CARBON-13 NUCLEAR MAGNETIC RESONANCE ASSIGNMENTS OF PACTAMYCIN AND RELATED COMPOUNDS

DWIGHT D. WELLER, ARTHUR HABER and KENNETH L. RINEHART, Jr.

Roger Adams Laboratory, University of Illinois, Urbana, Illinois 61801, U.S.A.

PAUL F. WILEY

The Upjohn Company, Kalamazoo, Michigan 49001, U.S.A.

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All resonances observed in the ^{13}C NMR spectrum of the antitumor antibiotic pactamycin and its degradation product pactamyçate have been assigned, employing off-resonance and specific proton decoupling as well as comparison with the ^{13}C NMR spectra of the model compounds *m*-aminoacetophenone and ethyl 6-methylsalicylate.

The structure[1,2] of the antitumor antibiotic pactamycin (**1**, Fig. 1),[3~5] elaborated by *Streptomyces pactum*,[5] is unique, as its nucleus involves a cyclopentane ring in which every ring atom is substituted, and usually disubstituted.[1] We have undertaken a study of the biosynthesis of pactamycin involving ^{13}C labeling,[6] for which the first requirement is the assignment of individual carbons in the ^{13}C NMR spectrum of **1**. In this paper we describe the ^{13}C NMR assignments of pactamycin, as well as those of pactamyçate (**3**),[1] a chemical degradation product isolated along with pactamycin (sometimes in better yields) from fermentations.

The most favorable solvent for the ^{13}C NMR study of **1** and **3** was found to be deuterioacetone. Only pactamycin possesses significant solubility in deuteriochloroform and, although both **1** and **3** are readily soluble in deuteriodimethyl sulfoxide, this solvent was, for **1**, inferior to deuterioacetone for spectral interpretations. The use of deuterioacetone was approached with caution, since pactamycin is known to form a complex readily with acetone.[1] In order to employ acetone successfully, the pactamycin acetone adduct had to be prepared and its structure determined. Comparison of the adducts' ^{13}C NMR spectrum in deuteriochloroform with that of pactamycin and several aliphatic ketimines established the ketimine structure **2** for the acetone adduct. We shall, therefore, begin the discussion with the ^{13}C NMR assignments of pactamycin (**1**) and its acetone complex (**2**) in deuteriochloroform. The adduct subsequently proved to be useful in that the effect of ketimine formation upon the ^{13}C NMR spectrum of **1** was used in the assignment of the carbons contained in the cyclopentane ring of **1** and **2**.

Pactamycin in Deuteriochloroform

Chemical shifts for the carbon atoms in pactamycin (**1**) and related compounds (Fig. 1) were determined on proton decoupled spectra. Assignments, summarized in Table 1, were made by comparison of the spectra with proton off-resonance decoupled spectra, from single frequency proton decoupled spectra, from standard chemical shift data,[7~9] and by comparison with the chemical shifts of model compounds. For convenience, carbons were divided initially into those signals due to the aliphatic carbons and those signals due to the aromatic and carbonyl carbons, based upon standard

Table 1.[a] ^{13}C NMR Signals of pactamycin (**1**), its acetone adduct (**2**), pactamyçate (**3**) and model compounds (**4**, **5**)

