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ON THE ROLE OF "CYCLIC CYSTEINYLVALINE" IN PENICILLIN BIOSYNTHESIS

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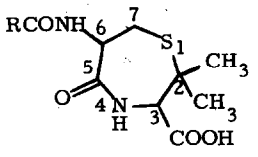
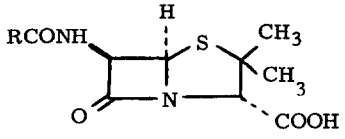
SOME years ago Arnstein and Clubb (1) reported that the N-acetyl and and N-phenylacetyl derivatives of a synthetic DL-6-amino-2, 2-dimethyl-5-oxo-hexahydro-1, 4-thiazepine-3-carboxylic acid ("cyclic



I



II

cysteinylvaline") (I, R = CH_3– or $C_6H_5CH_2$–) did not behave as *in vivo* precursors of penicillin produced by *P. chrysogenum*. However, the

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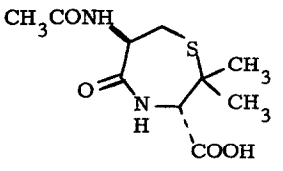
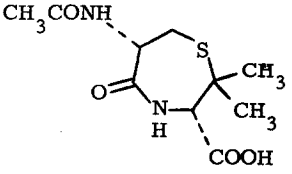
stereochemistry of the synthetic lactam remained unspecified, despite the clear necessity (1) of testing the particular isomer (III) which possesses the C_3 - C_6 stereochemistry corresponding to that in natural penicillin (3 D - 6 L) (II).[c] By means of a modification of the Arnstein-Clubb synthesis



III



IV

method, we have been able to prepare each of the cyclic cysteinylvaline diastereomers (III) (3 D - 6 L) and (IV) (3 D - 6 D) in pure form; and we have tested each, ^{14}C-labeled in the 6-position, in feeding experiments with P. chrysogenum.

Preparation of the key heptacycles (III) and (IV) started with the addition of D-penicillamine (V) to methyl α-acetamidoacrylate (VI), a reaction, first used (with DL-penicillamine) by Arnstein and Clubb (1), which generates a diastereoisomeric mixture of thioethers (VII). Rather

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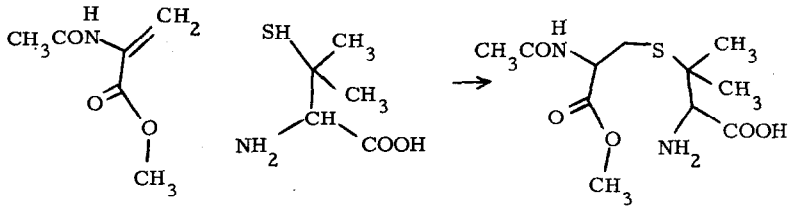
[c] This point was emphasized by N. J. Leonard and G. E. Wilson, Jr., Tetrahedron Letters, No. 23, 1465 (1964), who were, however, unable to secure the requisite 3 D - 6 L isomer.



$\longrightarrow$ III + IV

than effect lactam formation by subjecting intermediate (VII) to the lengthier azide method of peptide synthesis, as was done by Arnstein and Clubb (1), we ring closed directly by treating the mixture of epimers (VII) as hydrochlorides with triethylamine in methanol (2). The cyclic peptides (III) and (IV) were separated by silicic acid chromatography, and possessed the following properties:

Isomer A. Amorphous, but forms unstable, crystalline solvates with acetone and methylene dichloride. $[\alpha]_D^{24}$ -32.6° (95% ethanol). Yield, 27%. Methyl ester of A, m.p. 145.5 - 146°.

Isomer B. m.p. 214-215°. $[\alpha]_D^{24}$ -64.3° (95% ethanol). Yield, 25%. Methyl ester of B, amorphous but forms unstable, crystalline benzene solvate of indefinite m.p.

