ACTINOMYCIN MONOLACTONE. III

A CHEMICAL APPROACH TO IDENTIFICATION OF THE MICROBIAL METABOLITE*

R. W. RICKARDS, K. L. PERLMAN and D. PERLMAN

The Research School of Chemistry, Australian National University, Canberra, Australia
and
The School of Pharmacy, University of Wisconsin, Madison, Wisconsin, U.S.A.

(Received for publication December 21, 1972)

In recent publications from this laboratory we have reported that actinomycin monolactone is formed from actinomycin by an enzyme from *Actinoplanes missouriensis*[1] and as a 'natural' metabolite in the biosynthesis of actinomycin by *Streptomyces antibioticus*.[2,3] The identification of the monolactone in these instances was made by paper chromatographic and paper ionophoretic comparison with actinomycin monolactone produced by alkaline hydrolysis of actinomycin[4] and we were unable to distinguish which of the lactone rings of actinomycin (Fig. 1) had been cleaved and which was intact.

During their work on the structure of actinomycin BULLOCK and JOHNSON[5] oxidized actinomycin with alkaline hydrogen peroxide and obtained in about 2% yield a benzoxazolone-4-carboxylic acid-pentapeptide from ring A of actinomycin D. Unfortunately, under the conditions used, the pentapeptide lactone ring was hydrolyzed and the benzoxazolone carboxylic acid was a rearranged product originating from rings A and B of the actinomycin.

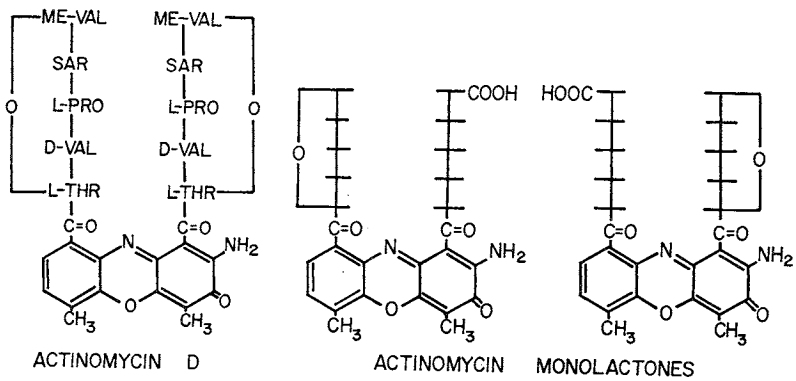
BROCKMANN and BOLDT[6] overcame the obvious disadvantage of the alkaline conditions by using hydrogen peroxide in acetic acid, but were unable to isolate in pure form the degradation products originating from rings A and B, and were only able to isolate the pentapeptide lactone attached to oxalic acid (from ring C).

We concluded that use of ozone might avoid the disadvantage of the alkaline hydrolysis and if the reaction was carefully controlled, the pentapeptide lactone ring would not be destroyed.

In a model experiment we ozonized actinocinyl-bis-glycine-methylester and obtained in 2% yield a compound which proved to be 2-oxo-(2H)-3-methoxy-8-methyl-(benzo-1,4-oxazine)-5-carbonyl-glycine methyl ester (Fig. 2). Though this method would be suitable for identification of the two pentapeptide lactone rings of the actinomycins, unfortunately the very low yield does not make it a very practical approach to be applied on a milligram scale to the metabolites available from the fermentations.[1,2]

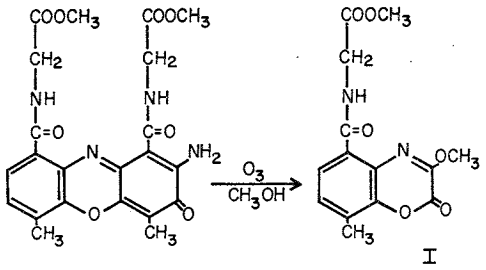
The following procedure was found satisfactory: 0.70 g of actinocinyl-bis-glycine-methyl ester (prepared by the BROCKMANN and MUXFELDT method[7]) was suspended in 100 ml of MeOH and cooled to 0°C. Ozone-

Fig. 1. Structures of actinomycin D and actinomycin monolactones.



* For previous papers in this series consult references 2 and 3.