Type	Carbon number	Solvents, compounds and chemical shifts											
		$CDCl_3$				CD_3COCD_3				CD_3SOCD_3			
		4	**5**	**1**	**2**	**1**[b]	**3**[b]	**4**	**5**	**1**	**3**	**4**	**5**
$-CH_3$	6			21.1	21.3	21.9	17.6*			21.2	17.3		
	8			18.1	18.5*	18.7	17.2*			18.3	16.3		
	11, 12			36.8	36.8	36.7	—			36.1	—		
	14				18.9*								
	15				30.3								
	7′		24.1	23.8	23.8	23.2	22.6		23.6	19.3	19.5		19.5
	8″	26.7		26.7	26.7	26.6	26.6	26.7		26.5	26.6	26.4	
$-CH_2-$	9			65.3	64.3	65.6	66.5			64.7	67.2		
$-\overset{|}{C}H-$	2			63.3	71.5	73.1	68.9			62.8	57.6		
	3			68.9	68.1	69.4	68.9			67.4	70.2		
	7			74.2	73.8	74.0	76.7			72.2	75.7		
$-\overset{|}{\underset{|}{C}}-$	1			71.5	73.7	74.0	73.2			70.3	69.8		
	4			84.7	84.8	84.7	83.6			82.1	80.3		
	5			88.8	90.0	89.9	84.6			87.5	82.0		
$=\overset{|}{C}-H$	3′		115.6	115.5	115.7	115.6	115.5		115.8	113.0	113.3		113.1
	4′		134.1	134.2	134.1	133.9	134.0		134.3	130.2	130.2		130.2
	5′		122.9	123.0	122.9	123.0	123.3		123.2	120.1	120.2		120.2
	2″	114.0		110.9	110.2	112.2	112.3	114.2		112.0*	111.4*	112.4*	
	4″	119.6		118.8	119.6**	118.9	118.2	119.7		116.7	116.6	118.1	
	5″	129.4		129.6	129.8	129.2	130.0	129.9		128.6	128.6	128.5	
	6″	118.9		118.3	118.9**	118.2	117.9	117.7		115.6*	115.6*	115.6*	
$=\overset{|}{C}-$	1′		112.5	112.4	113.0	114.9	— [c]		114.0	119.3	119.9		119.9
	2′		162.9	162.4	162.5	161.5	160.7		162.6	155.0	154.8		154.9
	6′		141.3	141.2	141.5	141.4	141.4		141.4	136.4**	136.9**		135.9
	1″	138.3		138.2	138.3	138.8	139.0	139.0		137.2**	137.2**	137.2	
	3″	146.8		146.7	146.6	147.8	149.2	149.4		146.9	149.9	148.3	
$-\overset{|}{C}=O$	10			159.2	158.9	159.6	158.8			158.2	158.1		
	13				171.5†	171.3	171.3**						
	8′		171.7	172.2	172.0†	170.8	171.4**		171.7	167.6	167.9		167.6
	7″	198.4		198.7	198.5	197.7	198.4	198.6		197.3	198.4	197.4	

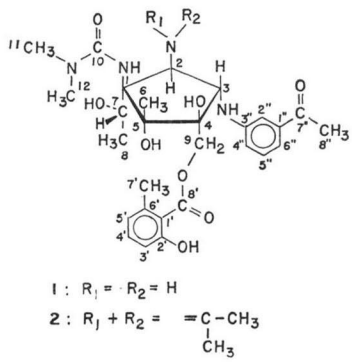
[a] Chemical shifts are given in ppm downfield from internal TMS. Chemical shift assignments marked with the same symbol are interchangeable.

[b] Actually, the spectrum of the deuterioacetone adduct (see text), in which the trideuteriomethyl groups are not observed. [c] Not observed.

chemical shift data. As shown in Table 1, these classes were further segregated into groups according to the number of attached hydrogens determined from the proton off-resonance decoupled spectrum. On this basis, the ketonic carbonyl carbon, C–7″, the ester carbonyl carbon, C–8′, the methylene carbon, C–9, and the urea N-methyl carbons, C–11 and C–12, were assigned unambiguously from their off-resonance multiplicities and the characteristic chemical shifts.[7~9] In the ^{13}C NMR spectrum of **1** in deuteriochloroform these signals appear at 198.7, 172.2, 65.3 and 36.8 ppm, respectively. Assignment of the remaining carbons was made through more detailed analyses.

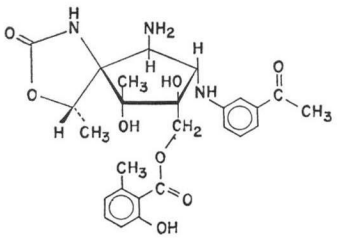
Fig. 1. Structures of pactamycin (**1**), its acetone adduct (**2**), pactamyçate (**3**) and model compounds (**4, 5**)

The numbering system employed for **1** and **3** is slightly different from that employed previously.[1]



1 : $R_1 = R_2 = H$

2 : $R_1 + R_2 =$



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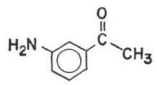

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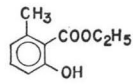

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CH_3– There are six methyl groups in pactamycin. The resonance at 36.8 ppm is due to the two urea N-methyl carbons, as noted above. The signals at 26.7 and 23.8 ppm were assigned to the C–8″ and C–7′ carbons, respectively, due to the excellent agreement (Table 1) with the chemical shifts of the analogous resonances in the model compounds *m*-aminoacetophenone (**4**) and ethyl 6-methylsalicylate (**5**). Unambiguous assignment of the signals at 18.1 and 21.1 ppm was provided through single frequency proton decoupling. Irradiation of the proton resonance at 1.03 ppm (H–8), the only methyl doublet in the ^1H NMR spectrum of **1**, collapses the quartet at 18.1 ppm in the ^{13}C NMR spectrum, assigning this resonance to C–8, while irradiation of the singlet at 1.54 ppm (H–6), the only aliphatic methyl singlet, causes collapse of the 21.1 ppm resonance and this signal must be due to C–6.

