Cu(II)- bzw. Ni(II)-Polymyxin-System unterdrückt die metallkatalysierte Peroxid-Abbaureaktion.

Der Abbau des Peptides durch $H_2O_2$ erfolgt offenbar immer dann, wenn ·OH- oder ·OO⁻-Radikale, die bei der metallkatalysierten Zerlegung von $H_2O_2$ primär entstehen[5], mit dem im katalytisch aktiven Komplex gebundenen Peptid direkt weiterreagieren können. Bei der Reaktion zwischen Cu(II)-Polymyxin und $H_2O_2$ ist zu beobachten, dass die Lösung bereits nach etwa 10 min kein unzersetztes $H_2O_2$ mehr enthält.

Die Ausbildung der Cu(II)- und Ni(II)-Komplexe sensibilisiert das Peptid weiterhin auch gegen die Einwirkung von UV-Strahlung: Während wässrige Lösungen von Polymyxin allein gegen 100stündige Einwirkung der Strahlung einer 125-Watt-Hochdruck-Quecksilberdampflampe völlig stabil sind, tritt unter gleichen Bedingungen in Lösungen der Cu(II)- bzw. Ni(II)-Komplexe der in der Figur 2 wiedergegebene Abbau ein. Auch hier ist nicht allein Anwesenheit eines Metallions, sondern wirkliche Ausbildung des Peptidkomplexes für den Eintritt der Spaltungsreaktion notwendig: Bei pH 6, bei dem die Metallkomplexe noch nicht ausgebildet werden, sind auch metallionenhaltige Lösungen von Polymyxin gegen die UV-Bestrahlung völlig stabil.

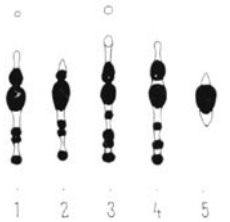



Fig. 2. Radikalische Spaltungen von Polymyxin B (Px): 1, Spaltung von CuPxH durch UV-Strahlung (100 h, 125 Watt). 2, Spaltung von NiPxH durch UV-Strahlung (100 h, 125 Watt). 3, Spaltung von CuPxH durch $H_2O_2$ (12facher molarer Überschuss). 4, Spaltung von NiPxH durch $H_2O_2$ (12facher molarer Überschuss). 5, Unbehandeltes Polymyxin B als Vergleich. Es wurden jeweils 2 $\mu$Mol des Cu- bzw. Ni-Polymyxin-Komplexes, gelöst in 3,5 ml Wasser, zur Reaktion gebracht (pH 8,5 bzw. 9,3). Chromatographie unter Zusatz von Komplexon III mit Butanol-Pyridin-Eisessig-Wasser (50:20:6:24) als Fliessmittel auf Kieselgel S.

Die Abklärung der Aminosäuresequenz der einzelnen Spaltprodukte ist im Gang. Über die Identität der Spaltstellen und über die Natur der in diesen Komplexen vorliegenden Koordinationsbindungen soll demnächst ausführlicher berichtet werden[6].

*Summary.* The decapeptide polymyxine B can be split into sub-units by hydrogen peroxide or by UV-irradiation when complexed with copper(II) or nickel(II); the splitting points are probably related to the points of attachment of the metal ions.

H. ERLENMEYER, H. BRINTZINGER, H. SIGEL und H. C. CURTIUS

*Institut für Anorganische Chemie der Universität Basel und Laboratorium der Universitäts-Kinderklinik, Zürich (Schweiz), 1. April 1965.*

[5] J. H. BAXENDALE, Advances in Catalysis *4*, 31 (1952). - N. URI, Chem. Rev. *50*, 375 (1952).

[6] Für die Überlassung von Substanzen und für wertvolle Ratschläge sind wir den Herren Dr. K. VOGLER und W. LERGIER, Hoffmann-La Roche & Co. AG, Basel, zu Dank verpflichtet. - Dem Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung danken wir für Unterstützung dieser Arbeit.

