# The Structure of Pactamycin[1]

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*Received September 22, 1969*

The structure of the antibiotic, pactamycin, has been shown to be that represented by I by physical studies and chemical degradation.

The isolation of the antibiotic, pactamycin, and its characterization were reported some years ago by Argoudelis, Jahnke, and Fox.[2] The present paper discusses the determination of the structure of pactamycin and of some of its degradation products and presents evidence that the structure is as represented in I in which the hydroxyl groups at C-4 and C-5 are *trans* to each other and the two nitrogen atoms at C-1 and C-2 are *cis* to each other and *trans* to the anilino system.

The originally reported molecular formula for pactamycin was $C_{28}H_{40}N_4O_8$.[2] As a result of mass spectra derived from degradation products it has been necessary to revise the molecular formula to $C_{28}H_{38}N_4O_8$. As mentioned earlier[2] pactamycin exhibits ultraviolet absorption having a strong maximum at 239 m$\mu$ with a shoulder at 264 m$\mu$ and weak maxima at 313 and 356 m$\mu$. There is almost no change in dilute acid solutions, but the 313 m$\mu$ maximum moves to 320 m$\mu$ in alkaline solutions. The infrared spectrum is quite complex showing bands indicative of OH–NH, carbonyl, and aromatic rings. However, it was found that in rigorously purified pactamycin the band reported[2] at 1718 cm$^{-1}$ is absent. A basic function is present (p$K_a'$ 7.25) as is a weakly acidic function (p$K_a'$ 9.35) with the basic function presumably being due to a primary amino group indicated to be present by a Van Slyke nitrogen determination. Pactamycin has been reported[2] to have a rotation of +79° in ethanol changing to +23° on standing. It has been found that pactamycin upon standing in acetone changes its rotation from +25° at zero time to +76° after 24 hr. Pactamycin which has been exposed to acetone has two new singlets in the nmr spectrum at $\delta$ 2.00 and 2.26 with each singlet representing a C-methyl group. Solution of such material in ethanol followed by reisolation gives pactamycin having the usual nmr spectrum. From these results it is concluded that pactamycin reacts with acetone to form a complex having a rotation of +79° in ethanol, and that the complex is readily destroyed to give pactamycin which has a rotation of +23° in the same solvent. This acetone product was studied only to clarify the discrepancy in rotation and no attempt was made to determine its structure. The chemical shifts of the protons on carbon of pactamycin are shown in Table I. The significance of the data shown will be discussed exhaustively in connection with the nmr spectra of degradation products. At this point it is sufficient to point out that four methyl groups attached to carbon are present with only one having a proton on an adjacent carbon atom. The singlet appearing at $\delta$ 2.94 indicates that two N-methyl groups are present.

A solution of pactamycin in 2 *N* hydrochloric acid heated on the steam bath for a short time gives rise to two products. One of these was identified as dimethylamine by reaction with phenyl isothiocyanate to form N-phenyl-N′-dimethylthiourea. The second product was a new compound designated pactamycate (II) and having the molecular formula $C_{26}H_{31}N_3O_8$ differing from pactamycin in its molecular formula by the elements of dimethylamine. Analyses of pactamycate and its diacetyl derivative and a molecular weight determination of the latter compound by mass spectrum established the molecular formula. In contrast to pactamycin, pactamycate is crystalline and is only slightly soluble in most common organic solvents. Its rotation, p$K_a$ values, and ultraviolet spectrum are almost the same as those of pactamycin. However, the infrared spectrum has a band at 1739 cm$^{-1}$ which was not present in the pactamycin spectrum. The 1739-cm$^{-1}$ band indicates, in view of the reaction conditions, that a new carbonyl system has been created concomitant with expulsion of dimethylamine. The primary amino group is still present.

Mild basic hydrolysis of pactamycate formed a further degradation product, desalipactamycate (IIIa). The same product was readily formed by base treatment of pactamycin. In addition there was formed 6-methylsalicyclic acid from both pactamycin and pactamycate. The acid was identified by conversion into its methyl ether and comparison of the physical properties of the two compounds with those reported in the literature. The molecular formula of desalipactamycate was established as $C_{18}H_{25}N_3O_6$ by analysis and high resolution mass spectra. Desalipactamycate is an amorphous material, highly soluble in water and methanol but sparingly soluble or insoluble in less polar solvents. No titratable groups are present in desalipactamycate suggesting that the acidic function of the preceding compounds is the phenolic hydroxyl group and that reaction had occurred at the basic group originally present. The ultraviolet spectrum of desalipactamycate is similar to those of pactamycin and pactamycate except that the maximum at 313 m$\mu$ is no longer present. The infrared spectrum differs significantly in the carbonyl region from those of pactamycin and pactamycate. A new band previously lacking appears at 1705 cm$^{-1}$, and the 1739-cm$^{-1}$ band is absent. Three derivatives of desalipactamycate (IIIb, IIIc, and IIId) were prepared by standard procedures. Characterization data are provided in Table II.

The presence of an infrared band at 1705 cm$^{-1}$ and the absence of a primary amino group in desalipacta-

(1) A portion of this material was presented at the 4th International Symposium on the Chemistry of Natural Products, Stockholm, Sweden, June 26–July 1, 1966. This work was supported by Contract PH43-68-1023, Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md.

