The Chemistry of Cephalosporins. XII. Configuration of the Carboxyl Group in Δ^2-Cephalosporins

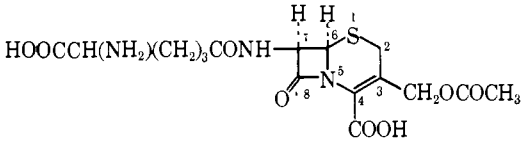
E. VAN HEYNINGEN AND LINDA KAY AHERN

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206

Received May 8, 1968

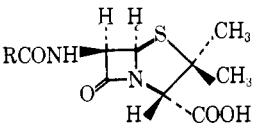
Although Δ^3-cephalosporins are potent antibiotics, Δ^2-cephalosporins possess little or no microbiological activity. The work herein described establishes that the 4-carboxyl in Δ^2-cephalosporins has the same absolute configuration as the 3-carboxyl in penicillins. Consequently, the lack of antimicrobial activity is ascribed to stability of the β-lactam ring in Δ^2-cephalosporins rather than a disadvantageous configuration of the carboxyl group.

Abraham and Newton[1] showed that cephalosporin C (I), the cephalosporin produced naturally by *Cephalosporium acremonium*, contained a dihydrothiazine ring in which the double bond was in the Δ^3 position. Since that time several groups of investigators have prepared



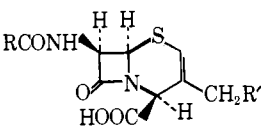
I

cephalosporin derivatives in which the double bond has been in the Δ^2 position.[2] Whereas many of the analogous Δ^3-cephalosporanic acids possessed good antimicrobial activity, the isomeric Δ^2-cephalosporins were almost completely inactive. Although seemingly trivial changes in molecular structure often do cause dramatic changes in biological activity, such results are only infrequently predictable. The inactivity of Δ^2-cephalosporins, because of their very close similarity to Δ^3-cephalosporins, was unanticipated and surprising. Once known, however, this inactivity requires explanation. Cooper[3] and Collins and Richmond[4] postulated that β-lactam antibiotics inhibit cell wall synthesis because they combine irreversibly with and thus inactivate an enzyme crucial to the synthesis. The covalent bond is formed by β-lactam acylation of the active site. Cocker and coworkers have observed that Δ^2-cephalosporins have a β-lactam ring that is much more stable toward basic hydrolysis than the β-lactam ring in Δ^3-cephalosporins. Morin[5] has correlated β-lactam stability with both antibiotic activity and β-lactam absorption in the infrared: in β-lactam antibiotics the more labile the β-lactam ring the lower the wavelength of β-lactam absorption and in general the greater the biological activity. Δ^2-Cephalosporin esters have β-lactam absorptions at high wavelength compared to Δ^3-cephalosporin analogs. To conclude that Δ^2-cephalosporin inactivity is attributable to an unreactive β-lactam is therefore eminently reasonable. There does exist, however, another possible factor that must also be considered. Penicillins contain a D-valine residue as proved by X-ray crystallographic analysis[6] and by degradation.[7] The molecular configuration of a penicillin is designated as shown in II where the protons on the β-lactam ring and the carboxyl all lie below the plane of the penam ring system which is concave with respect to the plane of the paper.

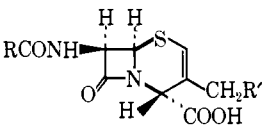


II

X-Ray analyses of a penicillin[6] and a cephalosporin,[8] as well as the conversion of a penicillin into a cephalosporin through a process in which the β-lactam ring remained intact,[9] proved that the β-lactam rings in both antibiotics are stereochemically identical. Cocker and coworkers[2d] deduced from nmr data that the carboxyl group in Δ^2-cephalosporins was in an axial position, but flexibility of the dihydrothiazine ring permits an axial configuration for either an α- or β-carboxyl so nmr analysis does not provide conclusive assignment of carboxyl configuration. Consequently, Δ^2-cephalosporins may have an α-carboxyl as in III, opposite to that in penicillin, or a β-carboxyl as in IV, with the same configuration as in penicillin II.