## Some Additional Observations on the Chemical Nature of Neutramycin

Accurate molecular weights are generally difficult to obtain for neutral macrolide antibiotics. Both members of the class for which complete structures have appeared, chalcomycin (I)[1] and lankamycin (II)[2], illustrate this problem, for in neither case was a reliable molecular formula available until the structure determination was far advanced. Similar difficulties have been encountered with neutramycin[3]. The Rast method failed, isothermal distillation gave widely divergent values (513, 551, 589, 607, 621 and 660), saponification gave nonspecific decomposition, hydrogenation produced mixtures, etc. X-ray analysis gave a value of 686 $\pm$ 35. The X-ray method was less reliable than usual because the crystals were small and the resulting photographs indistinct. These problems have now been overcome by use of direct inlet mass spectroscopy, which gave a value of 686 $\pm$ 0 for neutramycin[4] and therefore established the molecular formula as $C_{34}H_{54}O_{14}$. As was the case with lankamycin[2] microanalyses obtained under normal drying conditions[3] were misleading, and only after a reliable molecular weight became available was it possible to devise sufficiently vigorous drying conditions so that correct microanalytical data were obtained.

*Anal.* Calcd. for $C_{34}H_{54}O_{14}$: C, 59.46; H, 7.93; O, 32.61. Found (dried 3 days at 56°): C, 58.78; H, 7.97; O, 32.81. Found (dried 7 days at 100°): C, 59.17; H, 8.22; O, 32.31.

Tenacious binding of solvent could explain the unusually divergent molecular weight values obtained by isothermal distillation. In fact a value of 685 was obtained (chloroform solution) using a sample which had been dried under strenuous conditions.

The neutral antibiotics contain a variety of unusual sugars of which chalcose (III), mycinose (IV) and 4-o-acetylarcanose (V) have been characterized structurally. It has now been found that methanolysis of neutramycin produces two methyl glycosides and that these are identical with methyl chalcoside and methyl mycinoside (microanalysis, infrared, nmr, optical rotation, melting

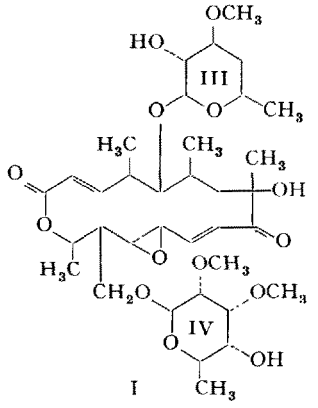
[1] P. W. K. WOO, H. W. DION, and Q. R. BARTZ, J. Am. chem. Soc. *86*, 2726 (1964).

[2] W. KELLER-SCHIERLEIN and G. RONCARI, Helv. chim. Acta *47*, 78 (1964).

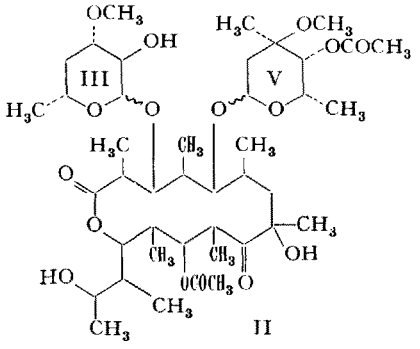
[3] D. V. LEFEMINE, F. BARBATSCHI, M. DANN, S. O. THOMAS, M. P. KUNSTMANN, L. A. MITSCHER, and N. BOHONOS, *Antimicrobial Agents and Chemotherapy* (1963), p. 41.

[4] Professor K. L. RINEHART, private communication.

point, mixture melting point, and thin layer chromatography). Careful methanolysis of neutramycin diacetate at low temperatures produced methyl o-acetylchalcoside and methyl o-acetylmycinoside (thin layer chromatography). This finding locates both acetylatable hydroxyls in neutramycin and means that the sugars are not bound to one another in the molecule. If the sugars were joined in a disaccharide linkage only one of the methyl glycosides could have carried an acetyl group.