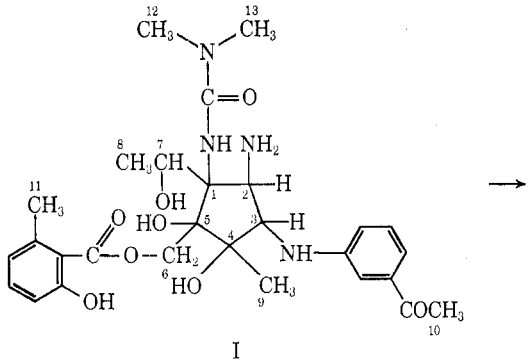
(2) A. D. Argoudelis, H. K. Jahnke, and J. A. Fox, *Antimicrobiol. Agents Chemother.*, 191 (1961).

TABLE I

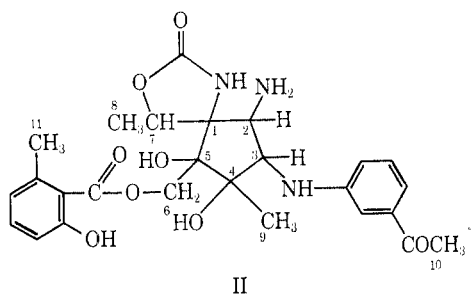
NUCLEAR MAGNETIC RESONANCE SPECTRAL DATA[a]

| Proton | Pactamycin (I) | Pactamycate (II) | Desalipactamycate (IIIa) | Diacetyldesalipactamycate (IIIe) |
|---|---|---|---|---|
| H-8 | 0.98 (3 H, d) | 1.54 (3 H, d) | 1.21 (3 H, d) | 1.20 (3 H, d) |
| H-9 | 1.49 (3 H, s) | 1.38 (3 H, s) | 1.52 (3 H, s) | 1.57 (3 H, s) |
| H-11 | 2.20 (3 H, s) | 2.24 (3 H, s) | | |
| H-10 | 2.50 (3 H, s) | 2.46 (3 H, s) | 2.53 (3 H, s) | 2.55 (3 H, s) |
| H-12, H-13 | 2.94 (6 H, s) | | | |
| H-3 | 3.97 (1 H, s) | 3.73 (1 H, d) | 3.85 (1 H, s) | 4.04 (1 H, s) |
| H-2 | 4.00 (1 H, s) | 3.56 (1 H, d) | 3.41 (1 H, s) | 3.59 (1 H, s) |
| H-7 | 4.03 (1 H, q) | 4.73 (1 H, q) | 4.21 (1 H, q) | 5.48 (1 H, q) |
| H-6 | 4.54, 4.78 (2 H, dd) | 4.65, 4.39 (2 H, dd) | 3.98, 3.72 (2 H, dd) | 4.30, 4.49 (2 H, d) |
| Aromatic | 6.7–7.3 (7 H, m) | 6.72–7.12 (7 H, m) | 6.98–7.3 (4 H, m) | 6.9–7.45 (4 H, dm) |
| O ‖ $CCH_3$ | | | | 1.90 (3 H, s)<br>2.02 (3 H, s) |

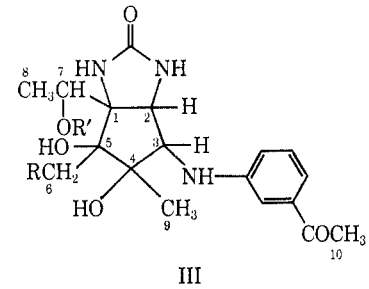
[a] Chemical-shift values are expressed in $\delta$ units (parts per million) relative to internal tetramethylsilane. The solutions are approximately 10% by weight in $d_7$-DMF with $D_2O$ added for exchange. All spectra were run at 100 MHz.



I



II



III

a, R = OH; R′ = H

b, R = $CH_3$–C$_6$H$_4$–$SO_3$; R′ = H

c, R = $O_2N$–C$_6$H$_4$–$SO_3$; R′ = H

d, R = I; R′ = H

e, R = $CH_3COO$; R′ = $CH_3CO$

mycate are consistent with the reaction of a carbonyl group already present to form a cyclic amide.[3] Treatment of desalipactamycate with 5 *N* sodium hydroxide under reflux forms carbon dioxide establishing that the cyclic amide is either a cyclic urea or a 2-oxazolidone.

(3) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962, p 47.

TABLE II

| Compd | Mp, °C | Calcd, % C | Calcd, % H | Calcd, % N | Found, % C | Found, % H | Found, % N |
|---|---|---|---|---|---|---|---|
| IIIb | 155 dec | 56.27 | 5.86 | 7.87 | 56.42 | 6.09 | 7.86 |
| IIIc | | 51.07 | 5.00 | 9.92 | 50.72 | 5.40 | 9.87 |
| IIId | 211 dec | 44.18 | 4.95 | 8.59 | 44.53 | 5.24 | 8.47 |

That it is probably a cyclic urea is indicated by the infrared band at 1705 $cm^{-1}$ which would be expected for such compounds.[4]

Oxidation of desalipactamycate with performic acid formed two products, *m*-nitroacetophenone and *m,m′*-diacetoazoxybenzene. Such products can arise only from a *m*-acetoanilino moiety. The presence of such a moiety is also indicated by the similarity of the ultraviolet spectrum of desalipactamycate to that of *m*-aminoacetophenone which also has a strong maximum at a low wavelength (231 m$\mu$ in methanol), a shoulder (257), and a weak maximum at a longer wavelength (330). The nmr signal appearing as a singlet at $\delta$ 2.53 would be as expected for the methyl group of such a system.