–CH– The three aliphatic methine carbons of **1** are found at 63.3, 68.9 and 74.2 ppm. The farthest downfield signal, at 74.2 ppm, should be attached to oxygen and is assigned to C–7. This value agrees well with the chemical shift of the carbinol carbon of 3-methyl-2-butanol, which appears at 72.3 ppm.[7] The nitrogen-bearing carbons, C–2 and C–3, are in similar environments, which differ mainly in having the aryl group attached to the C–3 nitrogen. The C–3 carbon should be shifted slightly downfield by the aryl group and the adjacent carbons upfield.[7~9] On this basis, C–2 and C–3 can be tentatively assigned to the resonances at 63.3 and 68.9 ppm, respectively. Direct evidence in support of these assignments was obtained by comparison with the ^{13}C NMR spectrum of the acetone adduct (**2**, Fig. 1), as will be discussed below.

–C– There are three aliphatic quaternary carbons in pactamycin, which absorb at 71.5, 84.7 and 88.8 ppm. Since C–1 of cyclopentanol is found at 73.6 ppm[7] and C–4 and C–5 should appear downfield from this, the nitrogen-bearing quaternary carbon of **1**, C–1, is assigned to the peak at 71.5 ppm. The oxygenated carbons, C–4 and C–5, are in similar environments and unambiguous

differentiation was not possible.

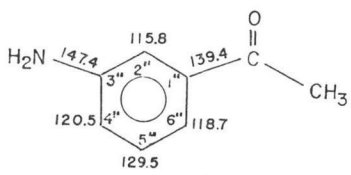
=C–H Assignment of the seven aromatic methine carbons was made by comparison to the ^{13}C NMR spectra of model compounds **4** and **5*** and was confirmed by specific proton decoupling. Only C–2″ deviates significantly (and C–4″ to a lesser degree) from the models' chemical shifts (probably due to the lack of an N-alkyl substituent in the models), so assignment of the models' carbons assigns those of **1**. On the other hand, certain resonances can not be unambiguously assigned by chemical shift comparison alone, and specific proton decoupling was employed.

The applicability of specific proton decoupling toward assigning unambiguously the close signals at 118.3 and 118.8 ppm, tentatively C–4″ and C–6″ in **1**, as well as the resonances at 115.5 and 110.9 ppm, tentatively C–3′ and C–2″ in **1**, was first tested in the model compounds **4** and **5**. In these compounds, H–3′, H–5′ and H–4″ appear significantly upfield from the other aromatic protons due to the influence of the adjacent hydroxyl and amine substituents. Irradiation of the broad upfield triplet (overlapping pair of doublets) at 6.75 ppm (H–3′ and H–5′) in the spectrum of **5** collapses the resonances at 115.6 and 122.9 ppm, and these signals must be due to C–3′ and C–5′, respectively. Similarly, irradiation of the upfield multiplet at 6.8 ppm in the ^{1}H NMR spectrum of **4** collapses the carbon signal at 119.6 ppm, assigning it to C–4″; thus, C–6″ must be at 118.9 ppm, as predicted by the calculated values.

A similar segregation of the aromatic protons exists in the 60 MHz ^{1}H NMR spectrum of pactamycin. An upfield multiplet integrating for three protons is centered at 6.75 ppm and is assigned to H–3′, H–5′ and H–4″. The downfield multiplet, consisting of four protons centered at 7.2 ppm, is assigned to H–4′, H–2″, H–5″ and H–6″. Irradiation (at several frequencies) of the upfield three-proton multiplet collapses, in stages, the resonances at 115.5, 118.8 and 123.0 ppm in the ^{13}C NMR spectrum of **1**, in accord with their assignments as C–3′, C–4″ and C–5′, as given in Table 1. In addition, irradiation (at several frequencies) of the downfield four-proton multiplet decouples, in stages, the carbon resonances at 110.9, 118.3, 129.6 and 134.1 ppm, in accord with their assignments to C–2″, C–6″, C–5″ and C–4′. Thus, C–6″ is at 118.3 ppm and C–4″ at 118.8 ppm, while C–3′ is at 115.5 ppm and C–2″ at 110.9 ppm.