Fig. 2. Conversion of actinocinyl-bis-glycine-methylester to 2-oxo-(2H)-3-methoxy-8-methyl-(benzo-1,4-oxazine)-5-carbonyl-glycine methylester.



I

oxygen mixture (from a Welsbach ozonizer at 60 volts and 4 lbs oxygen pressure) was bubbled into the suspension with stirring for 8 hours. By the end of this time all of the red actinocinylglycine methylester had dissolved, and the solution was a pale yellow. The reaction mixture was flushed with nitrogen and 5.0 ml of dimethylsulfide was then added and the mixture stirred for 16 hours. At the end of this time, the KI-starch test for ozonides was negative. The solvent was removed and the solids dissolved in $CHCl_3$ and extracted twice with water to remove dimethylsulfide and other water-soluble products. The $CHCl_3$ phase was dried over Na_2SO_4 and the solution evaporated to dryness (0.4 g). This mixture was chromatographed on a silica gel column (5 % Celite) and eluted with a benzene-ethylacetate mixture (first a 7 : 3 mixture was used and later a 1 : 1). A colorless material was eluted first followed by several yellow proucts as single bands.

The colorless material, m.p. 158~160°C, was crystallized from ethyl ether (yield: 16 mg, 2.5 %) and shown by mass spectral analysis to be I (Fig. 2). It had the following characteristics: u.v.: λ_{max} 293 (ε, 12,251); 232 (ε, 18,377) nm in MeOH. nmr ($CDCl_3$, TMS) C_8-CH_3, 2.6 (3) (s); OCH_3, 3.8 (3) (s); OCH_3, 4.1 (3) (s); CH_2, 4.3 (2) (d); aromatic H-s C_6 and C_7-H, 7.5 and 8.2 (1-1) (d). Analysis: Calculated for $C_{14}H_{14}N_2O_6$ (306.0852); C 54.90, H 4.61 and N 9.15. Found: C 54.81, H 4.61 and N 9.10; *m/e* (mass spectrometer), 306.0852 molecular ion.

Although this method would leave the pentapeptide lactone ring of actinomycin monolactone intact, and would be a useful method for structure proof of which monolactone is formed by enzyme inactivation of actinomycin[1], the very low yield of the benzoxazine derivative does not make it practical for our studies.[1,3]

Acknowledgements

This investigation was supported by a grant from the Eli Lilly Company and the US Public Health Service (grant AI-08230 from the National Institute of Allergy and Infectious Diseases). We are indebted to Dr. R. L. Foltz, High Resolution Mass Spectrometry Laboratory, Battelle Columbus Laboratories for the mass spectral analysis.

References

1) Hou, C. T. & D. Perlman: Microbial transformations of peptide antibiotics. V. Purification and properties of the actinomycin lactonase from *Actinoplanes missouriensis*. J. Biol. Chem. 245 : 1289~1295, 1970
2) Perlman, D. & A. Capek: Actinomycin monolactone, an intermediate in the biosynthesis of actinomycin. Appl. Microbiol. 16 : 1258, 1968
3) Perlman, K. L.; J. Walker & D. Perlman: Actinomycin monolactone, a metabolite of *Streptomyces antiboticus* 3720. J. Antibiotics 24 : 135~136, 1971
4) Perlman, D.; A.B. Mauger & H. Weissbach: Microbial transformations of peptide antibiotics. I. Degradation of actinomycins by *Actinoplanes* species. Antimicr. Agents & Chemoth. -1966 : 581~586, 1967
5) Bullock, E. & A. W. Johnson: Actinomycin. IV. An oxidative degradation of actinomycin B. J. Chem. Soc. 1957 : 1602~1607, 1957
6) Brockmann, H. & P. Boldt: Actinomycin. XXXII. Oxidative cleavage of the β-pentapeptide lactone ring from actinomycin C_2 and C_3. Confirmation of the bis-pentapeptide lactone structure of actinomycin. Chem. Ber. 101 : 1940~1947, 1968
7) Brockmann, H. & H. Muxfeldt: Antibiotics from actinomycetes. XL. Actinomycins. 19. Constitution and synthesis of the actinomycin chromophore. Chem. Ber. 91 : 1242~1265, 1958