The nmr spectrum of desalipactamycate (Table I) shows the presence of two C-methyl groups ($\delta$ 1.21 and 1.52) in addition to the one in the *m*-acetoanilino moiety. The higher field signal is coupled with a proton at $\delta$ 4.21 ($J$ = 6.4 Hz). Such a pattern establishes that one C-methyl group is attached to a carbon atom having a single proton and most probably an oxygen substituent. Desalipactamycate forms a diacetate (IIIe) whose nmr spectrum has a quartet centered at $\delta$ 5.48 arising from a proton adjacent to C-methyl. The 1.27-ppm downfield shift of the quartet chemical shift upon acetylation indicates acetylation of a hydroxyl group on the same carbon atom. There, then, must be a $CH_3C(OH)H$ system in desalipactamycate.

Periodate oxidation of desalipactamycate results in very rapid consumption of 2 mol of periodate/mol with a slow overoxidation to a total of a little more than 3 mol of periodate. The only product isolated was formaldehyde although electrometric titration of the reaction mixture indicated formation of a carboxyl group. The absence of acetaldehyde formation shows that the secondary alcohol was not involved in the oxi-

(4) (a) Biotin and dehydrobiotin have infrared bands in the region of 1700 ± 15 $cm^{-1}$. These data were provided by Mr. Paul Meulman of The Upjohn Co. (b) J. Altman and D. Ben-Ishai, *J. Heterocycl. Chem.*, **5**, 679 (1968).

TABLE III

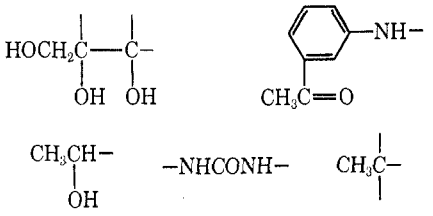
HIGH RESOLUTION MASS MEASUREMENTS[a]

| Desalipactamycate | | | Diacetyldesalipactamycate | | |
|---|---|---|---|---|---|
| Measured *m/e* | Calcd *m/e* | Composition | Measured *m/e* | Calcd *m/e* | Composition |
| 379.1744 | 379.1743 | $C_{18}H_{25}N_3O_6$ | 463.1957 | 463.1955 | $C_{22}H_{29}N_3O_8$ |
| 334.1401 | 334.1403 | $C_{16}H_{20}N_3O_5$ | 403.1735 | 403.1743 | $C_{20}H_{25}N_3O_6$ |
| 276.1342 | 276.1348 | $C_{14}H_{18}N_3O_3$ | 358.1396 | 358.1403 | $C_{16}H_{20}N_3O_5$ |
| 250.1090 | 250.1079 | $C_{13}H_{16}NO_4$ | 298.1193 | 298.1192 | $C_{16}H_{16}N_3O_3$ |
| 190.0873 | 190.0868 | $C_{11}H_{12}NO_2$ | 292.1155 | 292.1185 | $C_{15}H_{18}NO_5$ |
| 135.0690 | 135.0684 | $C_8H_9NO$ | 232.0976 | 232.0974 | $C_{13}H_{14}NO_3$ |
| 120.0446 | 120.0449 | $C_7H_6NO$ | 190.0864 | 190.0868 | $C_{11}H_{12}NO_2$ |
| | | | 135.0682 | 135.0684 | $C_8H_9NO$ |

[a] Not all of the data are included.

dation. As the nitrogen atoms are neutral and no products expected from oxidation involving the nitrogen atoms present were isolated, it appears that these too were not involved in the periodate oxidation. Since the speed of the reaction is consistent only with a normal periodate oxidation, the only remaining explanation is that a 1,2,3-trihydroxy system is present in desalipactamycate, and none of the three hydroxyls can be the secondary hydroxyl adjacent to the C-methyl group. The formation of formaldehyde must be due to the presence of one primary carbinol. The nmr spectrum of desalipactamycate and that of its diacetate are also indicative of the presence of such a primary hydroxyl. The signals centered at $\delta$ 3.72 and 3.98 in desalipactamycate and at 4.26 and 4.54 in its acetate are characteristically those of a primary carbinol which has then been acetylated. The AB pattern of doublets indicates the absence of a hydrogen atom adjacent to the methylene protons. The other two hydroxyl groups must be tertiary as there is no nmr evidence for hydrogen on carbon bearing a hydroxyl group and the two groups are not acetylatable.

The previous discussion leads to the conclusion that the following moieties are present in desalipactamycate and that no other functional groups can be present.




This information in conjunction with the molecular formula of desalipactamycate requires the presence of two ring systems, in addition to the aromatic ring, one of which must contain the urea system. These two rings are necessarily formed from six carbon atoms and two nitrogen atoms since twelve carbon atoms, one nitrogen atom, and all of the oxygen atoms have been shown to be outside any ring system.

The high resolution mass spectra of desalipactamycate and its diacetate (Table III) demonstrated that the moieties already indicated can only be combined to lead to the structure represented by IIIa or its isomer in which the $CH_3CHOH$ substituent is at C-2. A choice between these two structures will be presented later in this paper. Desalipactamycate fragments with loss of $C_5H_9N_2O_2$ (379.1744 $\xrightarrow{m^*}$ 250.1090) followed by loss of $C_2H_4O_2$ (250.1090 $\xrightarrow{m^*}$ 190.0873) to give a $C_{11}H_{12}NO_2$ ion, both paths being established by the presence of metastable ion peaks in the spectrum. The composition of the eleven-carbon fragment is only consistent with retention of the aromatic system. The diacetate exhibits a completely parallel pathway except that the loss at each stage is $C_2H_2O$ larger

