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GAS CHROMATOGRAPHIC DETERMINATION OF THE CONFIGURATION OF AMINO ACIDS IN ANTIBIOTICS OF THE VERNAMYCIN B GROUP*

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Recently, it has been shown (1) that enantiomeric amino acids can be identified and analysed by gas chromatography of certain diastereoisomeric derivatives. In the present communication we wish to describe the application of such a gas chromatographic procedure to the configuration of amino acids in two antibiotics of the vernamycin B group.

Doricin (2) and vernamycin Bα (3) are peptidic lactones, which were isolated and identified by Bodanszky and coworkers. The tentative structure of doricin is given in Fig. 1. Vernamycin Bα** differs from doricin only in as far as aspartic acid is replaced by β-ketopipecolic acid. The constituting amino acids of both

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** Identical with ostreogrycin B (F.W. Eastwood, R.K. Snell and L. Todd, J. Chem. Soc., 2286, 1960) and with micamycin B (K. Watanabe, J. Antibiot. (Tokyo), Ser. A 14, 1,1961).

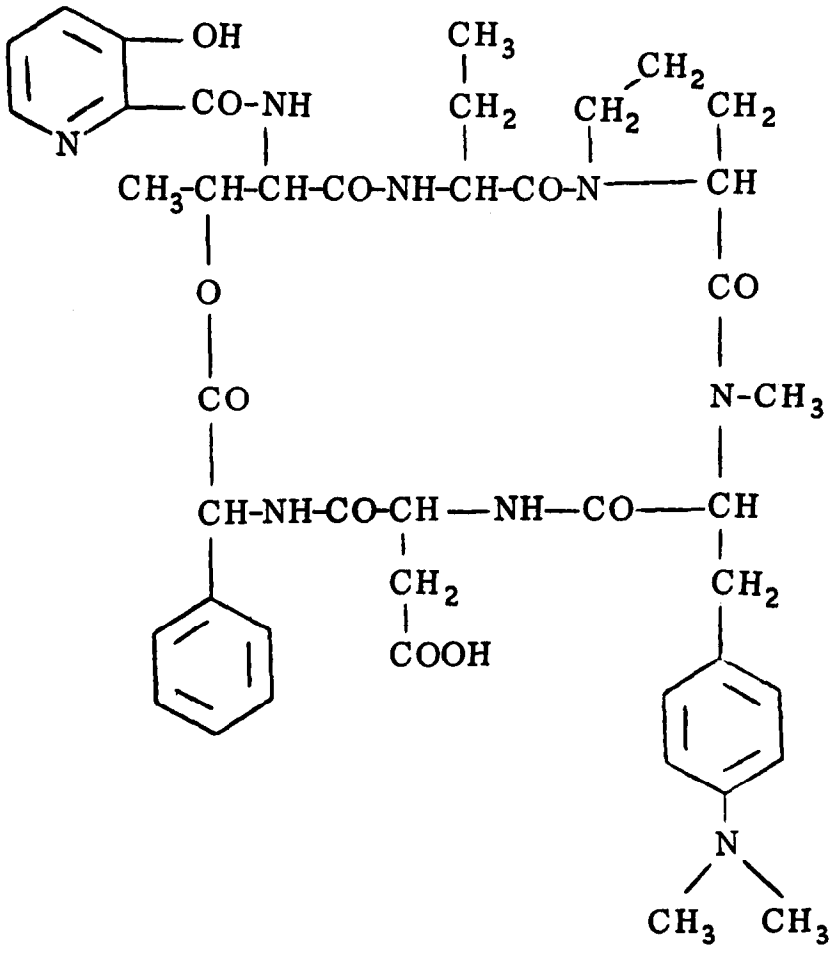

FIG. 1.

Tentative structure of doricin.

substances, with the exception of α-amino butyric acid (I) and aspartic acid (II), are known to have the L configuration;(I) was shown to be in the D form, and the configuration of(II)had not been established.