The infrared and proton magnetic resonance spectral characteristics were in complete accord with the structures assigned isomers A and B,

as demonstrated by the following data and assignments:

	Isomer A	Isomer B
Infrared absorption (μ) (chloroform)	2.9 (single)(NH)	2.9 (split) (NH)
	5.75 ($COOCH_3$)	2.95 (shoulder)
	5.95-6.05 (CONH)	5.75 ($COOCH_3$)
	6.7 (amide II)	6.0 (CONH)
		6.7 (amide II)
p-m-r (τ) (60 mc. in deuterochloroform; hexamethyl disiloxane internal reference)	8.70 s 3H, 8.63 s 3H } gem diMe	{ 8.61 s 3H, 8.49 s 3H
	7.97 s 3H CH_3CON	8.02 s 3H
	7.20 d 1H CH_2(C-7) (j = 6 cps)	7.22 s 1H
	7.08 d 1H CH_2(C-7) (j = 4 cps)	7.09 d 1H
	6.18 s 3H CH_3OOC	6.19 s 3H
	5.38 d 1H CH(C-3) (j = 8 cps)	5.60 d 1H (j = 7 cps)
	5.07 m 1H CH(C-6) (j = 6 cps)	5.35 m 1H
	3.45 d 1H CONH (j = 8 cps) (lactam)	2.94 d 1H (j = 7 cps)
	2.82 d 1H CONH (j = 6 cps) (acetamide)	2.67 m 1H

The elemental analyses of lactams A, B and their methyl esters were satisfactory.[d]

[d] Recently, Dr. K. Mallion and Mr. T. Cole (unpublished results secured at Stanford University, Department of Chemistry) elucidated the stereochemistry of isomer A as IV and isomer B as III, by identification (3D - 6L) of the β, β-dimethyllanthionine (i) produced by hydrolysis of lactam B. Hydrolysis of lactam A afforded the epimer (3D - 6D) of (i).

$HOOC–CH(NH_2)CH_2SC(CH_3)_2CH(NH_2)COOH$ (i)

Isotopically labeled peptides (III) and (IV) were prepared from pyruvic-2-^{14}C-acid (sodium salt), which was converted by reaction with acetamide to ^{14}C-2 labeled methyl α-acetamidoacrylate (1). Repetition of the synthetic sequence described above provided ^{14}C-6 labeled cyclic peptides (III) and (IV).

The radioactive peptides (III) and (IV) were tested as biochemical intermediates in two series of experiments. In the first, P. chrysogenum was grown in shake flasks on a complex medium to which had been added one or the other of the peptides (III) and (IV); and after seven days' incubation, the penicillin formed was isolated and purified. In the other series, washed and starved mycelium of P. chrysogenum was suspended for 20 hrs. in phosphate buffer containing peptide (III) or (IV), after which the penicillin produced was harvested. In parallel experiments, the respiration of the washed and starved mycelium was measured in a Warburg apparatus in the presence of phenylacetic acid alone, and also with added L-valine + L-cysteine; peptide (III); and peptide (IV), respectively. As determined by liquid scintillation counting techniques, no significant difference could be observed, either between the radioactivity level of penicillin fractions from various runs with labeled peptides (III) or (IV), or between these as a group and fractions produced in the absence of the labeled substances. In addition, samples of penicillins produced in the first fermentation series were converted by means of benzyl penicillin acylase to the corresponding 6-aminopenicillanic acid. Again, none of the samples exhibited significant presence of ^{14}C-labeled constituents.

Oxygen uptake by the washed and starved mycelium in the presence of the various added ingredients compared as shown in Table 1.

TABLE 1

Added Components	μl of O_2 Consumed in 4 hrs. at 25°C
1. No additions	36.3
2. Phenylacetic acid (10 μg/ml)	38.6
3. L-valine (40 μg/ml) L-cysteine (50 μg/ml) Phenylacetic acid (μg/ml)	84.1
4. Peptide (III) (90 μg/ml) Phenylacetic acid (10 μg/ml)	44.5
5. Peptide (IV) (90 μg/ml) Phenylacetic acid (10 μg/ml)	42.2

(Each experiment was carried out with an amount of mycelium corresponding to 10 mg dry weight, suspended in 3 ml of M/15 phosphate buffer, pH 6.5)

ACKNOWLEDGMENT

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REFERENCES

(1) H. R. V. Arnstein and M. E. Clubb, Biochem. J., 68, 528 (1958).

(2) B. C. Barrass and D. T. Elmore, J. Chem. Soc., 4830 (1957).