III



IV

If one were to assume that proper carboxyl configuration were crucial for antibiotic activity, then, almost certainly, the β configuration as in natural penicillins would provide the active form. The inactivity of Δ^2-cephalosporins may possibly be due, then, to the configuration of the carboxyl if it is in the α configuration. If, however, Δ^2-cephalosporins have a β-carboxyl, then their inactivity is probably not due to "wrong" carboxyl configuration because molecular models show that a Δ^2-cephalosporin with β-carboxyl is more nearly super-

(1) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).

(2) (a) R. R. Chauvette and E. H. Flynn, *J. Med. Chem.*, **9**, 741 (1966); (b) A. B. Taylor, *J. Chem. Soc.*, 7020 (1965); (c) R. A. Archer and B. S. Kitchell, *J. Org. Chem.*, **31**, 3409 (1966); (d) J. D. Cocker, S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, *J. Chem. Soc., C*, 1142 (1966).

(3) P. D. Cooper, *Bacteriol. Rev.*, **20**, 28 (1956).

(4) J. F. Collins and M. H. Richmond, *Nature*, **195**, 142 (1962).

(5) R. B. Morin (as quoted by E. Van Heyningen), *Advan. Drug Res.*, **4**, 48 (1967).

(6) D. Crowfoot, C. W. Bunn, B. W. Rogers-Low, and A. Turner-Jones in "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., Princeton University Press, Princeton, N. J., 1949, p 310.

(7) E. Kaczka and K. Folkers, ref 6, p 243.

(8) D. C. Hodgkin and E. N. Maslen, *Biochem. J.*, **79**, 303 (1961).

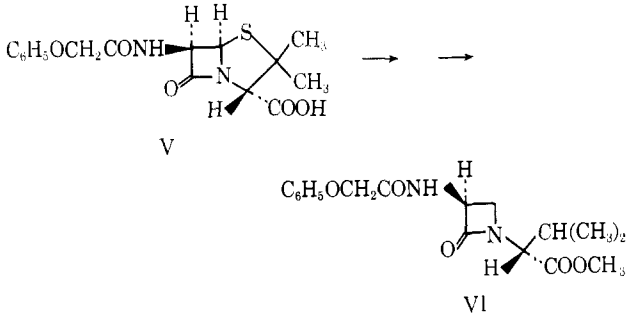
(9) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Am. Chem. Soc.*, **85**, 1897 (1963).

imposable upon a penicillin model than is a Δ^3-cephalosporin.

We considered it worthwhile to determine carboxyl configuration in a Δ^2-cephalosporin not only because it might help explain Δ^2-cephalosporin inactivity but also because we considered that, should the configuration be α, there still existed the possibility that inversion to the β configuration might provide biological activity.

Our plan was to desulfurize phenoxymethylpenicillin V and obtain, after esterification, phenoxymethyldesthiopenicillin methyl ester VI. Similarly we hoped to hydrogenate 7-phenoxyacetamido-Δ^2-desacetoxycephalosporanic acid to a tetrahydrothiazine and desulfurize it to VII, which may be either phenoxymethyldesthiopenicillin or an isomer. If VII proved to be identical with desthiopenicillin VI, obtained from the penicillin, we would have established that the carboxyls in penicillins and Δ^2-cephalosporins possess the same absolute configurations.

Desulfurization of potassium phenoxymethylpenicillin produced a crude desthiopenicillin that showed one major and several comparatively minor spots in a thin layer chromatogram (tlc). Diazomethane converted the acid into a mixture of compounds from which the major component, desthiopenicillin methyl ester (VI), was separated by column chromatography. None of the above processes should have affected the optical centers of the product.