The procedure used (1a,b) is based on conversion of the amino acids into N-trifluoroacetyl (N-TFA) esters of 2-butanol, and chromatography on a 150' stainless steel column of 0.01" I.D., coated with polypropylene glycol. An amount of 5-8 mg of hydrolysate*** of the antibiotics was converted into derivatives, as described previously. No recemization has been found to occur under the conditions of the procedure (1a,b,e). Each pair of diastereoisomers separates in a way such that the DD (or LL) isomer emerges before the DL (or LD) compound (1a,b and Table 1; for a discussion of peak assignment of aspartic acid derivatives, see 1b). Since the 2-butanol employed contained about 80% of the D enantiomer and 20% of the L antipode, and the time scale in Figs. 1 and 2 runs from right to left, the larger peak (80% area) of each pair appears on the right hand side for amino acids having the D configuration, and,conversely, the larger peak will be on the left for an L amino acid. If both enantiomers of an amino acid are present in a sample, the ratio of the areas of the peaks will differ from 4/1, but this was not the case within experimental errors for the hydrolysates tested. For the accurate determination of small amounts of antipodes, formed, for intance, by racemization during hydrolysis, optically pure alcohols

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should be used for the preparation of derivatives.

Peak assignment was made on the basis of retention data for pure N-TFA amino acid esters of 2-butanol of known configuration (N-TFA-leucine-L-2-butyl ester was used as reference substance, see Fig. 2). The chromatograms confirm clearly the L configuration of proline and phenylglycine and the D configuration of α-amino butyric acid in both antibiotics. Furthermore, aspartic acid in doricin has been shown to be the L enantiomer (Ratio of areas estimated at about 74/26, Fig. 3). It should be recalled (2) that the aspartic acid moiety seems to be related biogenetically to the 4-ketopipecolic acid, also of L configuration, in Vernamycin Bα.

The more complex amino acid components of the two antibiotic materials examined do not show up on the chromatograms, thus illustrating the present limitations of the method. The reasons for this behaviour have not been investigated. However, some difficulties arising with α-amino acids, having an additional functional group are discussed in reference 1b.

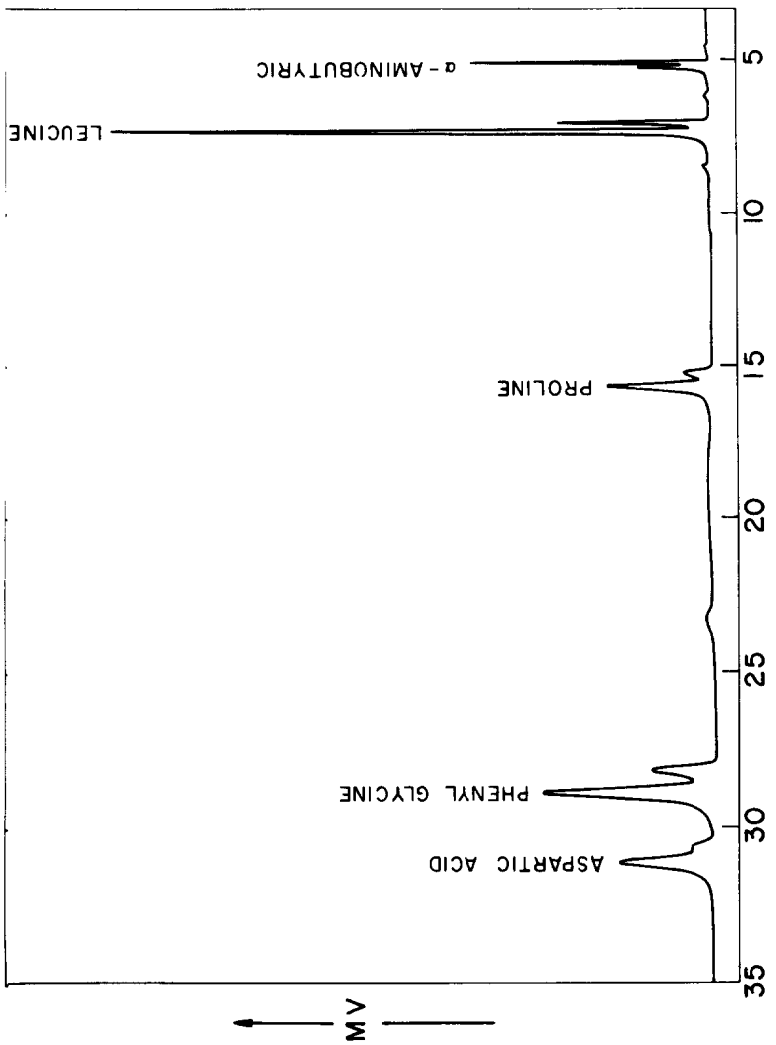

FIG. 2. Chromatogram of the N-TFA-amino acid esters of 2-butanol, derived from doricin, on a capillary column of 150'x 0.01" I.D. coated with polypropylene glycol. Temp. 150°; He 20 p.s.i; L-leucine was added to the sample as reference substance.