$$C_{22}H_{29}N_3O_8 \xrightarrow[m^*]{-C_7H_{11}N_2O_3} C_{15}H_{18}NO_5 \xrightarrow[m^*]{-C_4H_6O_3} C_{11}H_{12}NO_2$$

again established by metastable ions. The five-carbon fragment must contain an acetylated group in the acetate as does the two-carbon fragment. The $C_2H_4O_2$ includes the primary hydroxyl group as the secondary hydroxyl could not be lost in a fragment of this composition. The three-carbon sequence which has three hydroxyl groups can only be attached to a carbon substituted by the *m*-acetoanilino moiety as evidenced by the thirteen-carbon ion. The eleven-carbon fragment would necessarily include the C-methyl group on the ring to have eleven carbon atoms. Desalipactamycate also fragments by a different pathway with loss of $C_4H_7O_3$ (379.1744 → 276.1348). This loss establishes that the ring C-methyl group is on one of the carbon atoms bearing a hydroxyl group, and the loss of $C_2H_4O_2$ means that the C-methyl cannot be on the carbon atom adjacent to the primary carbinol. Otherwise a three-carbon fragment would be lost. Since the four-carbon fragment and the eleven-carbon fragment both contain the C-methyl group on the carbocyclic ring, and, in view of the presence of the primary hydroxyl group in the four-carbon fragment, the C-methyl group must be attached to a carbon atom which is attached to a carbon atom bearing the *m*-acetoanilino moiety. The remaining part of the desalipactamycate molecule is the five-carbon fragment which includes the secondary carbinol and the cyclic urea system. There are only two ways in which the five-carbon and the thirteen-carbon fragment can be combined. The two ways are as in IIIa or as the isomer of IIIa previously mentioned.

Pactamycate (II) is converted into desalipactamycate with addition of the elements of water and loss of 6-methylsalicyclic acid. However, changes other than hydrolysis occur, as shown by loss of the 1739-$cm^{-1}$ infrared band and appearance of a 1705-$cm^{-1}$ band in desalipactamycate. Such a change indicates that one of the carbonyl systems in pactamycate has been converted into the cyclic urea system of desalipactamycate. Also consistent with this interpretation is the presence of the primary amino function in pactamycate. An examination of the nmr spectrum of pactamycate shows that the chemical shifts due to the methylene group and the single proton on carbon of the secondary

carbinol system are downfield from the signals of desalipactamycate (Table I) indicating esterification of both the primary and secondary carbinols. Therefore, 6-methylsalicyclic acid must be attached to one of these groups and the 1739-$cm^{-1}$ carbonyl group must be esterified with the other hydroxyl group. That the 1739-$cm^{-1}$ carbonyl system is actually a 2-oxazolidone, although such a ring system could involve either the primary or the secondary oxygen, is indicated by loss of carbon dioxide by strong base hydrolysis of pactamycate. The 1739-$cm^{-1}$ infrared band is typical of substituted 2-oxazolidones such as 5-(*m*-trifluoromethylphenoxymethyl)-2-oxazolidone (1737 $cm^{-1}$) and 5-(3,4-dimethylphenyl)-2-oxazolidone (1735 $cm^{-1}$).[5] Pactamycate forms a neutral diacetyl derivative indicating that the acidic function is the phenolic hydroxyl group. Reduction of pactamycate with sodium borohydride gives a dihydro derivative which can be acetylated to a triacetate. Such behavior indicates reduction of a ketonic carbonyl, and the nmr spectrum of the triacetyl derivative shows that the carbonyl reduced was the carbonyl of the *m*-acetoanilino group. The signal in pactamycate representing the *m*-acetoanilino methyl appears at $\delta$ 2.46 (s), but this signal no longer appears after reduction while a new doublet representing three hydrogen atoms appears at $\delta$ 1.42. The free phenolic hydroxyl group indicating absence of an ether linkage, the absence of a ketonic carbonyl in the salicoyl moiety as shown by failure of the salicoyl carbonyl to reduce with sodium borohydride, and the nmr data showing that the protons on C-6 of pactamycate are downfield from the C-6 protons of desalipactamycate establish that 6-methylsalicyclic acid is present in pactamycate as an ester. Although the two hydrogen atoms (H-2 and H-3) giving chemical shifts of $\delta$ 3.41 and 3.85 in the nmr of desalipactamycate show no coupling, the same two hydrogen atoms in pactamycate (3.56 and 3.73) have a coupling constant of 8.5 Hz. This can only be interpreted to mean that they are adjacent. Consequently, the secondary carbinol system must be attached at C-1 in desalipactamycate and IIIa is the correct expression for the structure since the only possible isomer would necessitate a 1,3 relationship for these hydrogen atoms. The failure of pactamycate to consume periodate is somewhat surprising as there is no indication that the adjacent tertiary hydroxyl groups of desalipactamycate are no longer present. This will be discussed further.