=C– There are five quaternary aromatic carbons in pactamycin. The resonances at 112.4, 138.2, 141.2 and 146.7 ppm are assigned to C–1′, C–1″, C–3″ and C–6′, respectively, from their excellent agreement with the chemical shifts of the models (**4** and **5**). Although the resonance at 162.4 ppm of **1** agrees well with the chemical shift of the phenolic carbon C–2′ in **5** (162.9 ppm) it could also potentially be assigned to the urea carbonyl carbon, C–10, since urea and tetramethylurea show signals for

* The assignments of the aromatic carbons of **4** and **5** listed in Table 1 were made by comparison of observed with calculated values[7~9] (below) and by specific proton decoupling as described in the text. The calculated values in deuteriochloroform shown here for **4** and **5** were derived by adding increments for amino and methyl substituents to acetophenone and methyl salicylate values, respectively.[9]



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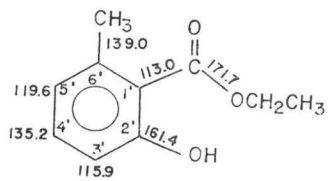

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their carbonyl carbons at 161.2 and 164.4 ppm, respectively.[8] The assignment of the phenolic carbon was most convincingly made by comparison of the two signals in the proton-coupled spectrum of **1**. As expected, one low-field resonance (162.4) shows the expected three-bond C–H coupling (9 Hz) to the *meta* hydrogen at C–4′; this can then be assigned as C–2′.

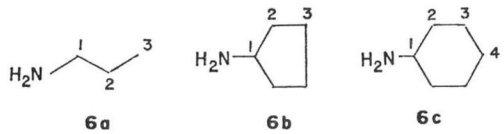
C=O The three carbonyl carbons of pactamycin absorb at 198.7, 172.2 and 159.2 ppm. The first two were assigned to C–7″ and C–8′ (*vide supra*); the last, by difference, must be C–10.

The only carbons for which direct evidence of the chemical shift was not obtainable were the quaternary carbons C–4 and C–5. A recent report has described the use of long range C–H couplings and nuclear OVERHAUSER enhancement (NOE) for carbon assignments.[11] We attempted a selective NOE of C–4 or C–5 by specific proton irradiations of H–9 and H–6, respectively, in the ^{1}H NMR spectrum of **1** in deuteriochloroform. Unfortunately, irradiation of either proton absorption led to greater enhancement of the peak at 84.7 ppm than that of the peak at 88.8 ppm. In view of this result and since no method to distinguish between two-bond and three-bond C–H couplings was obvious in our system, no selective decoupling experiments were performed.

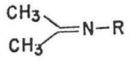
Pactamycin Acetone Adduct

Assignment of the carbon resonances of pactamycin allows determination of the structure of the pactamycin acetone adduct, **2**. The acetone adduct is most conveniently obtained by the chromatographic purification of pactamycin on Florisil using acetone-hexane as eluant.[4] Alternatively, solution of **1** in acetone and evaporation yields **2** as an unstable oil easily decomposed to **1** by water. The high resolution field desorption mass spectrum of **2** indicates that the molecular formula of the acetone adduct is C_3H_4 ($C_3H_6O-H_2O$) greater than that of pactamycin.

The chemical shifts of **2** in deuteriochloroform are listed in Table 1. Assignment of the individual resonances was made by comparison with those in the spectrum of **1** and by single frequency off-resonance decoupling. The additional resonances in the acetone adduct are found at 171.5 (or 172.0), 30.3 and 18.9 (or 18.5) ppm. The absorption at 171.5 ppm must be due to a carbonyl derivative (sp^2 hybridized carbon), ruling out ketal-like structures in which C–2 of acetone has become sp^3 hybridized. These absorptions are, in fact, in agreement with the chemical shifts anticipated for aliphatic ketimines. Since no data have been reported on the ^{13}C NMR characteristics of ketimines, three representative aliphatic amines (**6a**～**c**) were converted to their isopropylidene ketimines (**7a**～**c**) by an established procedure.[10] The chemical shifts of the *syn* and *anti* ketimine methyl carbons (assigned by analogy to oximes[9]) are in excellent agreement with the extra methyl carbons in the spectrum of **2**, as seen in Table 2; however, the imine carbon in **2** is downfield relative to the models.



