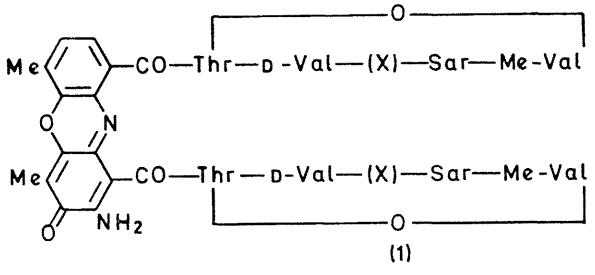
Degradation of Peptides to Diketopiperazines: Application of Pyrolysis–Gas Chromatography to Sequence Determination in Actinomycins

By A B MAUGER

(*Research Foundation of the Washington Hospital Center, Washington, D C* 20010)

Summary Pyrolysis–gas chromatography of peptides permits identification of diketopiperazines derived from adjacent amino-acid pairs, utilization of this technique for obtaining sequence information is illustrated by its application to four actinomycins

FORMATION of diketopiperazines (cyclic dipeptides) during thermal degradation of peptides has been reported [1] This phenomenon was employed to obtain information on the amino-acid sequence of actinomycin C_1 (=D) (**1a**), from which Val-Pro and Sar-*N*-Me-Val diketopiperazines were



(1)

Actinomycins

(**a**) D X = proline
(**b**) C_3 X = proline D Val replaced by D-*allo* Ileu
(**c**) II X = sarcosine
(**d**) Pip 2 X = pipecolic acid

obtained [2] The assumption was made that diketopiperazines (DKP's) can only emerge from neighbouring pairs of amino-acids in the peptide The studies described here support this supposition in the case of actinomycins, as does further work on other peptides to be reported elsewhere

With the development of g c of diketopiperazines,[3] it became possible to reinvestigate the thermal degradation of actinomycin D conveniently on a micro-scale, and to demonstrate the formation of Pro-Sar DKP in addition to the two DKP's previously observed This result would be adequate to confirm the entire amino-acid sequence,[4] provided it is demonstrated that *N*-methylvaline is *C*-terminal This requirement is a consequence of the fact that the reverse sequence would be expected to afford the same DKP's Extension of the thermolysis method to three additional actinomycins is also described here

Whereas thermolysis and g c were initially conducted separately, the procedure was much simplified and reduced to a single step by pyrolysis–g c (Hamilton Probe Sampling System†) The actinomycin sample (25 μg) was held at 400° for 15 s in the flowing carrier gas and the resulting pyrolysis–gas chromatograms are shown in the Figure

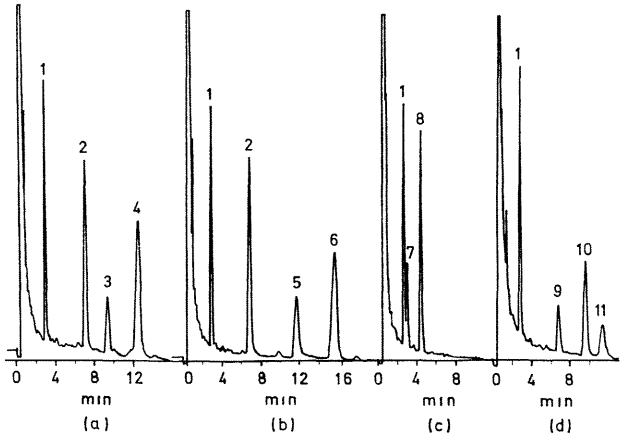


FIGURE *Pyrolysis–gas chromatograms of* (a) *actinomycin* D, (b) *actinomycin* C_3, (c) *actinomycin* II, *and* (d) *actinomycin Pip* 2 *Peaks represent the diketopiperazines of* (1) Sar Me Val, (2) Pro-Sar, (3) Val Pro (*cis*), (4) Val-Pro (*trans*), (5) Ileu-Pro (*cis*), (6) Ileu Pro (*trans*), (7) Sar-Sar, (8) Val-Sar, (9) Pip Sar, (10) Val Pip (*trans*), (11) Val Pip (*cis*)

(column 6 ft × 3 4 mm of 3% EGSP-Z on Gas Chrom Q, 100—120 mesh, carrier gas argon at 45 ml/min, column temperature 210°, detector hydrogen flame ionization)

No DKP's containing threonine or the derivable dehydrobutyrine were observed, but DKP's were obtained from all other adjacent pairs of amino-acids The amino-acid compositions of actinomycins II (**1c**)[5] and Pip 2 (**1d**)[6] were previously known, but it was merely an assumption that their amino-acid sequences were analogous to that of

† As supplied by Hamilton Co , Whittier California

actinomycin D (**1a**). This assumption is verified by the data presented here, although it remains to identify *N*-methylvaline as *C*-terminal in order to define these sequences absolutely.

The various chromatogram peaks (Figure) were assigned to the DKP's indicated by comparison of retention times with those of synthetic DKP's‡ theoretically derivable from all possible sequences. Good diastereoisomeric separations were observed for Val-Pro, Ileu-Pro, and Val-Pip DKP's (Pip = pipecolic acid), the *trans* (DL) isomer predominating in each case. For actinomycin D pyrolysate, peak identity was further verified by combined g.c.–m.s. (instrument: LKB 9000). All four peaks gave mass spectra having the expected fragmentation patterns and identical with those obtained from the authentic DKP's.‡ By addition of an internal standard (Gly-Leu DKP) to actinomycin D it was possible from peak area ratios in the resulting pyrolysis–gas chromatograms to calculate yields of the DKP's obtained. The results, expressed in mol DKP per mol of actinomycin, were: Sar-Me-Val, 0·28; Pro-Sar, 0·54; Val-Pro (*cis*), 0·15, and Val-Pro (*trans*), 0·59.

Extension of the procedure described here to other peptides will be reported elsewhere. While obviously not of universal applicability, the technique is rapid, simple, and requires only micrograms of peptide. The sample size can be much diminished, permitting application of the procedure to peptides extracted from spots on paper or thin-layer chromatograms.

I thank Dr. H. Fales and Mr. W. Comstock for the experiment involving the LKB 9000 mass spectrometer, and the National Institutes of Health for a grant.

(*Received, November 2nd,* 1970; *Com.* 1895.)

‡ The synthesis of new diketopiperazines, m.s. data, and other experimental details will be reported in *J. Chem. Soc.*

[1] N. Lichtenstein, *J. Amer. Chem. Soc.*, 1938, **60**, 560; K. T. Poroshin, *Bull. Acad. Sci*, U.S.S.R., 1959, 2055.
[2] H. Brockmann, G. Bohnsack, and C. H. Suling, *Angew. Chem.*, 1956, **68**, 66.
[3] A. B. Mauger, *J. Chromatog.*, 1968, **37**, 315.
[4] E. Bullock, and A. W. Johnson, *J. Chem. Soc.*, 1957, 3280.
[5] A. W. Johnson and A. B. Mauger, *Biochem. J.*, 1959, **73**, 535.
[6] J. F. Formica, A. J. Shatkin, and E. Katz, *J. Bact.*, 1968, **95**, 2139.