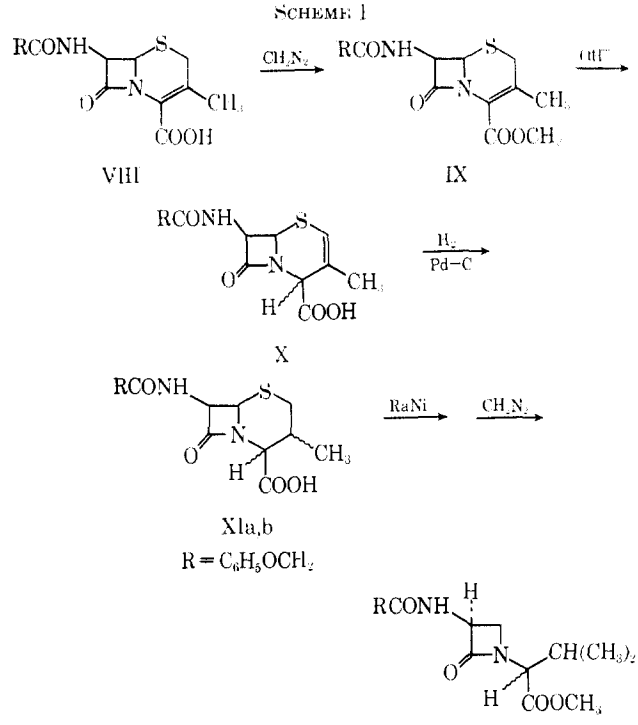


V

VI

We then proceeded to convert 7-phenoxyacetamidodesacetoxycephalosporanic acid into a desthiopenicillin or an isomer. We chose to start with a desacetoxycephalosporin rather than the natural 3-acetoxymethyl analog in order to avoid possible acetoxyl containing impurities even though it is known that the acetoxyl is removable by hydrogenolysis.[10,11] 7-Aminodesacetoxycephalosporanic acid, prepared by the method of Stedman, *et al.*,[11] was acylated with phenoxyacetyl chloride and the acid VIII so produced was converted into its methyl ester (IX) (Scheme I). Saponification in aqueous pyridine isomerized the methyl ester to its Δ^2 isomer which was preferentially saponified to 7-phenoxyacetamido-Δ^2-desacetoxycephalosporanic acid (X). Only one isomer resulted from this treatment. Hydrogenation reduced the Δ^2 double bond to yield a mixture containing dihydrocephalosporins XIa and b. Tlc of the mixture revealed the presence of a major and two minor components. Analysis of the nmr spectrum of this mixture indicated that one of the minor components was unreduced X and that the reduced

SCHEME I



VII

products, XIa and XIb, retained the same configuration at C-4, but were isomeric at C-3.

A Raney nickel desulfurization of the isomer mixture, XIa and b, yielded a crude product which, when treated with diazomethane, gave a methyl ester (VII). Tlc showed one very predominant spot accompanied with faint spots of trace contaminants, substantiating the conclusion that XIa and XIb differed only at C-3 which becomes a symmetrical isopropyl group in the desulfurized product. The methyl ester VII was purified by column chromatography.

The methyl phenoxymethyldesthiopenicillinates VI and VII possess identical nmr, ir, and uv spectra. Their X-ray powder diagrams coincide exactly. Although VI melted at 110° and VII at 112°, their mixture melting point was not depressed. Samples VI may have had trace contaminants. In order to be certain that our assignment was correct, we subjected VII to acid hydrolysis and isolated the valine so obtained. Although its rotation was low compared to pure D-valine, the rotation was strongly negative like D-valine. The hydrolysis may have caused some racemization. Tlc showed only one spot for valine. Consequently, the assignment above must be correct.

We can only conclude, therefore, that it is not the 4-carboxyl configuration but β-lactam stability that explains Δ^2-cephalosporin biological inactivity.

Experimental Section[12]

Methyl 6-Phenoxymethyldesthiopenicilloate (VI).—The desulfurization method described for benzyl penicillin was applied to phenoxymethylpenicillin.[13] Phenoxymethylpenicillin potassium salt (3 g, 7.74 mmoles) was dissolved in H_2O (250 ml) and approximately 18 g of Raney Ni (stored under H_2O) was added. The mixture was heated to reflux in an oil bath (165°) for 15 min and then quickly cooled. The reaction mixture was care-

(10) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **62**, 658 (1956).

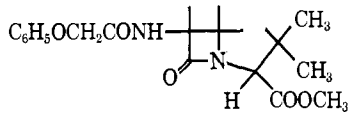
(11) R. J. Stedman, K. Swered, and J. R. E. Hoover, *J. Med. Chem.*, **7**, 117 (1964).