6a **6b** **6c**



7a: R = n-C_3H_7
7b: R = cyclo-C_5H_9
7c: R = cyclo-C_6H_{11}

Additional support for the ketimine structure was obtained by other spectral methods. The ^{1}H NMR spectrum of **2** revealed additional methyl absorptions at 2.18 and 1.93 ppm, while the infrared spectrum showed loss of intensity at 3326 cm^{-1} and an increase in intensity near 1680 cm^{-1} attending the conversion of **1** to **2**.

Table 2. ^{13}C NMR Signals of model primary amines and their acetone-derived ketimines

Carbon	Compounds, chemical shifts*, and increments									
	6a	**7a**	Δ**	**6b**	**7b**	Δ**	**6c**	**7c**	Δ**	Δ ave.
1	44.3	53.5	+9.2	53.4	61.1	+7.7	50.5	59.1	+8.9	+8.6
2	27.0	24.2	−2.8	36.3	33.9	−2.4	37.0	33.7	−3.3	−2.8
3	11.3	12.1	+0.8	23.9	24.7	+0.5	25.1	25.1	0.0	+0.4
							25.7	25.8	+0.1	+0.1
syn-CH_3		18.5			18.5			18.0		
anti-CH_3		29.2			29.3			29.4		
imino		166.5			164.7			164.0		

* Ppm downfield from internal TMS.

** Δ=change in chemical shift (in ppm) upon ketimine formation. Positive values indicate downfield displacement.

The effect of ketimine formation on the chemical shifts of the carbons in the amine portions of **6a**~**c** is of value in assigning carbons of **1** and **2**. A large downfield displacement (+8.6 ppm) is found for the α-carbon (that attached to nitrogen), a moderate upfield displacement (−2.8 ppm) for the β-carbons, and only a small effect for more distant carbons. These changes parallel those obtained by methylation of an amine.[7~9] It is apparent that in the conversion of **1** to **2** the resonance at 63.3 ppm in the spectrum of **1** has been shifted downfield in **2** and is best assigned to the signal at 71.5 ppm (Δ=8.2). The signal at 68.1 ppm for **2** is, therefore, related to the 68.9 ppm absorption of pactamycin (Δ=−0.8). The signal at 63.3 ppm in the ^{13}C NMR spectrum of **1** must then be assigned to C–2 since this carbon bears the primary amine in **1** and the ketimine in **2**, and the signal at 68.9 ppm in the spectrum of **1** must be assigned to C–3. It may be noted that C–1 of pactamycin is shifted downfield upon conversion to **2** (Δ=+2.2), an effect not observed in the simpler systems.

Pactamycin in Deuterioacetone Solution

With the structure and chemical shifts of the acetone adduct **2** assigned, the ^{13}C NMR spectra of pactamycin and model compounds **4** and **5** in deuterioacetone were recorded; the chemical shifts are listed in Table 1. The agreement of the spectrum of **1** in deuteriochloroform with the spectra of the models in the same solvent and with the spectrum of **2** in deuteriochloroform allowed straightforward assignment of the carbon resonances. To remove any ambiguities the assignments of the methyl, aromatic and carbonyl carbons of **1** in deuterioacetone were confirmed in a manner identical to that used for **1** in deuteriochloroform. Some of the carbons in the central ring appear slightly downfield from their deuteriochloroform positions, apparently due to more complex secondary interactions with the solvent. That this is the case was demonstrated by obtaining the ^{13}C NMR spectrum of **2** in deuterioacetone. The chemical shifts of the carbons of **2** in this solvent are identical to the chemical shifts observed for these carbons when pactamycin is dissolved in deuterioacetone. Additional peaks at 171.3 and 19.2 ppm demonstrated the continued presence of the protioacetone adduct in deuterioacetone. The other methyl absorption of **2** is apparently obscured by the solvent peaks. After 36 hours, all protioacetone adduct had disappeared, replaced by the deuterioacetone adduct. The peak at 171.3 ppm was diminished and broadened, while the peak at 19.2 ppm had disappeared, replaced by an acetone peak at 29.9 ppm. Thus, the imine structure best represents the form of pactamycin in deuterioacetone.

