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PERIMYCIN

THE STRUCTURE OF SOME DEGRADATION PRODUCTS

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Abstract—Retro-aldolization and methanolysis of the polyene antibiotic perimycin yield N-methyl-*p*-aminoacetophenone and methyl perosaminide, respectively. Their structural elucidation, mainly by spectroscopic techniques, is presented.

INTRODUCTION

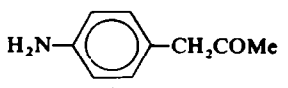
A LARGE number of antifungal antibiotics have been produced by *Actinomycetes*. Among them the most important group are the polyenes. They include the tetraenes, pentaenes, hexaenes, and the heptaenes, depending on the number of their conjugated double bonds.[2] From a practical, clinical viewpoint the heptaenes command the most interest because they possess the most potent antifungal activities.[3]

The heptaenes have been classified[4] into two subgroups: Subgroup I contains an amino sugar but no aromatic moiety, subgroup II yields not only an amino sugar upon acid hydrolysis, but also an aromatic ketone upon alkaline treatment. Partial structures have been advanced for the heptaenes amphotericin B[5] and trichomycin A.[6] The former has mycosamine[7] (3-amino-3,6-dideoxy-D-mannose) as the amino sugar moiety, while the latter yields *p*-aminoacetophenone in addition to mycosamine as degradation products.[8]

Perimycin[9] (syn: fungimycin), a heptaenic antifungal antibiotic isolated from cultures of *Streptomyces coelicolor* var. *aminophilus*, was found to belong to subgroup II of the above classification. Besides its specific biological activity,[11] it is also chemically unique among the polyenes for which structural studies have been made, in both its aromatic and amino sugar moieties. In this communication we wish to report the structural elucidation of these moieties mainly by spectroscopic techniques.

N-*Methyl*-p-*aminoacetophenone*

Alkaline treatment of perimycin yielded a crystalline compound for which structure **1** was previously proposed, based on elemental analysis and on the chromic acid oxidation of its N-acetyl derivative to N-acetyl-*p*-aminobenzoic acid.[10a,*] However, detailed spectroscopic analysis now led to the conclusion that **2** instead is the correct structure.



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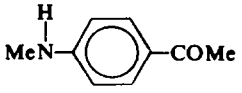


2

* The Me group on nitrogen probably was removed by chromic acid oxidation.[10b]

Consistent with structure **2**, the IR spectrum of this compound showed bands at 1650 cm^{-1} and 1278 cm^{-1} (CO conjugated to an aromatic ring). The UV absorption revealed λ_{max} at 328 mμ and $E^{1\%}_{1cm}$ = 1740 (EtOH). Considering that mono N-methylation of aniline (λ_{max} = 284 mμ) gives rise to a bathochromic shift of 10 mμ, it was reasonable that structure **2** had its λ_{max} at a wavelength 11 mμ longer than *p*-aminoacetophenone (λ_{max} = 317 mμ).[12]

Evidence for structure **2** rather than structure **1** was further furnished by NMR spectroscopy. The NMR spectrum in $CDCl_3$ of the compound showed the expected 3-proton singlet (7·53 τ) for the acetyl group, the 3-proton singlet (7·16 τ) for the N-Me group, and two doublets (3·48 τ, 2·20 τ; J = 8 c/s) easily recognized as due to the *para*-substituted benzene ring. The proton attached to nitrogen gave rise to a very broad singlet centering at 5·45 τ.

The mass spectrum of the compound was relatively simple but unequivocal. The molecular ion appeared at *m/e* 149 (11%). The ion at *m/e* 134 (100%) was due to the loss of a Me group, while the *m/e* 43 peak (16%) was assignable to the CH_3CO^+ ion. Most significant was the peak at *m/e* 30 (13%), clearly due to the ion $CH_2{=}NH_2^+$ fragmented from structure **2** but not structure **1**.

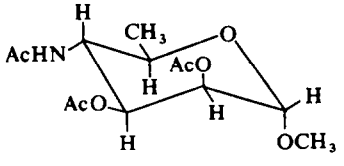
Final proof for structure **2** was provided by synthesis. When *p*-aminoacetophenone was treated with one molar equivalent of methyl iodide in the presence of silver oxide, a product was obtained identical with the aromatic ketone isolated from perimycin by mixed m.p., IR, UV, NMR and mass spectroscopy.

It is of interest to note that N-methyl-*p*-aminoacetophenone has been found also as a moiety in the antibiotic candimycin.*

Methyl perosaminide peracetate

Acid hydrolysis yields an amino sugar with chromatographic mobility and color reactions different from mycosamine. We have previously reported[14] degradational and rotational data indicating that the amino sugar, perosamine, is 4-amino-4,6-dideoxy-D-mannose.† This communication presents the NMR data on a perosamine derivative which independently led to the identification of its relative stereochemistry.