(12) All evaporations employed a rotary evaporator at less than 50°.

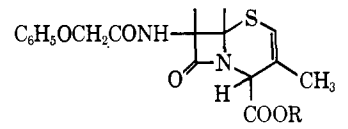
(13) E. Kaczka and K. Folkers, ref 6, p 256.

TABLE I

NMR SPECTRA[a-c]



No.	NH, C_6H_5–H	OCH_2–H	6-H	5-H	2-H	3-H	2-CH_3	OCH_3
VI	409–455 m	269 s	295 m	237 t (5.5) 211 q (3.0)	120–160 h[d]	255 d (7.5)	61 d (6.5) 59 d (6.5)	223 s
VII	407–462 m	269 s	294 m	237 t (5.5) 212 q (3.0)	120–160 h[d]	255 d (7.5)	62 d (6.5) 60 d (6.5)	233 s



No.	NH	C_6H_5–H	OCH_2	7-H	6-H	2-H	3-H	3-CH_3	4-H	R (OCH_3 or H)
IX[e]	457 d (9.5)	406–450 m	271.5 s	349 q (5.0)	297.5 d (5.0)	200 q (18)	...	127 s	...	228 s
X[f]	477 d (9.0)	410–453 m	274 s	339 q (4.0)	319.0 d (4.0)	357 m	...	117 d (1.0)	282 m	...
XIa[f]	451 d (9.5)	405–450 m	272 s	339 q (4.0)	322.5 d (4.0)	295 d (12)	137–160 m	64 d (7.0)	272 d (6.0)	...
XIb[g]	*h*	405–451 m	275 s	338 d (4.2)	314.5 d (4.2)	*h*	*h*	76 d (7.0)	261 d (2.0)	...

[a] $DCCl_3$ was solvent. The instrument was a HA-60. [b] The desthiopenicillin is numbered as if it were a penicillin. [c] Chemical shifts in cps; coupling constants in parentheses. [d] Heptuplet. [e] Δ^3-Cephalosporin. [f] $CDCl_3$ + 0.02 ml of DMSO-d_6. [g] $CDCl_3$–D_2O. [h] The multiplets do not permit assignment.

fully filtered through a Büchner funnel coated with talc and the catalyst was washed four times with 20-ml portions of H_2O. The catalyst was then suspended in 500 ml of 0.05 *M* NaOH solution and stirred for 0.5 hr and again filtered from the solution. The aqueous filtrates were layered with EtOAc and acidified to pH 2 with 1 *N* HCl, and the organic layers were separated and dried (Na_2SO_4). Evaporation of the combined EtOAc solutions *in vacuo* left a colorless oil that weighed 2.18 g, an 88% yield of crude desthiopenicillin. The product did not crystallize readily but tlc (silica; Et_2O–AcOH–H_2O, 18:3:1) showed one major and one minor spot.

The oil, dissolved in EtOAc (50 ml), was chilled in an ice bath and CH_2N_2 in CH_2Cl_2 was added portionwise until the yellow color persisted. After the solution had stirred in the cold for 15 min, glacial AcOH was added, dropwise, until the yellow color of excess CH_2N_2 disappeared. The oily residue obtained after evaporation of the solvents *in vacuo* crystallized on standing. Tlc (silica; EtOAc) separated the material into one major spot and several very minor spots. The product was therefore chromatographed over 70 g of silica. The eluting solvent progressed from C_6H_6 to 30% EtOAc–C_6H_6. The residue after evaporation of solvents, which showed only one spot in tlc, was recrystallized from benzene–petroleum ether (bp 60–65°), 250 mg, mp 108°. *Anal.* Calcd for $C_{17}H_{22}N_2O_5$: C, 61.06; H, 6.63; N, 8.38. Found: C, 60.79; H, 6.75; N, 8.24.

The product was recrystallized three times from benzene–petroleum ether and the melting point was raised to 109–110°. The chemical shift values for the protons in the nmr spectrum of VI are in Table I.