Pactamyçate in Deuterioacetone Solution

Pactamyçate (**3**) is the chemical hydrolysis product of **1** and neither **3** nor its acetone adduct is soluble in chloroform. Sufficient solubility for the ^{13}C NMR experiment was obtained with deuterioacetone and the chemical shifts of **3** in this solvent appear in Table 1. The assignments of the aromatic and carbonyl carbons were confirmed in a manner identical to that used for **1**. [Carbon 1′ could not be observed, even in the presence of 0.5 M chromium (III) acetylacetonate.] Assignment of the resonances of the carbons of the cyclopentane ring and the aliphatic methyl carbons C–6, C–8 and C–7′ required more detailed analyses.

$\underline{CH_3}$— The methyl carbons in the ^{13}C NMR spectrum of **3** in deuterioacetone appear at 26.6, 22.6, 17.6 and 17.2 ppm. By comparison to the model compounds, the peak at 26.6 ppm is easily assignable to the acetophenone methyl, C–8″, and the peak at 22.6 ppm, with less certainty, to the salicylate methyl, C–7′. The two upfield absorptions were convincingly shown to be due to C–6 and C–8, since irradiation of the aliphatic methyl resonances in the ^1H NMR spectrum of **3** collapses the resonances at 17.6 and 17.2 ppm. Unfortunately, the attached hydrogens, H–6 and H–8, are separated by only 13 Hz in the ^1H NMR spectrum of **3**, and C–6 and C–8 are not distinguished by specific proton decoupling.

$\underline{-CH_2-, -CH-, -\overset{|}{C}-}$ The resonances in the region 90 to 60 ppm in the ^{13}C NMR spectrum of **3** in deuterioacetone were assigned by their chemical shifts and single frequency off-resonance multiplicities. The only triplet in the off-resonance spectrum is centered at 66.5 ppm and must arise from the methylene carbon C–9. There are three singlets in the off-resonance spectrum of **3** in this region, and the downfield absorptions, at 84.6 and 83.6 ppm, must be due to the carbinol carbons in the ring (C–4 and C–5), though they are not distinguishable, while the resonance at 73.2 must arise from C–1. Although there are three methine carbons which absorb in this region, only two doublets are present in the off-resonance spectrum. The peak at 76.7 ppm is certainly due to C–7, which as a consequence of carbamate formation has been shifted slightly downfield relative to its position in **1**. The resonance at 68.9 is slightly broadened and more intense than the other peaks in this region and is, therefore, assigned to both C–2 and C–3. It is of some interest that C–2 is shifted more than three ppm upfield from its position in **1**.

Pactamyçate in Deuteriodimethyl Sulfoxide Solution

$\underline{CH_3-, -CH_2-, -CH-, -\overset{|}{C}-}$ Since the C–6 and C–8 and the C–4 and C–5 resonances in the ^{13}C NMR spectrum of **3** in deuterioacetone are not unambiguously assigned and C–2 and C–3 are not differentiated, the spectra of **3** and **1** were also determined in deuteriodimethyl sulfoxide. The chemical shifts of the carbons of **1** and **3** and of the model compounds **4** and **5** in this solvent are listed in Table 1. The methyl carbons of **3** are completely assigned here by comparison to the chemical shifts of the analogous carbons in **4** and **5** and by specific proton decoupling. The protons assigned to H–6 and H–8 are separated by 16 Hz in the ^1H NMR spectrum of **3** in deuteriodimethyl sulfoxide, and, with the greater chemical shift difference of C–6 and C–8 in the ^{13}C NMR spectrum, specific proton decoupling was successful in assigning these carbons. Irradiation at low power and off-center of either H–6 or H–8 suggested that the protons (H–8) in the doublet at δ 1.44 were coupled to the peak at 17.3 ppm and that the protons (H–6) in the singlet at δ 1.21 were coupled to the peak at

16.3 ppm. This correlation was based upon the magnitude of the residual C–H couplings of C–8 and C–6 in the ^{13}C NMR spectra upon irradiation downfield or upfield of both proton resonances. The resonances in the 90 to 60 ppm region are assigned in the same manner as the same resonances in the deuterioacetone spectrum. Now, however, the signals for C–2 and C–3 are well separated; since no ketimine is present, C–2 is found upfield at 57.6 ppm.