In view of the data already discussed, it has been established that pactamycin has been converted into pactamycate by loss of the elements of dimethylamine with incorporation of a carbonyl group into a 2-oxazolidone system. Such a transformation would be consistent with the attachment of the dimethylamine group to a carbonyl, and such a linkage is confirmed by the signal at $\delta$ 2.94 in the nmr due to the dimethylamino moiety. However, the presence of an N,N-dimethylamide should give rise to a doublet while the signal at $\delta$ 2.94 is a singlet. It would seem more probable that the dimethylamino group was part of a urea system as the nmr spectrum of tetramethylurea has a singlet at $\delta$ 2.85 representing all the methyl groups. Such a supposition was confirmed by hydrolysis of pactamycin with either strong acid or strong base to form carbon dioxide. The urea system must be attached at C-1 since attachment at C-2 would require a molecule which could be readily oxidized with periodate to form acetaldehyde. Such reaction does not occur. Furthermore, urea attachment at C-2 would not give an oxazolidone. The nmr signal due to hydrogen adjacent to methyl (C-8) has moved back upfield from its position in pactamycate. Therefore the hydroxyl on the same carbon atom must be free in pactamycin and part of the oxazolidone ring in pactamycate. In such case the 6-methylsalicoyl group can only be attached at the oxygen which becomes a primary carbinol in desalipactamycate.

The two single hydrogen atoms (H-2 and H-3) which show coupling in the nmr spectrum of pactamycate and fail to show such coupling in desalipactamycate again are not coupled in pactamycin. Pactamycin shows only slow and nonspecific oxidation with periodate although two tertiary hydroxyl groups are present. These rather unusual phenomena can be explained on the basis of stereochemistry. The large $J_{H\text{-}2,H\text{-}3}$ shown in pactamycate is usually taken as an indication that such hydrogen atoms are *cis*. However, in pactamycin the coupling constant is 0 Hz indicating a dihedral angle of about 90°, and the maximum angle possible with *cis* hydrogen atoms would be about 45°.[6,7] However, a *trans* arrangement of H-2–H-3 could give the coupling constant observed if the dihedral angle were large enough (about 160°), and such coupling constants have been observed in very rigid systems.[8] When the five-membered carbocyclic ring is part of a less rigid system, conformational change could result in an H-2–H-3 dihedral angle giving rise to H-2 and H-3 signals which exhibit no vicinal coupling. In view of the usually accepted mechanism for periodate oxidation involving a cyclic ester,[9] it appears that *trans* hydroxyl groups would be oxidized with difficulty. Such a view is supported by experiment.[10] Furthermore, it has been shown that in some *trans* five-membered-ring diols oxidation is very slow, and, in certain cases involving extremely rigid bicyclic systems, oxidation does not occur.[11] In the case of pactamycin, periodate oxidation is slow and nonspecific. If the *vic*-diol system were *trans*, the slow periodate oxidation of such a system could allow oxidation at other sites resulting in the mixture of products obtained with pactamycin and the slow rate of reaction. In pactamycate the great rigidity of the spirobicyclic system and its conformation could result in a *trans* system in which two adjacent hydroxyl groups are so far apart that oxidation is precluded. Desalipactamycate is readily oxidized, but, when the primary hydroxyl is no longer present (IIIb,

(5) (a) These data were provided by Mr. Paul Meulman of The Upjohn Co. (b) J. E. Herweh, *J. Heterocycl. Chem.*, **5**, 687 (1968).

(6) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience Publishers, a Division of John Wiley & Sons, Inc., New York, N. Y., 1965, p 203.

(7) L. M. Jackman, "Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, New York, N. Y., p 87.

(8) (a) K. L. Rinehart, Jr., W. S. Chilton, and M. Hichens, *J. Amer. Chem. Soc.*, **84**, 3216 (1962); (b) H. W. Heine, R. Peary, and A. J. Dubetaki, *J. Org. Chem.*, **31**, 3924 (1966); (c) M. E. Munk, C. S. Sodano, R. L. McLean, and L. H. Haskil, *J. Amer. Chem. Soc.*, **89**, 4158 (1967); (d) B. K. Tidd, *J. Chem. Soc., C*, 218 (1967).

(9) R. Breslow, "Organic Reaction Mechanisms," W. A. Benjamin, Inc., New York, N. Y., 1966, p 198.

(10) R. Criegee, E. Buchner, and W. Walther, *Chem. Ber.*, **73**, 571 (1940).

(11) H. H. Wasserman in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley & Sons, Inc., New York, N. Y., 1956, p 385.

IIIc, and IIId), no oxidation occurs. This can happen because with structure IIIa oxidation first leads to a ketone which could then be oxidized further quite readily as the *trans*-diol system would have been destroyed. In those cases (IIIb, IIIc, and IIId) in which the only periodate oxidizable system present is a *trans*-diol, the situation would be very similar to that in pactamycate.

These considerations lead to the assignment I for the structure of pactamycin with the proviso that the stereochemistry of C-4 and C-5 relative to the other carbocyclic carbon atoms is not known, and the absolute stereochemistry is unknown.

## Experimental Section[12]

**Acetone Complex with Pactamycin.**—A sample of pactamycin was prepared by the Florisil chromatography procedure of Argoudelis, Jahnke, and Fox.[2] The nmr spectrum of the Florisil sample, which had been exposed to acetone, was run in $d_6$-acetone. The spectrum was identical with that of pactamycin except for the presence of two new signals, each representing three protons, at $\delta$ 2.00 and 2.26. In the same system acetone gave a signal at $\delta$ 2.17.

A solution of 1 g of the acetone complex in 25 ml of 95% ethanol was allowed to stand at room temperature for 4 days. The ethanol was removed by evaporation, and an nmr was run on the residue. The peaks at $\delta$ 2.00 and 2.26 were absent.

**Rotation of Pactamycin in Various Solvents.**—The rotation of pactamycin was run in acetone, 95% ethanol, and chloroform each at a concentration of 1%. The rotations were run immediately and after 24 hr (Table IV).