Methanolysis of perimycin gave methyl perosaminide which was acetylated with acetic anhydride in pyridine to yield a peracetate, presumedly **3**, whose NMR data are summarised in Table 1.



Ac = CH_3CO—

3

The three singlets (7·83, 7·98, 8·04 τ) may be assigned[16] as axial acetoxy, equatorial acetoxy, and equatorial acetamido groups respectively, the last of which shifted to 7·98 τ on addition of a trace of trifluoroacetic acid.[17] Earlier detailed consideration of the NMR spectrum of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-β-L-

* We thank Drs. K. L. Rinehart, Jr., S. Horii and S. Tatsuoka for relating the information to us prior to publication. For the original report on candimycin, see Ref. 13.

† Methyl 4-acetamido-4,6-dideoxy-α-D-mannopyranoside and its di-O-acetate have recently been synthesized and were found to be identical with N-acetyl methyl perosaminide and its peracetate, respectively.[15]

TABLE 1. NMR (100 Mc) DATA OF METHYL PEROSAMINIDE PERACETATE IN $CDCl_3$

Pattern	Chemical shift (τ)	Number of protons	Coupling constant (c/s)	Assignment
Doublet	4·17	1	10·0	—NH
Multiplet	4·8–5·1	2		H-2 & H-3
Doublet	5·39	1	1·5	H-1
Quartet	5·80	1	10·0	H-4
Octet	6·23	1	6·0, 10·0	H-5
Singlet	6·61	3		—O—CH_3
Singlets	7·83	3		
	7·98	3		—CO—CH_3
	8·04	3		
Doublet	8·72	3	6·0	—C—CH_3

glucopyranoside[18] using proton–proton spin decoupling and isotopic exchange techniques facilitated assignment of ring protons in **3**. The large coupling constant between H-4 and H-5 showed them to be diaxially situated. The quartet centering at 5·80 τ, which on deuterium exchange became a triplet (J = 10 c/s) with simultaneous disappearance of the amide proton doublet, clearly was due to H-4 (split by H-3 and H-5) and the large coupling constants indicating the axial disposition of H-3 as well. The equatorial acetamido group was, therefore, placed on C-4, the equatorial acetoxy group on C-3, leaving the axial acetoxy group on C-2. However, the small coupling constant between H-1 and H-2 did not unequivocally establish orientation of the anomeric proton since coupling constants of equatorial-axial and di-equatorial protons fall within this range. Fortunately, model compounds[18, 19] displayed different absorptions for axial and equatorial glycosidic methoxy groups and we could conclude that the 6·61 τ 3-proton singlet indicated the axial disposition of the glycosidic methoxy group, thus identifying **3** as the α-form.

Although a 4-amino-4-deoxy hexose derivative was synthesized as early as 1946,[20] it was only recently that 4-amino sugars have been isolated[21, 22] from natural products as components of antibiotics and cell walls of several species of bacteria.

EXPERIMENTAL

General. M.ps were taken with a Fischer-Johns m.p. apparatus and are uncorrected. Optical rotations were determined with an O.C. Rudolph polarimeter Model No. 209. IR spectra were obtained from a Perkin–Elmer 521 Grating Infrared Spectrophotometer. NMR spectra were taken with a Varian Associates A-60 or HA-100 spectrometer, using TMS as internal standard in $CDCl_3$. Chemical-shift values are given on the τ-scale. Mass spectra were obtained with a Bendix Time-of-Flight Mass Spectrometer, Model 12. Elemental analysis was performed by Alfred Bernhardt Microanalytical Laboratory, Ruhr, Germany. Preparative TLC was performed with Silica Gel G as the adsorbent, the layer being 1 mm thick. The developing solvent was 6% MeOH in benzene.

N-*Methyl* p-*aminoacetophenone*

A. *Isolation from perimycin.* The ketone was isolated by alkaline retro-aldolization as described.[10] Recrystallization from water gave m.p. 102–103° [lit.[10] 101–102°].

B. *Synthesis from* p-*aminoacetophenone.* 6·75 g of *p*-aminoacetophenone in 50 ml benzene was treated with 7·1 g MeI and 11·6 g Ag_2O. The suspension was stirred at room temp for 17 hr. After filtration, the soln was evaporated to dryness. TLC, indicated by I_2 vapour, showed only one major component (R_f 0·42) beside the starting material (R_f 0·27), the former having the same mobility as the ketone isolated from perimycin. NMR spectrum of the crude mixture indicated a proton ratio of 1 : 3 for N-Me and Ac groups, respectively;

therefore, the molar ratio of starting material to reaction product was 2:1. No attempt was made to increase the yield. The reaction product was isolated by preparative TLC and recrystallized from water. It is identical with the ketone from perimycin by mixed m.p. IR, NMR and mass spectra.