$\underline{=CH}$, $\underline{=C-}$ The chemical shifts measured in deuteriodimethyl sulfoxide of the peaks due to the aromatic carbons are significantly shifted relative to the resonances for the same carbons in deuteriochloroform or deuterioacetone. As a consequence, the resonances at 112.4 and 115.6 ppm in the ^{13}C NMR spectrum of **4**, due to C–2″ and C–6″, cannot be unambiguously assigned. In addition, the very close peaks at 137.2 and 136.9 ppm in the spectrum of **3** are presumably due to C–1″ and C–6′, respectively, based on comparisons with the model compounds **4** and **5**, but this assignment could be interchanged. The remainder of the aromatic carbon resonances in the spectrum of **3** are assigned by comparison to the chemical shifts of the carbons in the model compounds and by specific proton decoupling.

Pactamycin in Deuteriodimethyl Sulfoxide

The peaks due to the aromatic carbonyl and aromatic methyl carbons of **1** in deuteriodimethyl sulfoxide are assigned solely on the basis of the excellent correlation with the peaks in the spectra of **3**, **4**, and **5**. The resonances in the 90 to 60 ppm region and the methyl carbons, C–6 and C–8, of **1** are assigned by their good agreement with the values for these carbons in the ^{13}C NMR spectrum of **1** in deuteriochloroform and by the multiplicities in the off-resonance proton decoupled spectrum.

C–4 and C–5 of Pactamyçate

Finally, the assignment of the C–4 and C–5 resonances in pactamyçate must be considered. Examination of Table 1 reveals that both C–4 and C–5 are shifted upfield in **3** but C–5 more than C–4 so that the difference between the chemical shifts for C–4 and C–5 in **3** is much smaller in both deuterioacetone and deuteriodimethyl sulfoxide (1.0 and 1.7 ppm, respectively) than the corresponding difference in **1**. The observed upfield shifts of C–4 and C–5 in the ^{13}C NMR spectra attending conversion of **1** to **3** are not unique to these carbons in the 90 to 60 ppm region, as can be seen in Table 3. It appears that carbons (*e.g.*, C–2 and C–6) close in space to the oxazolidinone are shifted upfield extensively, while more remote carbons (*e.g.*, C–3 and C–8) are less affected, and C–7 and C–9 are shifted downfield in both solvents. The downfield shift of C–7 is expected due to acylation. The downfield shift of C–3 of **3** in deuteriodimethyl sulfoxide is unique, but the carbon is also uniquely assigned. One of the cyclopentane carbinol carbons (C–4 or C–5)

Table 3. Chemical shift changes Δ upon conversion of **1** to **3**

Carbon	Δ*	
	Acetone-d_6	DMSO-d_6
1	−0.8*	−0.5
2	−4.2	−5.2
3	−0.5	+3.2
4	−0.9	−1.9
5	−5.4	−5.5
6	−4.3, −4.7	−3.9
7	+2.7	+2.5
8	−1.5, −1.1	−2.0
9	+0.9	+2.5

* Δ=shift in ppm of **1** on conversion to **3**; a downfield shift is positive.

is significantly shifted while the other is less affected. Since C–2 is shifted more than C–3, the carbinol carbon with the larger shift is assigned to C–5 and must be due to the resonances in **1** at 89.9 and 87.5 ppm in deuterioacetone and deuteriodimethyl sulfoxide, respectively. In order for the magnitude of the change in chemical shift to correspond to the C–2 example (~4 ppm in acetone, ~5 ppm in dimethyl sulfoxide), the C–5 carbon of **3** is assigned to the resonances at 84.6 and 82.0 ppm, respectively, in deuterioacetone and deuteriodimethyl sulfoxide, as shown in Table 1. The alternative assignments, to 83.6 and 80.3 ppm, respectively, would represent a too large shift for C–5 (and a too small shift for C–4).

The carbon atoms of pactamycin and pactamyçate have now been completely assigned. Though some ambiguity remains for a few carbons in a single solvent, the use of several solvents allows every carbon to be unambiguously assigned in at least one solvent.

Experimental*

Pactamycin (**1**) and Pactamycin Acetone Adduct (**2**).