TABLE IV

| Solvent | $[\alpha]_D$ at 0 hr, degree | $[\alpha]_D$ at 24 hr, degree |
|---|---|---|
| Acetone | 25 | 76.1 |
| 95% ethanol | 22 | 17.8 |
| Chloroform | 36.5 | 41.1 |

**Pactamycate (II).**—Concentrated hydrochloric acid (2 ml) and 10 ml of water were added to a solution of 1 g of pactamycin in 1 ml of absolute ethanol. The solution was heated on the steam bath for 2 hr. A precipitate which formed during heating was dissolved by adding methanol. The clear solution was adjusted to pH 8.0 with sodium hydroxide solution. After a short period of standing, a light yellow precipitate formed. The precipitate was removed by filtration and recrystallized from ethanol. The yield of crystalline pactamycate, mp 207–210°, was 425 mg: $[\alpha]_D$ +26° (*c* 0.84, DMF); $\lambda_{max}^{EtOH}$ 239.5 mμ (ε 26,800), 313 (2016), and 356 (1744); $\lambda_{sh}^{EtOH}$ 264 mμ (ε 7870); $\nu_{max}$ 3390, 1739, 1660, 1635, 1595, 1320, 1265, 1250, 1205, 1165, 1125, 1105, 1068, 1040, 980, 948, 884, 805, 775, 728, 704, and 683 cm$^{-1}$; p$K_a$ = 6.00 and 8.83.

*Anal.* Calcd for $C_{26}H_{31}N_3O_8$ (dried at 150°): C, 60.81; H, 6.09; N, 8.18. Found: C, 60.72; H, 5.83; N, 8.16.

**Dimethylamine from Pactamycin.**—One gram of pactamycin was dissolved in 5 ml of methanol, and 20 ml of 10% sodium hydroxide was added. The solution was heated on a steam bath in a stream of nitrogen which was bubbled through 50 ml of 0.1 *N* hydrochloric acid. The acidic solution was evaporated to dryness, 1.03 g. The residue was dissolved in 2 ml of ethanol, and 0.7 ml of phenyl isothiocyanate and 1.5 ml of 1 *N* sodium hydroxide were added. After the mixture had stood for 10 min, water was added, and the mixture was extracted with ethyl acetate. The organic phase was evaporated to dryness, and the crystalline residue was recrystallized from ethanol, mp 132–134° (lit. mp 135° for N-phenyl-N′,N′-dimethylurea). The infrared spectrum was identical with that of an authentic sample.

**Diacetylpactamycate.**—One gram of pactamycate was dissolved in 30 ml of acetic anhydride and 60 ml of pyridine. After the solution had stood at room temperature for 24 hr, it was evaporated to dryness under reduced pressure. The residue was dissolved in 15 ml of absolute ethanol at 70°, and water was added until the solution was cloudy. Slow cooling resulted in a precipitate which was collected and dried, yield 950 mg. Recrystallization in the same way gave a 90% recovery: mp 173–176°; $[\alpha]_D$ +31° (*c* 0.97, 75% EtOH); $\lambda_{max}^{EtOH}$ 240 mμ (ε 25,900) and 359 (1900); $\lambda_{sh}^{EtOH}$ 265 mμ; $\nu_{max}$ 3540, 3400, 3310, 1755, 1710, 1675, 1605, 1590, 1520, 1295, 1275, 1235, 1200, 1115, 1100, and 1070 cm$^{-1}$. Potentiometric titration showed the absence of titratable groups.

*Anal.* Calcd for $C_{30}H_{35}N_3O_8$: C, 60.35; H, 6.90; N, 7.03; O, 26.78; acetyl (2), 14.4; mol wt, 597.6. Found: C, 60.42; H, 6.58; N, 6.95; O, 27.27; acetyl, 15.38; mol wt (mass spectrum), 597.

**Triacetyldihydropactamycate.**—A solution of 450 mg of pactamycate in 150 ml of absolute methanol was mixed with a solution of 200 mg of sodium borohydride in 10 ml of water and 5 ml of saturated sodium bicarbonate solution. After the reaction mixture had stood at room temperature for 3 hr, it was adjusted to pH 1.5 with hydrochloric acid and evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of methanol and hydrochloric acid and again evaporated to dryness under reduced pressure followed by three repetitions. The final residue was dissolved in a mixture of 20 ml of acetic anhydride and 40 ml of pyridine, and the solution was allowed to stand at room temperature overnight. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was partitioned between water and chloroform. The chloroform solution was concentrated to a small volume, and the solute was precipitated by the addition of Skellysolve B. The precipitate was collected and dried, yield 320 mg. The product was chromatographed on 20 g of alumina pretreated with ethyl acetate and packed in Skellysolve B. The material was added in the minimum amount of chloroform, and the column was developed using 10 ml of each of the following solvent systems: benzene–Skellysolve B (4:6), benzene–Skellysolve B (8:2), benzene, benzene–ethyl acetate (8:2), benzene–ethyl acetate (6:4), benzene–ethyl acetate (4:6), benzene–ethyl acetate (2:8) and 30 ml of ethyl acetate. Twelve 10-ml fractions were collected. On the basis of a weight analysis, the last four fractions were combined and evaporated to dryness under reduced pressure. The residue was crystallized from a mixture of chloroform and Skellysolve B: yield 200 mg; mp 148–153°; $\lambda_{max}^{EtOH}$ 250 mμ (ε 14,750) and 300 (1920); $\nu_{max}$ 3370, 1740, 1655, 1605, 1590, 1525, 1245, 1200, 1100, 1065, and 1025 cm$^{-1}$. Potentiometric titration showed the absence of titratable groups.