7-Phenoxyacetamido-3-methyl-3-cephem-4-carboxylic Acid (VIII).—A solution of 21.4 g (0.1 mole) of 7-aminodesacetoxycephalosporanic acid[11] in a mixture of 800 ml of H_2O and 600 ml of acetone containing 28 g (0.332 mole) of $NaHCO_3$ was cooled in an ice–alcohol bath and treated by dropwise addition with 17 g (0.1 mole) of phenoxyacetyl chloride in 200 ml of acetone. After 3 hr in the cold, the solution was evaporated *in vacuo* to remove acetone, layered with EtOAc, and acidified to pH 2.7 with concentrated HCl. The EtOAc layer was washed (H_2O), dried ($MgSO_4$), and evaporated *in vacuo*. The crystalline residue was recrystallized from Me_2CO–Et_2O–C_6H_6, yield 25 g (74%), mp 187–188° dec. Another sample made similarly had the same melting point. *Anal.* Calcd for $C_{16}H_{16}N_2O_5S$: C, 55.17; H, 4.63; N, 8.04. Found: C, 55.16; H, 4.77; N, 8.01.

Methyl 7-Phenoxyacetamido-3-methyl-3-cephem-4-carboxylate (IX).—7-Phenoxyacetamido-3-methyl-3-cephem-4-carboxylic acid (12 g, 0.035 mole) suspended in 300 ml of EtOAc and cooled in an ice bath was treated with CH_2N_2 in CH_2Cl_2 by dropwise addition until the acid dissolved and the solution became yellow. After 1 hr in the cold the reaction mixture was allowed to warm to room temperature. Excess CH_2N_2 was decomposed by careful addition of glacial AcOH. The residue that remained after vacuum evaporation of solvents was dissolved in C_6H_6, washed (5% $NaHCO_3$ solution, H_2O), and after drying ($MgSO_4$), recovered by evaporation. The product crystallized in an 8.85 g yield (71%), mp 137–139°. The signals for an nmr spectrum are reported in Table I. *Anal.* Calcd for $C_{17}H_{18}N_2O_5S$: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.39; H, 5.09; N, 7.68.

7-Phenoxyacetamido-3-methyl-2-cephem-4-carboxylic Acid (X).[14]—Methyl 7-phenoxyacetamido-3-methyl-3-cephem-4-carboxylate (2 g, 5.53 mmoles) in 50 ml of H_2O and 50 ml of pyridine was chilled in an ice bath and 5.53 ml of 1 *N* NaOH was added. After 5 hr in the cold the solution was diluted (H_2O), layered with EtOAc, and while still cold acidified to pH 3 with concentrated HCl. The ethyl acetate layer over water was adjusted to pH 8.0 and the water layer was separated, again acidified to pH 2.5, and extracted with ethyl acetate. The organic layer after being washed (H_2O) and dried ($MgSO_4$) was evaporated *in vacuo* to give the Δ^2-acid which crystallized from chloroform–hexane, 1.45 g (75%), mp 167–169°. Its chromatogram on silica (Et_2O–AcOH–H_2O, 15:3:1) showed one spot. The nmr chemical shift values for X are in Table I. *Anal.* Calcd for $C_{16}H_{16}N_2O_5S$: C, 55.17; H, 4.63; N, 8.04. Found: C, 55.28; H, 4.81; N, 8.08.

7-Phenoxyacetamido-3-methylcepham-4-carboxylic Acid (XI).—A solution of 7 g (0.02 mole) of 7-phenoxyacetamido-3-methyl-2-cephem-4-carboxylic acid in 700 ml of EtOH containing 14 g of 5% Pd–C was heated and shaken at 60° for 7 hr under 3 atm of H_2. After filtration to remove the catalyst, the product was isolated by evaporation of the solvent. Tlc (silica; Et_2O–AcOH–H_2O, 15:3:1) showed one major spot and two more mobile, minor spots. Of the two minor spots, the slower had the same R_f value as starting material. The material corresponding to that in the forerunning minor spot crystallized from a warm CH_3CN solution of the product mixture, mp 187–189°, 50 mg (XIa). An nmr spectrum showed this material to be one of the expected tetrahydrothiazine products, isomeric to the main product at C-3. (See Table I.) *Anal.* Calcd for $C_{16}H_{18}N_2O_5S$: C, 54.85; H, 5.18; N, 8.00. Found: C, 54.66; H, 5.21; N, 7.96.