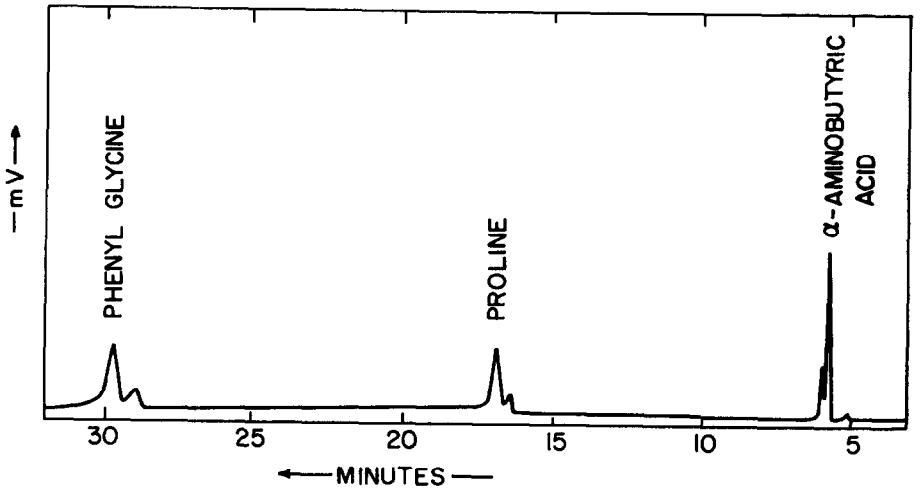

FIG. 3.

Chromatogram of the N-TFA amino acid ester of 2-butanol, derived from Vernamycin, Bα on a capillary column of 150'x0.01" I.D., coated with polypropylene glycol. Temp. 150°; He 20 p.s.i.

TABLE I

Relative Retention Volume of Diastereoisomeric

N-TFA-Phenylglycine Esters

Compound	Column	Temp. °C	Relative Retention Volume LL-Leucine-2-n-alkanol ester=1.00[c]		
			LL or DD[d]	LD or DL[d]	$r_{LD/LL}$
(±) 2-Butanol	B[a]	150	3.99	4.08	1.02
	C[b]	160	3.63		1.00
(±) 2-Octanol	C[b]	195	3.45	3.32	0.96

[a] Capillary column 150' x 0.01"" I.D., coated with polypropylene glycol (LB 550X, Perkin Elmer column R).

[b] Capillary column as above, coated with butanediol succinate (Perkin Elmer column BDS).

[c] Retention time for N-TFA-L-leucine-L-2-butyl ester: Column B - 150°C, 7.3 min; column C - 160°C, 7.4 min. Retention time for N-TFA-L-leucine-L-2-octyl ester: Column C - 195°, 5.83 min.

[d] L-phenylglycine employed for preparation of derivatives for peak assignment was kindly supplied by Dr. M. Bodanszky; the D-phenylglycine was a commercial product.

The separation of diastereoisomeric N-TFA esters of α-amino butyric acid, proline and aspartic acid has been reported before (1a,b,e), but the resolution of phenylglycine by gas chromatography has not been studied previously. Data on the gas chromatographic behaviour of 2-butyl and 2-octyl esters are summarized in Table I. Whereas on polypropylene glycol the 2-butyl ester emerge in the "normal" order ($r_{LD/LL} > 1$) and can be readily separated, the behaviour on the polyester phase (column C, Table I) is unusual. Thus, the peaks of the diastereoisomers of the 2-butyl ester could not be resolved on this phase, and a reversal of peak order was observed for the 2-octyl esters. This is the first exception to the empirical rule of emergence of 2-n-alkanol esters of N-TFA-α-amino acids reported so far.≠

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2. M. Bodanszky and J.T. Sheehan, Antimicrobial Agents and Chemotherapy, 38 (1963).

3. M. Bodanszky and M.A. Ondetti, ibid., 360 (1963).

≠ In reference 1b, p. 58, line 11, left hand column, read "N-TFA-phenylglycine-2-octyl" instead of "N-TFA-Phenylglycine-2-butyl".