

ON THE CONFIGURATION  
OF THE N-METHYLALLOISOLEUCINE  
CONTAINED IN QUINOXALINE  
ANTIBIOTICS

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Several N-methylamino acids are constituents of quinoxaline antibiotics, namely N, N'-dimethyl-L-cystine, N-methyl-L-valine, N-methyl-alloisoleucine and N,  $\gamma$ -dimethyl-L-alloisoleucine as components of both the quinomycin and triostin series of antibiotics.<sup>1)</sup> Configurations of the  $\alpha$ -carbons of N, N'-dimethyl-L-cystine<sup>2)</sup> and N-methyl-L-valine<sup>3)</sup> were determined by comparison of their  $[\alpha]_D$  values with those of authentic compounds. That of N,  $\gamma$ -dimethyl-L-alloisoleucine<sup>4)</sup> was deduced by its increasing of positive  $[\alpha]_D$  value on acidification. The positive rotation shift on acidification has been observed in natural L-amino acids by LUTZ and JIRGENSONS<sup>5)</sup>, and has also been found in some N-methyl-L-amino acids by PL. A. PLATTNER and U. NAGER<sup>6)</sup>.

In an early experiment<sup>7)</sup> on quinoxaline antibiotics, these N-methylamino acids were tested with D-amino acid oxidase without giving any oxidation. However, the effect of the oxidase on N-methylated amino acids is quite obscure. At that time, the quantity of N-methylalloisoleucine needed for  $[\alpha]_D$  rotation studies was not available. However, it is now possible to determine the configuration of the N-methylal-

loisoleucine by ORD and CD measurements.

A triostin complex which contained mainly component B and trace amounts of components A and D was prepared from a fermentation of strain S-2-210 L in a sodium nitrate-maltose medium containing DL-isoleucine<sup>8)</sup>. By preparative employment of modified circular TLC<sup>9)</sup>, triostin B was isolated as colorless needles, degenerates above 263°,  $[\alpha]_D^{23.5} = -149.4 \pm 2.3^\circ$  (c, 0.822, CHCl<sub>3</sub>). Anal. Found: C, 56.06; H, 5.89; N, 14.87; S, 6.02; M. W., 1118 (Osmometry, CHCl<sub>3</sub>). C<sub>52</sub>H<sub>66</sub>O<sub>12</sub>N<sub>12</sub>S<sub>2</sub> requires: C, 56.00; H, 5.97; N, 15.07; S, 5.75; M. W., 1115.28.

Triostin B (10 mg) was hydrolyzed with 6 N HCl at 105°C for 20 hours. The hydrolyzate gave 4 ninhydrin-positive spots corresponding to N, N'-dimethylcystine, serine, alanine and N-methyl-alloisoleucine on a paper chromatogram (Toyo Roshi No. 51, BuOH-AcOH-H<sub>2</sub>O (4:1:2)). It was subjected to preparative paper chromatography (Toyo Roshi, No. 51, 60 × 60 cm) developed with the some solvent by descending for 16 hours. A zone containing N-methylalloisoleucine was cut out, and extracted with water. The extract was adsorbed on a small column of Dowex 50 (NH<sub>4</sub><sup>+</sup> form) and eluted with 0.5 N NH<sub>4</sub>OH. Lyophilization of the eluate afforded 0.25 mg of N-methylalloisoleucine. It showed identity with synthetic N-methyl-DL-alloisoleucine on amino acid analysis and also a positive COTTON effect at 210 m $\mu$  in its CD curve. CD (c, 0.0127, 0.5 N HCl),  $[\theta]_{230} 0$ ,  $[\theta]_{210} +7660$  (peak),  $[\theta]_{205} +6510$ . Though the measurement of amplitude was not reliable because of the low concentration, the L-configuration for the amino acid was

Table 1. COTTON effect in ORD and CD measured in 0.5 N HCl.

Amino acid	ORD		CD	
	$[\phi]$	$\lambda$ (m $\mu$ )	$[\theta]$	$\lambda$ (m $\mu$ )
N-Methyl-L-valine (synthesized)	+3442	224.5 (c, 0.1045)	+5081	211
N-Methyl-L-isoleucine (synthesized)	+3553	225 (c, 0.1054)	+5146	210.5
N-Methyl-alloisoleucine <sup>10)</sup> (from actinomycin E)	+3795	226 (c, 0.0650)	+4906	212
N, $\gamma$ -Dimethyl-L-alloisoleucine (from triostin C)	+2009	225.5 (c, 0.0965)	+3332	212
N, N'-Dimethyl-L-cystine (from triostin B)	+2870	226 (c, 0.1707)	+4603	216.5

deduced from this studies.

Recently, the configuration of the N-methylamino acid contained in actinomycin E has been determined by E. KATZ and the present author<sup>10)</sup>, showing that it is also N-methyl-L-alloisoleucine.

J. P. JENNINGS, W. KLYNE and P. M. SCOPES<sup>11)</sup> have observed that a series of L- $\alpha$ -amino acids shows positive COTTON effects with peaks at about 215 m $\mu$  (in water) and about 225 m $\mu$  (in 0.5 N HCl) in their ORD curves. Similar observations have been reported by other workers<sup>12)</sup>.

In this experiment, synthesized N-methyl-L-valine and N-methyl-L-isoleucine; and N-methyl-L-alloisoleucine, N,  $\gamma$ -dimethyl-L-alloisoleucine and N, N'-dimethyl-L-cystine derived from natural sources were compared by ORD and CD measured in 0.5 N HCl. These N-methyl-L-amino acids exhibited positive COTTON effects with peaks at about 225 m $\mu$  in ORD and about 211 m $\mu$  in CD, being exhibited at 216 m $\mu$  only with N, N'-dimethyl-L-cystine as listed in Table 1. These results make the determination of configurations of N-methylamino acids derived from natural sources a simple procedure.

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