Purification of crude pactamycin *via* Florisil chromatography using acetone-hexane as eluant[4] furnished pure acetone adduct **2** as a foam: ^{1}H NMR ($CDCl_3$) δ 7.3~6.8 (m, 4 H), 6.8~6.5 (m, 3 H), 4.83 (d, 1 H, J=12 Hz), 4.64 (d, 1 H, J=12 Hz), 3.99 (dd, 1 H, J=10, 5 Hz), 3.9 (q, 1 H, J=7 Hz), 3.61 (d, 1 H, J=10 Hz), 2.96 (s, 6 H), 2.50 (s, 3 H), 2.38 (s, 3 H), 2.18 (s, 3 H), 1.93 (s, 3 H), 1.57 (s, 3 H), 1.02 (d, 3 H, J=7 Hz); IR ($CHCl_3$) 3367, 2923, 1709, 1667 (broad), 1624, 1601, 1580 (sh) cm^{-1}; $[\alpha]_D^{25}$ +36.8° (*c* 1, $CHCl_3$) (lit.[14] $[\alpha]_D^{25}$ +36.5°).

Anal. Calcd. for $C_{31}H_{42}N_4O_8$: mol. wt, 598.3002. Found: mol. wt, 598.3014 (HRFDMS).

The pure adduct **2** was dissolved in ethyl acetate and extracted into 0.1 N sulfuric acid. The aqueous solution was adjusted to pH 5.5 by addition of citrate buffer (pH 6), then further basified with 2 N sodium hydroxide to pH 8.0. Extraction with ethyl acetate and evaporation furnished pure **1** as a glass: IR ($CHCl_3$) 3355, 3226, 1730, 1675, 1647 (sh), 1601, 1580 (sh) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.3~7.1 (m, 4 H), 6.8~6.5 (m, 3 H), 4.47 (s, 2 H), 3.92 (d, 1 H, J=9 Hz), 3.9 (q, 1 H, J=7 Hz), 2.99 (s, 6 H), 2.95 (s, 1 H), 2.53 (s, 3 H), 2.38 (s, 3 H), 1.54 (s, 3 H), 1.03 (d, 3 H, J=7 Hz). This material was identical in every respect to authentic pactamycin.

Pactamyçate (**3**)[1].

Pactamyçate was recrystallized from ethanol; mp 205~208°C (lit.[1] 207~210°C). Before each use the yellow crystals were dried *in vacuo* (150°C, 0.1 mm).

m-Aminoacetophenone (**4**).

m-Aminoacetophenone (Aldrich) was crystallized twice from water containing added Norit; mp 96~97°C (lit.[12] 98~99°C).

Ethyl 6-methylsalicylate (**5**).

6-Methylsalicylic acid, prepared by the method of ELIEL[13] from *m*-cresol, had mp 170~172°C (from hexane, lit.[13] 170~171°C). The acid (645 mg, 4.24 mmol) was converted in refluxing anhydrous ethanolic hydrogen chloride to the ester. Recrystallization from ether – hexane (1 : 4) yielded 226 mg (30%) of **5**; mp 43~45°C (lit.[14] 42.5°C).

Amines **6a~c**.

n-Propylamine (Eastman), cyclopentylamine (Aldrich) and cyclohexylamine (Matheson, Coleman and Bell) were used without purification.

* Carbon magnetic resonance spectra were obtained using a JEOL FX-60 FT-NMR spectrometer with a dual probe (^{13}C, ^{1}H). Proton magnetic resonance spectra were obtained on a Varian EM–390 spectrometer with the aid of Dr. S. ULRICH and his associates. All chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were obtained on Varian MAT CH5–DF (EIMS) and Varian MAT 731 (FDMS) spectrometers by Mr. J. C. COOK, Jr., and his associates. Microanalyses were obtained at the University of Illinois by Mr. J. NEMETH and his associates. Melting points and boiling points are uncorrected.

Ketimines **7a**~**c**.

The isopropylidene ketimines **7a**~**c** were prepared from acetone and the amines **6a**~**c** with a catalytic amount of hydrogen chloride according to a known procedure.[10] Compound **7a** had bp 107°C (lit.[10] 108°C), **7c** had bp 177°C (lit.[10] 180°C). The cyclopentylisopropylidene ketimine **7b**, bp 157°C, is previously undescribed: ^{1}H NMR δ 3.80 (quintet, 1 H), 1.97 (s, 3 H), 1.84 (s, 3 H), 1.7 (m, 4 H), 1.5 (m, 4 H); IR 1678 cm^{-1}; EIMS 41 (100%), 125 (44%).

Anal. Calcd. for $C_8H_{15}N$: C, 76.73; H, 12.10; N, 11.19.
Found: C, 76.90; H, 11.90; N, 11.08.

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