*Anal.* Calcd for $C_{32}H_{39}N_3O_{11}$: C, 59.90; H, 6.12; N, 6.54; O, 27.43; acetyl (3), 20.1. Found: C, 59.03; H, 6.29; N, 6.74; O, 26.96; acetyl, 17.9.

**Desalipactamycate (IIIa). A. From Pactamycin.**—A solution of 25 g of pactamycin in 500 ml of saturated barium hydroxide was heated on the steam bath under nitrogen for 1 hr. The reaction mixture was refrigerated and filtered, and the insoluble material was washed with 25 ml of cold water. The $Ba^{2+}$ was precipitated by the addition of 1 *N* sulfuric acid. The precipitate was removed by centrifugation and washed in the same way. The combined supernatant and washings were washed with two 200-ml portions of ether, and the aqueous solution was freeze dried. The yield of crude material was 11.2 g. The product was chromatographed on 525 g of silica gel packed in ethylene dichloride–methanol (8:2) and washed with 500 ml of the same solvent mixture and then with 500 ml of ethylene dichloride. The sample was added in ethylene dichloride–methanol (9:1) and eluted with 600 ml of the same solvent system and then with ethylene dichloride–methanol (8:2) until a total of 125 20-ml fractions had been collected. On the basis of an ultraviolet spectrum analysis, fractions 70–120 were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 100 ml of 50% *t*-butyl alcohol, and the solution was freeze dried, yield 1.3 g.

Five grams of material prepared as above was purified by countercurrent distribution in a cyclohexane–*n*-butyl alcohol–water (1:9:10) system for 450 transfers. Tubes 250–320 were combined and concentrated under reduced pressure to an aqueous residue which was freeze dried: yield 3.76 g; mp 125–145° $[\alpha]^{25}_D$ −14° (*c* 0.64, $H_2O$); $\lambda_{max}^{H_2O}$ 238 mμ (ε 26,550) and 350 (1700); $\lambda_{sh}^{H_2O}$ 264 mμ; $\nu_{max}$ 3300, 1705, 1680, 1600, 1583, 1510, and 1100 cm$^{-1}$. Potentiometric titration showed the absence of titratable groups.

(12) Infrared spectra were run as Nujol mulls on a Perkin-Elmer 421 instrument. Nmr spectra were taken on a Varian A-60A instrument. The melting points are corrected.

*Anal.* Calcd for $C_{18}H_{25}N_3O_6$: C, 56.98; H, 6.65; N, 11.08; mol wt, 379.1743. Found: C, 57.06; H, 6.93; N, 10.45; mol wt (mass spectrum), 379.1744.

**B. From Pactamycate.**—A solution of 203 mg of pactamycate in a mixture of 1 ml of ethanol and 4 ml of 10% sodium hydroxide was heated on a steam bath under nitrogen for 2 hr. The solution was adjusted to pH 2 with hydrochloric acid and extracted with ether. The aqueous phase was evaporated to dryness under reduced pressure. The residue was extracted with 500 ml of hot absolute ethanol. Evaporation of the ethanol gave 382 mg of residue which was extracted with pyridine. The pyridine extract was evaporated to give 190 mg of crude desalipactamycate identified by paper chromatography and infrared spectrum.

**6-Methylsalicyclic Acid from Pactamycate.**—The ether extract from the above experiment was evaporated to dryness, yield 61 mg. The product was purified by sublimation at 130° under reduced pressure, mp 164–168° (lit.[3] mp 168°). The melting point and infrared spectrum established that the product was 6-methylsalicyclic acid. A sample was converted into its methyl ether by the procedure of Anslow and Raistrick.[13] The melting point was 137–138° (lit.[13] mp 139°).

**Diacetyldesalipactamycate (IIIe).**—Desalipactamycate (500 mg) was dissolved in 15 ml of dry pyridine, and 5 ml of acetic anhydride was added. The solution was allowed to stand at room temperature overnight, diluted with 5 ml of methanol, and allowed to stand 0.5 hr. The solution was concentrated under reduced pressure to a volume of about 3 ml, and 20 ml of water was added. The mixture was extracted with three 10-ml portions of ethyl acetate. The combined extracts were dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in water, acidified with 1 *N* hydrochloric acid, and again extracted in the same way with ethyl acetate. The combined extracts were dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure. The residue was crystallized from ethanol: yield 190 mg; mp 147–151°; $[\alpha]^{25}D$ −7° (*c* 0.37, ethyl alcohol); $\lambda_{max}^{EtHO}$ 239 mμ (ε 26,350) and 354 (1850); $\lambda_{sh}^{EtOH}$ 260 mμ (ε 9050); $\nu_{max}$ 3400, 1755, 1745, 1705, 1685, 1600, 1580, 1510, 1265, 1240, 1115, 1095, 1075, 1055, 1050, and 1030 $cm^{-1}$.

*Anal.* Calcd for $C_{22}H_{29}N_3O_8$: C, 57.03; H, 6.32; N, 9.07; acetyl (2), 18.93; mol wt, 463.1955. Found: C, 57.02; H 6.90; N, 8.87; acetyl, 16.77; mol wt (mass spectrum), 463.1957.