Methyl perosaminide. After refluxing 6 g of perimycin with 250 ml of 0·3 N methanolic HCl for 1 hr, 200 ml MeOH was added, the soln neutralized with lead carbonate, the ppts centrifuged out, and the light brown supernatant evaporated to dryness. Extraction of the residue with 100 ml water yielded 650 mg solid, which was applied to an IRA-400 (OH^-) column and eluted with water. Fractions of the first ninhydrin-positive peak were pooled and evaporated to 370 mg of white amorphous solid. Slow crystallization from EtOAc yielded methyl perosaminide: m.p. 150–151°, $[\alpha]_D^{23} = +80°$ (*c*, 1·6; MeOH), $[\alpha]_D^{23} = +67°$ (*c*, 2·0; water). (Found: C, 47·45; H, 8·64; N, 7·98; OMe, 16·73. Calc. for $C_7H_{15}NO_4$: C, 47·46; H, 8·47; N, 7·90; OMe, 17·51%).

Methyl perosaminide peracetate. This compound was obtained by acetylation of methyl perosaminide in pyridine and subsequent crystallization from benzene–chloroform: m.p. 157–159° $[\alpha]_D^{23} = +82°$ (*c*, 3·0; MeOH). (Found: C, 51·85; H, 7·22. Calc. for $C_{13}H_{21}NO_7$: C, 51·48; H, 6·98%).

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REFERENCES

[1] Present address: Corporate Research Department, Technical Center, General Foods Corporation, Tarrytown, New York 10591.
[2] W. Oroshnik and A. D. Mebane, *Fortsch. Chem. Org. Naturst.* **21**, 17 (1963).
[3] S. A. Waksman, H. A. Lechevalier and C. P. Schaffner, *Bull. Wld. Hlth. Org.* **33**, 219 (1965).
[4] C. P. Schaffner and E. Borowski, *Antibiot. and Chemother.* **11**, 724 (1961).
[5] [a] A. C. Cope, U. Axen, E. P. Burrows and J. Weinlich, *J. Am. Chem. Soc.* **88**, 4228 (1966);
[b] E. Borowski, W. Mechlinski, L. Falkowski, T. Ziminski and J. D. Dutcher, *Tetrahedron Letters* 473 (1965).
[6] K. Hattori, *J. of Antibiotics, Tokyo* Ser. B, **15**, 39 (1962).
[7] D. R. Walters, J. D. Dutcher and O. Wintersteiner, *J. Am. Chem. Soc.* **79**, 5076 (1957).
[8] H. Nakano, *J. Antibiotics, Tokyo* Ser. A, **14**, 68 (1961).
[9] R. R. Mohan, R. S. Pianotti, J. F. Martin, S. M. Ringel, B. S. Schwartz, E. G. Bailey, L. E. McDaniel and C. P. Schaffner, *Antimicrob. Ag. and Chemother.* 462 (1963).
[10] [a] E. Borowski, C. P. Schaffner, H. Lechevalier and B. S. Schwartz, *Antimicrob. Ag. Ann.* 532 (1960);
[b] A. T. Bottini and R. E. Olsen, *J. Org. Chem.* **27**, 452 (1962).
[11] E. Borowski and B. Cybulska, *Nature, Lond.* **213**, 1034 (1967).
[12] Data from *Organic Electronic Spectral Data* (Edited by M. J. Kamlet), Vol. I, Interscience, New York (1960).
[13] M. Shibata, M. Honjo, Y. Tokui and K. Nakazawa, *J. Antibiotics, Tokyo* Ser. B, **7**, 168 (1954).
[14] C.-H. Lee and C. P. Schaffner, *Tetrahedron Letters* 5837 (1966).
[15] C. L. Stevens, S. K. Gupta, R. P. Glinski, K. G. Taylor, P. Blumbergs, C. P. Schaffner and C.-H. Lee, *Carbohydrate Research* **7**, 502 (1968).
[16] L. D. Hall, *Adv. in Carbohydrate Chem.* **19**, 51 (1964).
[17] O. Cedar and G. Eriksson, *Acta Chem. Scand.* **18**, 98 (1964).
[18] H. Agahigian, G. D. Vickers, M. H. von Saltza, J. Reid, A. I. Cohen and H. Gauthier, *J. Org. Chem.* **30**, 1085 (1965).
[19] H. H. Baer and F. Kienzle, *Canad. J. Chem.* **43**, 3074 (1965).
[20] S. P. James, F. Smith, M. Stacey and L. F. Wiggins, *J. Chem. Soc.* 625 (1946).
[21] J. D. Dutcher, *Adv. Carbohydrate Chem.* **18**, 259 (1963).
[22] C. L. Stevens, S. K. Gupta, R. P. Glinski, G. E. Gutowski and C. P. Bryant, *Tetrahedron Letters* 1817 (1968), and the refs cited therein.