2.2 and 2.80 ppm have a geminal relationship. The observed olefinic coupling of 15 Hz in the signal from H-16 at 6.4 ppm demonstrates the *trans* configuration for at least part of the tetraene system which has been shown to be all-*trans* on the basis of UV-data.

The chemical-shift values and coupling-constant data for the spin system B are summarized in Fig. 9. These results prove that the hemiketalic ring is 6-membered and exists in a chair-like conformation with H-11, H-12 and H-13 all in axial positions. The chemical-shift difference

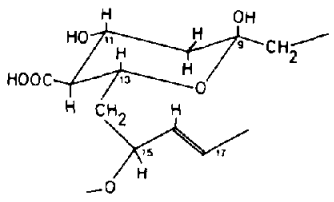

Fig. 9. Relative configurations, chemical-shift (ppm), and coupling-constant data for the C-10—C-16 proton spin system (pyridine-d_5).

H-10a = 1.85	$J_{10a-10e}$ = 12.1 Hz	$J_{13-14''} < 1$ Hz
H-10e = 2.60	J_{10a-11} = 10	$J_{14'-14''}$ = 15.4
H-11 = 5.25	J_{10e-11} = 4.7	$J_{14'-15} < 1$
H-12 = 2.95	J_{11-12} = 10.3	$J_{14''-15}$ = 4.4
H-13 = 5.25	J_{12-13} = 10.3	J_{15-16} = 8.1
H-14' = 2.20	$J_{13-14'}$ = ?	J_{16-17} = 15
H-14'' = 2.80		
H-15 = 5.00		
H-16 = 6.40		

between the two C-10 methylene protons is in accord with the general observation that equatorial protons resonate more down-field than the corresponding axial ones.[25]

Since the entire spectrum consists of only one set of signals it seems safe to assume that only one C-9 epimer is present. The configurations at C-9 and C-15 cannot be deduced from coupling-constant data. However, the high δ-values for H-11 and H-13 indicate that both of them are deshielded by an axially oriented C-9 OH group[25] which would leave C-8 in an equatorial position. This relative configuration was earlier proposed to explain the ready loss of carbon dioxide and two molecules of water from the molecular ion of pimaricin in our EI and FD MS-experiments.

It is interesting to note that the structure of the heptaene macrolide amphotericin B, which has been determined by X-ray crystallography,[12] between C-11 and C-20 is identical to that of pimaricin between C-7 and C-16. Both contain a pyranoid hemiketalic ring in a chair conformation with the larger substituents in equatorial positions.

We now turn to the last spin system, the protons on C-2—C-8. Two doublets displaying different coupling constants appear at 3.30 ppm (J = 7.4 Hz) and 3.15 ppm (J = 8.1 Hz), a region in which epoxy protons are known to resonate.[27] Irradiation of the lower-field doublet does not affect the other epoxy-proton signal but causes a four-line one-proton signal in the olefinic region at 6.7 ppm (J = 15.4 and 7.4 Hz) to change into an AB doublet (J = 15.4 Hz). Irradiation at 6.7 ppm changes the olefinic AB doublet at 6.3 ppm (J = 15.7 Hz) into a singlet and causes the 3.30 ppm epoxy-proton signal to collapse into a singlet.

Since the β-protons in α,β-unsaturated CO systems always absorb down-field relative to the corresponding α-protons,[28] we can conclude that: (a) H-2 and H-3 absorb at 6.3 and 6.7 ppm respectively, the coupling constant J_{2-3} = 15.4 Hz proves *trans* configuration and (b) H-4 and H-5 absorb at 3.30 and 3.15 ppm respectively. The coupling constant, $J_{4-5} < 1$ Hz, indicates *trans* relation also between the epoxy protons.[27]

Irradiation of the epoxy-proton resonance at 3.15 ppm causes no detectable changes anywhere in the spectrum recorded at 270 MHz. At 360 MHz, however, the same decoupling experiment changes the partially hidden multiplet at 1.45 ppm into a triplet. Irradiation at 1.45 ppm

finally causes the H-5 epoxy doublet to form a singlet ($J < 1$ Hz) and, in addition, affects the triplet at 4.75 ppm.

