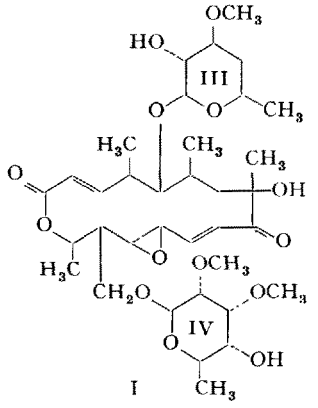
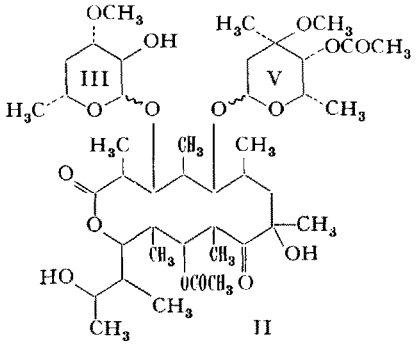
point, mixture melting point, and thin layer chromatography). Careful methanolysis of neutramycin diacetate at low temperatures produced methyl o-acetylchalcoside and methyl o-acetylmycinoside (thin layer chromatography). This finding locates both acetylatable hydroxyls in neutramycin and means that 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 α,β-unsaturated lactone (or ester) and an α,β-unsaturated-γ,δ-epoxyketone function is indicated on spectral grounds. The unsaturated lactone function is revealed by an UV-absorption band at 216 mμ (ε23,350) and IR-bands at 5.82 and 6.02 μ. The UV-band disappears upon hydrogenation and the IR-bands shift to a single peak at 5.78 μ. The substituted ketone function is indicated by an UV-absorption shoulder at 240 mμ (ε ca. 14,080), which is lost upon hydrogenation, and IR-peaks at 5.87 and 6.10 μ which shift to 5.83 μ 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 mμ band to approximately 270 mμ and by the appearance of bands at 5.91, 6.11 and 6.25 μ 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.

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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.

remains quantitatively in the aqueous phase and was estimated by micro Kjeldahl analysis of aliquot portions. The results are listed in the Table.

A study of the literature reveals that our results differ considerably from those obtained previously under conditions of both alkaline and acid-catalysed hydrolysis. Thus LEVENE et al.[5], who compared the rates of hydrolysis of glycine and sarcosine dipeptides *in the presence of 0.3N or 1N HCl*, found the order of reactivity to be gly-gly > sar-gly > gly-sar > sar-sar, and MORAWETZ et al.[6], in a study of *hydroxide ion-catalysed hydrolysis* of amides, found, for instance, second-order rate constants at 75.8° for propionamide $10.5 \cdot 10^{-4}$, N-methylpropionamide $1.44 \cdot 10^{-4}$, and N,N-dimethylpropionamide $2.85 \cdot 10^{-4}$ 1 mole^{-1} sec^{-1}.

Finally, under conditions most closely resembling ours with respect to pH and temperature (in the presence of 0.03*N* HCl), SCHULTZ et al.[7] found no indication of any preferential release of imino acid from proteins containing proline.

The reason for the particular behaviour of N-methyl hippuric acid is unknown. Inductive effects are unimportant since the two acids are of comparable strength (hippuric acid, pK 3.64[8], pK^{x}_{MCS} 5.74[9]; N-methyl hippuric, pK 3.50[8], pK^{x}_{MCS} 5.55[9]), and steric effects resulting in rate *acceleration* are also highly improbable. We are unable to offer a reasonable explanation, unless one postulates a rather exotic mechanism that will accommodate the special structural features and the pH dependence. The latter suggests that only undissociated acid undergoes hydrolytic cleavage. This mechanism (Figure) may be termed intramolecular nucleophilic catalysis[10], an unstable intermediate (III) being finally attacked by the water molecule.

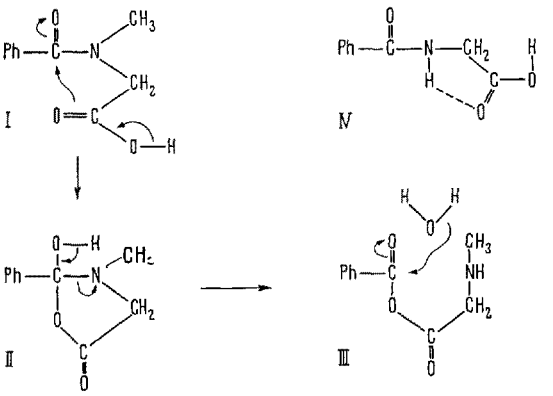
N-methyl hippuric acid, 0.025 *M* in H_2O, with added NaOH or HCl to initial pH, 9 days at 100° ± 0.2°

Exp. No.	1	2	3	4	5	6	7
pH*o*	2.45	2.21	2.57	2.77	3.06	3.50	10.68[a]
pH*t*	2.92	2.70	3.04	3.24	3.44	3.80	7.32
%h	57	63	53	52	46	34	7.9

All pH's measured at 25°. pH*o* initial, pH*t* final pH. %h = % amide hydrolysed. [a] Completely neutralized with NaOH.

By contrast, hippuric acid (IV) is thought to be stabilized by an intramolecular (or possibly intermolecular) hydrogen bond.

In conclusion, we are wondering what WITKOP[11] had in mind when he wrote: 'N-Peptides derived from proline and hydroxyproline are in a separate class because they are tertiary amides carrying no proton at the nitrogen atom. It may be possible to utilize this special feature for a preferential cleavage under proper conditions'.



Zusammenfassung. Bei 100° und pH 2,2 bis 3,5 wird die Amidbindung der N-Methylhippursäure weitgehend hydrolytisch gespalten, während Hippursäure unter denselben Bedingungen stabil ist.

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Medizinisch-chemisches Institut der Universität Bern (Switzerland), April 9, 1965.

[5] P. A. LEVENE, H. S. SIMMS, and M. H. PFALTZ, J. biol. Chem. *61*, 445 (1924).
[6] H. MORAWETZ and P. S. OTAKI, J. Am. chem. Soc. *85*, 463 (1963).
[7] J. SCHULTZ, H. ALLISON, and M. GRICE, Biochemistry, Washington D. C. *1*, 694 (1962).
[8] Our own measurements (potentiometric titration in pure water).
[9] As determined by PD Dr. W. SIMON, E.T.H., Zürich.
[10] M. L. BENDER, Chem. Rev. *60*, 53 (1960).
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An Apocynaceae-Alkaloid of a Novel Type

From the leaves of the New Caledonian Apocynacea *Melodinus scandens* Forst.[1] an amorphous alkaloid I could be isolated, which, when introduced directly into the ion source of the mass spectrometer, exhibits a molecular ion with the empirical formula $C_{20}H_{20}N_2O_2$. On distillation *in vacuo* or treatment with potassium *t*-butoxide in *t*-butanol I is transformed to a crystalline compound II, $C_{19}H_{20}N_2O$, m.p. 188–190°. Since all attempts to crystallize and properly purify I failed, we first turned our attention to the structure of II.

On the basis of UV, IR, NMR, and MS evidence[2] discussed below, the structures shown can be proposed for II and its derivatives III–VII.

The yet unknown structure of the natural alkaloid I will be the subject of a later publication.

Catalytic hydrogenation of II under various conditions gives the tetrahydro compound $C_{19}H_{24}N_2O$ (III). On oxi-

[1] We are indebted to Dr. H. U. STAUFFER, University of Zürich, for the collection and identification of the plant material.
[2] MS-9 mass spectrometer (AEI, Manchester).