**Performic Acid Oxidation of Desalipactamycate.**—One gram of desalipactamycate was added slowly with stirring to a mixture of 6 ml of 97% formic acid and 3 ml of 30% hydrogen peroxide. After the reaction mixture had cooled, it was diluted with 9 ml of water. Filtration gave 33 mg of product A, mp 124–126°. The filtrate was cooled in an ice bath and kept below 40° while the pH was adjusted to 6 with cold 50% sodium hydroxide. The crystalline precipitate was cooled and dried, yield 165 mg, mp 62–69°, designated product B. Repeated recrystallization of both products from ethy alcohol gave A melting at 136–137° and B melting at 73–76°. Product A had an infrared spectrum identical with that of *m,m*-diacetoazoxybenzene,[14] and the mixture melting point showed no depression. Product B had an infrared spectrum identical with that of *m*-nitroacetophenone, and the mixture melting point showed no depression.

**Carbon Dioxide from Acid Hydrolysis of Pactamycin.**—A solution of 2 g of pactamycin in 50 ml of 12 *N* sulfuric acid was refluxed for 6 hr while the gases from the reaction were led into 50 ml of saturated barium hydroxide solution. The precipitate was collected, washed thoroughly with water, and dried under reduced pressure at 130°. The yield was 508 mg, 78%. The product was identified as barium carbonate by its infrared spectrum.

**Carbon Dioxide from Pactamycin, Pactamycate, and Desalipactamycate from Base Hydrolysis.**—All three of the compounds were run in the same fashion. Starting material (0.5 g) dissolved in 10 ml of 5 *N* sodium hydroxide was refluxed for 48 hr. Nitrogen, which had been passed through barium hydroxide solution, was used to sweep out the reaction mixture while 10 ml of 6 *N* hydrochloric acid was added and then for 4 hr afterward. The nitrogen was bubbled through 100 ml of saturated barium hydroxide solution. The precipitate was isolated by centrifugation, washed thoroughly in the same way, and dried under reduced pressure at 140°. In each case the product was identified as barium carbonate by its infrared spectrum. The yields follow: pactamycin, 90 mg (52%); pactamycate, 78 mg (41%); and desalipactamycate, 74 mg (38%).

**Periodate Oxidation of Pactamycin (I).**—Pactamycin was titrated by the Fleury–Lange procedure[15] using a solution of 280 mg in 100 ml of a 1:1 mixture of dioxane and 0.1 *M* sodium periodate and titrating with 10-ml aliquots. The consumption of periodate in hours (moles) follows: 0 (0.2), 0.15 (0.3), 1 (0.6), 4 (0.6), and 8 (0.9).

No acetaldehyde was detected by the procedure reported in Dyer.[15]

**Periodate Oxidation of Desalipactamycate (IIIa). A. Titration.**—Desalipactamycate was titrated by the Fleury–Lange procedure[15] using a solution of 80 mg in 40 ml of 0.05 *M* sodium periodate and titrating 4-ml aliquots. The consumption of periodate in hours (moles) follows: 0 (0.75), 0.5 (2.0), 1 (2.25), 2 (2.62), 4 (3.0), 6 (3.0), and 10 (3.62). Titration with periodic acid in the same way gave very similar results except that 2 mol of periodate was consumed in less than 10 min.

**B. Determination of Formaldehyde.**—A solution of 400 mg of desalipactamycate in 100 ml of 0.05 *M* sodium periodate was allowed to stand for 4 hr. The solution was adjusted to pH 7 with 0.1 *N* sodium hydroxide and 150 ml of 0.05 *M* sodium arsenite was added. One-half of the solution was adjusted to pH 5.5 with 2 *N* acetic acid. The solution was mixed and 170 ml of 1:1 2 *N* sodium acetate and 1 *N* hydrochloric acid and 175 ml of 0.4% dimedon solution were added. After the solution had stood for 3 days, 71 mg of precipitate was collected. After two recrystallizations from ethyl alcohol, the product melted at 187–192°. The infrared spectrum and mixture melting point identified the product as the dimedon derivative of formaldehyde.

A periodate oxidation in the same way followed by the chromotropic acid procedure for determining formaldehyde[16] indicated 0.653 mol of formaldehyde/mol of desalipactamycate.

**C. Determination of Acetaldehyde.**—The other half of the solution from B was adjusted to pH 5.5 with 2 *N* acetic acid, and nitrogen was bubbled through it and then through 200 ml of 2,4-dinitrophenylhydrazine solution. After 4 hr no precipitate was obtained.

**D. Electrometric Titration.**—A solution of 380 mg of desalipactamycate in 100 ml of 0.05 *M* sodium periodate was allowed to stand at room temperature in the dark for 24 hr. Ethylene glycol (0.4 ml) was added. After 1 hr the mixture was titrated with 0.1 *N* sodium hydroxide. The first end point required 7.60 ml of sodium hydroxide indicating that a carboxyl group had been formed by the oxidation.

**E. Determination of Volatile Acid.**—The procedure reported by Dyer[15] was used on 190 mg of desalipactamycate. Only a trace (0.03 mmol) of volatile acid was detected.

**Periodate Oxidation of Pactamycate, Diacetylpactamycate, Desalipactamycate *p*-Nitrobenzenesulfonate, and 6-Deoxy-6-iododesalipactamycate.**—These were all run by the Fleury–Lange procedure.[15] No oxidation was detected after 4 hr.

**Registry No.**—I, 23668-11-3; II, 23754-55-4; IIIa, 23668-12-4; IIIb, 23754-56-5; IIId, 23668-13-5; IIIe, 23668-14-6.

**Acknowledgment.**—The authors would like to express their appreciation for the invaluable assistance of Dr. Kenneth L. Rinehart, Jr., on this problem. We also thank Dr. Marvin F. Grostic for assistance in obtaining and interpreting mass spectra.

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