# Improved method for the preparation of malonyl coenzyme A

Hans Mohrhauer, Kirsten Christiansen, Minerva Gan, Manfred Deubig, and Ralph T. Holman

*The Hormel Institute, University of Minnesota, Austin, Minnesota 55912*

SUMMARY Malonyl coenzyme A is synthesized by transacylating *S*-malonyl-*N*-decanoyl cysteamine with coenzyme A. A simplified procedure for the preparation of *S*-malonyl-*N*-decanoyl cysteamine, using cysteamine as starting material, is described.

KEY WORDS malonyl coenzyme A · transacylation · *S*-malonyl-*N*-decanoyl cysteamine

During an investigation of the chain elongation of linoleic acid and its inhibition by other fatty acids in vitro (1), the need arose for a convenient method for preparing malonyl CoA. Procedures for the chemical synthesis of malonyl CoA have been described by Wieland and Rueff (2), Trams and Brady (3), and Vagelos (4). Eggerer and Lynen (5, 6) transacylated *S*-malonyl-*N*-octanoyl cysteamine with coenzyme A, and their method gave the best yields of all these procedures in our hands. We report here a further simplification of their procedure.

The reaction leading to *N*-decanoyl cysteamine was carried out without isolating either of the intermediates, cysteamine or *N*,*N*′-didecanoyl cysteamine. Sodium borohydride was used instead of sodium amalgam as the reducing agent, because the borohydride is more convenient to handle and is readily available. Decanoyl chloride was used instead of octanoyl chloride because it raises the melting point of the intermediate 15°C, which facilitates its recrystallization without diminishing its reactivity.

*N-Decanoylcysteamine.* Cysteamine hydrochloride (Sigma Chemical Co., St. Louis, Mo.), 3.0 g (26 mmoles), was dissolved in 10 ml of distilled water and neutralized with 5 N NaOH. Hydrogen peroxide (30%) was added dropwise with stirring at 0°C until the nitroprusside test for the SH-group was negative. Alternatively, cysteamine dihydrochloride (Aldrich Chemical Co. Inc.) can be used after neutralization with 5 N NaOH. Decanoyl chloride (7), 5.3 g (28 mmoles), prepared from decanoic acid and thionyl chloride, was added slowly, the solution being kept at pH 10 by dropwise addition of 5 N NaOH. The slurry was heated to 55–60°C for 5 min and then transferred with 200 ml of methanol into a 500 ml, three-necked flask fitted with a reflux condenser and magnetic stirrer. The solution was refluxed under nitrogen while 16 g of sodium borohydride was added in small portions during 60–90 min. After addition of the sodium borohydride, the mixture was refluxed for 1 hr and cooled, and 200 ml of distilled water was added. The slurry was poured into a mixture of 100 g of ice and 100 ml of 5 N $H_2SO_4$. The precipitate formed was collected on a Buchner funnel and washed with water until the filtrate was neutral. The product was dissolved in the minimum volume of hot ethanol and was recrystallized by dropwise addition of water until the hot solution became cloudy. The crystallization was completed at 0°C and yielded 4.9 g (82%) of *N*-decanoyl cysteamine, mp 64–65°C. In one experiment the intermediate *N*,*N*′-didecanoyl cysteamine was isolated and its melting point was found to be 124–125°C. The comparable preparation of *N*-octanoyl cysteamine required a lower temperature, 45–50°C, for the reduction with sodium borohydride and yielded, after recrystallization, 4.2 g (80%), mp 50–51°C.

*S-Malonyl-N-decanoylcysteamine.* *S*-malonyl-*N*-decanoyl cysteamine was prepared according to the procedures of Eggerer and Lynen (5, 6). The yield was 60% and the mp 80–81°C. Malonyl CoA was prepared from this intermediate by the usual transacylating procedure (5, 6). The aqueous solution of malonyl CoA is stable at −30°C for several months.

This investigation was supported, in part, by USPHS Research Grants HE 10489 and HE 08214 from the National Institutes of Health, and The Hormel Foundation.

*Manuscript received 22 January 1968; accepted 14 February 1968.*

## References

1. Mohrhauer, H., K. Christiansen, M. Gan, M. Deubig, and R. T. Holman. 1967. *J. Biol. Chem.* **242:** 4507.
2. Wieland, T., and L. Rueff. 1953. *Angew. Chem.* **65:** 186.
3. Trams, E. G., and R. O. Brady. 1960. *J. Am. Chem. Soc.* **82:** 2972.
4. Vagelos, R. R. 1960. *J. Biol. Chem.* **235:** 346.
5. Eggerer, H., and F. Lynen. 1962. *Biochem. Z.* **335:** 540.
6. Lynen, F. 1962. *Methods Enzymol.* **5:** 443.
7. Bauer, S. T. 1946. *Oil and Soap.* **23:** 1.