The nmr spectrum of the residue after MeCN had been evaporated (4 g, 58%) showed the presence of a small amount of starting material X, based on vinyl proton and 4-proton and 3-methyl proton integrals. The residue also contained isomer XIa above as shown by its 3-methyl and 4- and 6-proton signals,

(14) We are indebted to Dr. R. B. Morin and Dr. B. G. Jackson for the directions for this experiment.

and calculations based on the integral showed that XIa composed approximately one-third of the mixture. Most of the nmr signals for XIb could be assigned from the spectrum and are in Table I. Because the desulfurization product would be identical from both isomers XIa and XIb, no attempt was made to purify the product further.

Desulfurization of 7-Phenoxyacetamido-3-methylcepham-4-carboxylic Acid to Methyl Phenoxymethyldesthiopenicillinate (VII).—The same procedure used to desulfurize the penicillin was used for the dihydrodesacetoxycephalosporin. Crude 7-phenoxyacetamido-3-methyl-3-cepham-4-carboxylic acid (1 g, 2.86 mmoles) was dissolved in 80 ml of H_2O by adjusting the solution to pH 7 with 1 *N* NaOH. Then 6 g of Raney Ni was added; the mixture was heated for 15 min in an oil bath at 165° and then quickly chilled. After the catalyst was filtered and washed (H_2O), the cooled filtrates were acidified to pH 2.5 below a layer of EtOAc. The EtOAc solution was washed (H_2O), dried ($MgSO_4$), and evaporated to give an oil which in tlc (silica; Et_2O–AcOH–H_2O, 15:3:1) showed three spots.

The catalyst above was extracted with cold 0.05 *N* NaOH (500 ml) from which an additional small amount of oily product could be recovered by acidification and extraction as above. In tlc this fraction showed the same spots as the first fraction.

The two fractions were combined in 15 ml of EtOAc and cooled in an ice bath while excess CH_2N_2 in CH_2Cl_2 was added. After 15 min the yellow color of CH_2N_2 was discharged with a few drops of AcOH and the solvent was removed by evaporation *in vacuo*. The residue was redissolved in EtOAc, washed (5% $NaHCO_3$, H_2O), and dried ($MgSO_4$). After the solvent was evaporated, the oily residue crystallized after standing overnight in the cold. A tlc (SiO_2; EtOAc) of this product compared with the methyl ester of the penicillin desulfurization showed a major spot moving as did that product, and in addition two quite minor, less mobile spots. The product (400 mg) was chromatographed over a column of SiO_2 (16 g), eluting with C_6H_6 containing increasing amounts of EtOAc. The recovered product (80 mg) corresponding to the major tlc spot was recrystallized from C_6H_6–petroleum ether and showed only one spot in tlc: mp 112°. *Anal.* Calcd for $C_{17}H_{22}N_2O_5$: C, 61.06; H, 6.63; N, 8.38. Found: C, 61.06; H, 6.36; N, 8.28.

The above product melted at 112°, that from the penicillin at 109–110°, and the mixture of the two at 109–112°, all observed at the same time in a Mel-Temp melting point apparatus.

The two 6-phenoxymethyldesthiopenicillin methyl esters possessed identical ir and nmr spectra, and X-ray diffraction powder diagrams. The X-ray pattern of the ester from the cephalosporin appeared to be sharper, indicating a more highly crystalline material. This is also supported by the slightly higher melting point of the cephalosporin-derived ester.