We can now assign the 1.45 ppm signal to the C-6 methylene protons and the 4.75 ppm signal, which is the only remaining unassigned signal in the down-field region, to H-7. Finally irradiation at 4.75 ppm changes the partially hidden two-proton doublet in the methylene-region at 1.95 ppm into a singlet. The last signal therefore represents the two methylene protons at C-8. This assignment is of considerable interest since it, together with the ^{13}C-NMR investigations, conclusively proves the correctness of structure **3** for N-acetylpimaricin and discards the structure with an OH-group at C-8. The chemical-shift values and relevant coupling-constant data for the protons in spin system C are summarized in Fig. 10.

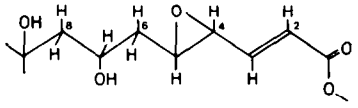

Fig. 10. Relative configurations, chemical-shift (ppm), and coupling-constant data for the C-2—C-8 proton spin system (pyridine-d_5).

H-2 = 6.3	H-6 = 1.45	J_{2-3} = 15.4 Hz
H-3 = 6.7	H-7 = 4.75	J_{3-4} = 7.4
H-4 = 3.30	H-8 = 1.95	$J_{4-5} < 1$
H-5 = 3.15		J_{5-6} = 8.1

The signal from the OH-protons has not been discussed so far. Temperature studies, as well as D_2O-exchange experiments, clearly show that the broad signal in the olefinic region belongs to these protons. This concludes the assignments of all signals in the ^{1}H-NMR spectrum of N-Ac-PM to the fourty-nine protons in structure **3**.

EXPERIMENTAL

Pimaricin used in this investigation was supplied by Gist-Brocades NV, Delft, Holland.

Preparation of N-*acetylpimaricin* (N-Ac-PM). To a soln of 5 ml Ac_2O in 60 ml MeOH, 1 g of pimaricin was added and dissolved. The soln was filtered and kept at room temp. for 3 hr. White crystals gradually formed, the soln was cooled and the crystals were filtered off, washed with MeOH and dried in vacuum for 24 hr, yield: 0.9 g of N-Ac-PM.

Preparation of N-*acetyldodecahydropimaricin* (N-Ac-12HPM). N-Ac-PM (0.5 g) was dissolved in 50 ml MeOH containing 0.1 g of 10% Pd on C catalyst. Hydrogenation at room temp. and atmospheric pressure for 2 hr yielded, after filtering off the catalyst and evaporation of the solvent, 0.5 g of N-Ac-12HPM.

The periodate oxidations were performed in basic (0.1 M $NaHCO_3$), neutral (H_2O-MeOH 1:1), and slightly acidic (0.01 M $NaIO_4$, pH = 5) soln. The consumption of periodate was measured by arsenite titration and UV spectrometry.[6,7]

The electron impact and field desorption mass spectra were recorded with the double focussing mass spectrometer Varian MAT 711 equipped with the field desorption/field ionization/electron impact combination ion source. The instrument parameters were as follows: *EI-mode*, accelerating voltage: 8 kV, electron energy: 70 eV, emission current: 1 mA, and resolution (10% valley definition): 1000 and 10,000 for exact mass determinations (performed with the peak matching technique and PFK as reference substance), *FD-mode*: accelerating voltage: 8 kV, extraction voltage: −6 kV, and wire heating current: 14–20 mA. The ^{13}C-NMR spectra were recorded on a Varian XL-100 instrument at the Swedish Tobacco Co., Stockholm using pyridine-d_5 (99.5%) as the solvent. Chemical-shift values are reported in ppm using internal TMS as the reference. Gated decoupling was used when the anomeric coupling constant, $J_{C\text{-}1—H\text{-}1}$, was determined. The ^{1}H-NMR spectra were recorded on a Bruker WH-270 instrument using FT-technique. Pyridine-d_5 (99.5% isotopical purity, Ciba-Geigy) was used as the solvent, and the peaks in the spectra at 7.20, 7.55 and 8.75 ppm are due to pyridine molecules not completely deuterated. The solutions were prepared by dissolving 20 mg of the sample in 0.5 ml of pyridine-d_5 from a freshly opened vial.

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