N-ACETYL-S-METHYLMALONYLCYSTEAMINE, AN INHIBITOR OF METHYLMALONYL COENZYME A ISOMERASE

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N-Acetyl-S-methylmalonylcysteamine has been synthesized and found to be an inhibitor of methylmalonyl coenzyme A isomerase.

Many of the substrates of enzymes that require coenzyme B_{12} as a co-factor are relatively simple organic molecules free of the additional structural appendages so often carried by biologically active molecules, e.g., propanediol (Brownstein and Abeles, 1961; Lee and Abeles, 1963) and glutamic acid (Barker, Weissbach, and Smyth, 1958). On the other hand, the isomerization of the carbon skeleton of methylmalonic acid to that of succinic acid catalyzed by methylmalonyl coenzyme A isomerase normally utilizes as substrates the coenzyme A esters of methylmalonic acid (I) or succinic acid (Smith and Monty, 1959; Stadtman, Overath, Eggerer, and Lynen, 1960; Gurnani, Misty, and Johnson, 1960; Lengyel, Mazumder, and Ochoa, 1960). We wish to report the synthesis of N-acetyl-S-methylmalonylcysteamine (II), a substance with the same functional groups near the ester carbon of the methylmalonate residue, but one that lacks the nucleotide residue and five atoms in the chain (and their attached groups) of coenzyme A.

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N-Acetyl-S-methylmalonylcysteamine

II

Methylmalonyl Coenzyme A

I

The substance was synthesized by addition of methylmalonyl dichloride (Crawhall, Elliott, and Hooper, 1956) in benzene to a solution of N-acetylcysteamine [prepared by reduction of N-acetylcysteamine with sodium in liquid ammonia (Rabjohn, 1963)] in pyridine followed by partial hydrolysis (in 50 ml. of 1 M sodium carbonate solution at room temperature for one day) of the resulting dithiolester. After purification, the monothiolester was crystallized from ethyl acetate-ligroin to give a white solid, m.p. 107.5-109. Anal. calcd. for $C_8H_{13}NO_4S$: C, 43.82; H, 5.97; N, 6.39; S, 14.63. Found: C, 43.90; H, 5.92; N, 6.47; S, 14.70.

As shown in Tables I and II, N-acetyl-S-methylmalonylcysteamine is not isomerized (its rate of isomerization is slower than that of methylmalonyl coenzyme A by a factor of at least 100) by methylmalonyl coenzyme A isomerase. On the other hand, II is bound (presumably to the active site) of the enzyme and can function as a rather weak (probably competitive) inhibitor of methylmalonyl coenzyme A itself. Thus, a 10 molar excess of N-acetyl-S-methylmalonylcysteamine over methylmalonyl coenzyme A lowers the rate of isomerization by 43%.

TABLE I. Attempted Isomerization of N-Acetyl-S-methylmalonyl-cysteamine by Methylmalonyl Coenzyme A Isomerase

Enzyme units[a]	Thiol ester (μ moles)[b]			Methylmalonyl coenzyme A[c] (μ moles)
	before reaction	after reaction	μ moles isomerized	
0	1.06±0.02	1.06±0.02	0	0
0.034	1.06±0.02	1.04±0.02	0.02±0.02	0.70±0.01
0.100	1.06±0.02	1.05±0.02	0.01±0.01	2.00±0.04

[a]1 unit = 1 μ mole methylmalonyl coenzyme A isomerized/min/mg protein

[b]N-acetyl-S-methylmalonylcysteamine

[c]Amount of methylmalonyl coenzyme A isomerized under identical conditions

TABLE II. Inhibition of Methylmalonyl Coenzyme A Isomerase by N-Acetyl-S-methylmalonylcysteamine

Mole Inhibitor[a]/mole substrate[b]	M.p. of recovered succinic acid	Total dpm	% Inhibition
0	186.5-187.5	1310±65	0
2	187-188	1430±72	0
10	189-190	745±35	43±5

[a]N-Acetyl-S-methylmalonylcysteamine

[b]4-^{3}H-Methylmalonyl coenzyme A

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