Valine from Cephalosporin-Derived Methyl Phenoxymethyldesthiopenicillinate (VII).—An initial experiment[15] designed to produce valine methyl ester from the desthiopenicillin ester VII (500 mg) gave instead an α,β-diaminopropionic acid derivative (205 mg) due to simple β-lactam opening as its water-soluble hydrochloride; an nmr spectrum possessed the requisite signals. A portion (150 mg) of this acid was hydrolyzed in 25 ml of 2 *N* HCl at reflux for 12 hr. After being cooled and extracted with ether to remove neutral, amphoteric, acidic materials, the reaction mixture was evaporated to dryness *in vacuo*. The residue in 2.3 ml of 1 *N* HCl was placed on a Dowex 50W-X4 (acid form) column (0.9 × 30 cm) and the amino acids eluted with 1 *N* HCl. Fractions (1–2 ml) enriched in valine were combined and evaporated to give 78 mg of product. The product was purified by chromatography[16] over Sephadex G-25 (25 g in a 2.5 × 25 cm column) using *n*-BuOH–AcOH–H_2O (62:15:25). Valine was readily separated. No attempt was made to crystallize the product. Its specific rotation in 0.01 *N* HCl was compared with that of pure D-valine at 250 mμ. D-Valine standard showed a specific rotation, $[\alpha]$ −563°, whereas the isolated product had a specific rotation of −245°. Therefore, less than 30% of the material racemized in the process if no account is taken of degree of solvation and experimental errors. The product is predominantly of the D configuration.

Acknowledgments.—We are grateful to R. R. Chauvette and I. G. Wright for their counsel and suggestions, to D. Wnolf and associates for physical measurements, to G. M. Maciak and associates for microanalyses, to M. M. Marsh for the optical rotation measurements, and to H. A. Rose for the X-ray powder diagrams.

(15) E. Kaczka and K. Folkers, *loc. cit.*, p 263.
(16) L. D. Zeleznick, *J. Chromatog.*, **14**, 139 (1964).

Acetyl Migration in Rifampicin[1]

N. MAGGI, A. VIGEVANI, G. G. GALLO, AND C. R. PASQUALUCCI

Research Laboratories, Lepetit S.p.A., Milan, Italy

Received May 23, 1968

Mild alkaline treatment of rifampicin affords 25-desacetylrifampicin and two other rifamycins, identified as 25-desacetyl-23-acetyl and 25-desacetyl-21-acetyl derivatives. From the antibacterial activity data of the two compounds, the presence of the C-21 and C-25 hydroxyls in rifamycins seems to be an essential prerequisite for their antibacterial activity.

Rifampicin (I) is a new semisynthetic rifamycin[2,3] selected from many derivatives of 3-formylrifamycin SV[4] for its high antibacterial activity *in vitro* and *in vivo*.[2,5,6] Clinical experiments on this antibiotic are presently underway and the data available indicate its effectiveness in the systemic diseases induced by gram-positive bacteria and in tubercular infections. Like other rifamycins, rifampicin undergoes desacetylation by alkaline treatment, affording the corresponding desacetyl derivative (II) without substantial loss of antibacterial activity.[7] Under milder alkaline conditions I yields two additional products whose identification and antibacterial activity evaluation was considered of interest for the knowledge of activity–structure relationships in rifamycins. To these two rifamycins structures III and IV were assigned on the basis of physical and chemical data.

By treating I in a pH 8.2 buffered aqueous solution at 90–95° for 5 hr, II–IV were detected by tlc as reaction products besides minor by-products and a fair amount of starting I. The reaction mixture was separated by

(1) Rifamycins LIX [part LVIII: C. R. Pasqualucci, A. Vigevani, P. Radaelli, and N. Maggi, *Farmaco, Ed. Prat.*, submitted for publication].
(2) N. Maggi, R. Pallanza, and P. Sensi, *Antimicrobial Agents Chemotherapy*, 765 (1965).
(3) N. Maggi, C. R. Pasqualucci, R. Ballotta, and P. Sensi, *Chemotherapia*, **11**, 285 (1966).
(4) N. Maggi, G. G. Gallo, and P. Sensi, *Farmaco, Ed. Sci.*, **22**, 316 (1967).
(5) S. Furesz, V. Arioli, and R. Pallanza, *Antimicrobial Agents Chemotherapy*, 770 (1965).
(6) V. Arioli, R. Pallanza, S. Furesz, and G. Carodi, *Arzneimittel-Forsch.*, **17**, 523 (1967).
(7) N. Maggi, V. Vigevani, and R. Pallanza, *Experientia*, **24**, 280 (1968).