I



II

The presence of an $\alpha,\beta$-unsaturated lactone (or ester) and an $\alpha,\beta$-unsaturated-$\gamma,\delta$-epoxyketone function is indicated on spectral grounds. The unsaturated lactone function is revealed by an UV-absorption band at 216 m$\mu$ ($\varepsilon$23,350) and IR-bands at 5.82 and 6.02 $\mu$. The UV-band disappears upon hydrogenation and the IR-bands shift to a single peak at 5.78 $\mu$. The substituted ketone function is indicated by an UV-absorption shoulder at 240 m$\mu$ ($\varepsilon$ ca. 14,080), which is lost upon hydrogenation, and IR-peaks at 5.87 and 6.10 $\mu$ which shift to 5.83 $\mu$ upon catalytic reduction. Treatment of neutramycin with potassium iodide in acetic acid[5] results in the liberation of iodine and the formation of an $\alpha,\beta,\gamma,\delta$-unsaturated ketone as revealed by the shift of the 240 m$\mu$ band to approximately 270 m$\mu$ and by the appearance of bands at 5.91, 6.11 and 6.25 $\mu$ in the IR-spectrum[6]. The presence of these two chromophoric systems satisfactorily accounts for the uptake of three moles of hydrogen in the hexahydro derivative[3]. The lactone ring, the two sugar rings and these two chromophoric systems contain all of the degrees of unsaturation (8) in the neutramycin molecule and leave eleven carbons and one oxygen to be accounted for. Future communications will deal with that portion of the molecule unrevealed by these studies.

*Zusammenfassung.* Für das Antibiotikum Neutramycin wird die Summenformel $C_{34}H_{54}O_{14}$ abgeleitet. Die Methanolyse lieferte die Methylglykoside der Chalcose und Mycinose.

M. P. KUNSTMANN and L. A. MITSCHER

*Biochemical Research Section, Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York (USA), January 21, 1965.*

[5] S. BODFORSS, Ber. dtsch. chem. Ges. *49*, 2801 (1916).

[6] P. W. K. WOO, H. W. DION, and Q. R. BARTZ, J. Am. chem. Soc. *86*, 2724 (1964).

## Unusual Lability of the Amide Bond in N-Methylhippuric Acid

In connection with our studies on enzyme-catalysed ester hydrolysis[1], it became highly desirable to obtain data also covering non-enzymatic 'neutral hydrolysis'[2] of acylated N-alkyl amino acid esters. However, all attempts to measure the rate of this hydrolysis reaction with N-methylhippurylcholine iodide failed consistently because it was found that the substrate undergoes simultaneous hydrolytic cleavage at both the amide and the ester bonds. Under the same conditions hippuric acid is perfectly stable, and 'neutral hydrolysis' of the choline ester at 100° gave good first-order rate constants[3] over the whole range of the reaction. The present paper gives some results on the decomposition of N-methylhippuric acid in water.

*Material.* N-methylhippuric acid was prepared from sarcosine, benzoyl chloride, and sodium hydroxide[4], and recrystallized from water. M.p. 91–96°; elementary analyses, neutralization equivalent, and IR-spectrum in good agreement with structure.

*Analytical procedure.* After 9 days at 100°, the sealed tubes were chilled and opened, the reaction mixtures were made strongly acid by addition of HCl, and then subjected to quantitative extraction with ethyl acetate in a four-stage counter-current distribution, and with extensive back-washing of the organic phases with water.

The procedure has been described in detail previously[3]. The amino acid (sarcosine) liberated during hydrolysis

[1] M. GEMPERLI, W. HOFMANN, and M. ROTTENBERG, Helv. chim. Acta, in press (1965).

[2] That is, hydrolysis of the substrate in pure water at initially neutral pH; after a few % reaction the pH remains approximately constant between 2 and 3. Our standard temperature is 100°.

[3] E. WENGER, H. URHEIM, and M. ROTTENBERG, Helv. chim. Acta *45*, 1013 (1962).

[4] J. P. GREENSTEIN and M. WINITZ, *Chemistry of the Amino Acids* (John Wiley, New York 1961), vol. 2, p. 1267.