SYNTHESIS OF 5'-(LIPOYL)ADENYLATE, A CARRIER COFACTOR FOR LIPOIC ACID

L. G. Chebotareva and A. M. Yurkevich

In continuation of the search for drugs of the coenzyme and nucleoside cofactor types, we have prepared and examined the properties of 5'-(lipoyl)adenylate (I), which functions as a carrier for lipoic acid (II) in the biosynthesis of lipoyl-dependent enzymes. It has been shown that the formation of I, which is a mixed anhydride of II and adenosine monophosphate (AMP), is an intermediate step in the reactions of II [1].

AMP transports II to the ε -amino-groups of the lysine residues of the enzymes of the pyruvate oxidase and ketoglutarate complexes [2, 3].

The use of II and its amide as hepatoprotective agents is well known [4-6]. It is quite possible that I, as a normal metabolite of lipoic acid, would be a still more active compound with similar properties. A practically important property of such a compound must be its hydrolytic stability. In order to investigate the properties of I we have checked the literature for its preparation [1], and we have further developed a more convenient variant of this synthesis. The literature method is based on exchange between the anhydride of II and the phosphate anion [7]. Compound I has been obtained in this way in 39% yield, calculated on AMP. The anhydride of II is in turn synthesized from II by treatment with N,N'-dicyclohexylcarbodiimide. We subsequently obtained I from the mixed anhydride of II and ethyl carbonic acid by reaction with AMP.



In spite of the fact that we failed to increase the yield of I from AMP, this is the preferred method, since in this case one mole of II is consumed per mole of AMP, which of course raises the yield of I from II from 14.86%, using the known method employing the anhydride of II, to 31.3% using our reaction sequence. The avoidance of the use of dicyclohexylcarbodiimide is also important.

Compound I was characterized by its elemental analysis, paper chromatography, and UV and IR spectra. The UV spectrum at pH 7.0 exhibits maxima at 259 and 332 nm, characteristic of the adenine chromophore of AMP and the dithiolane ring of II (see Fig.). The IR spectrum of I contains a strong band at 1760 cm^{-1} , characteristic of acid anhydrides.

We have examined the hydrolytic stability of I on storage in solution and in the crystalline state. It appears that I is completely degraded in the course of a few hours in a phosphate buffer at pH 6.94. The compound is also unstable on storage in the crystalline state, since traces of AMP appear after storage for 2-3 weeks at $3-5^{\circ}$ C (by chromatographic analysis).

EXPERIMENTAL

The UV spectra were recorded on an Hitachi M-124 spectrophotometer in a phosphate buffer at pH 6.94, and the IR spectra on a UR-10 spectrophotometer in vaseline oil. Chromatography was carried out on FN · 11 paper using the solvent system isopropanol-water (7:3).

All-Union Vitamin Research Institute, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 8, No. 12, pp. 11-12, December, 1974. Original article submitted October 30, 1973.

© 1975 Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$15.00.



Fig. 1. UV Spectrum of I.

5'-(Lipoyl) adenylate (I) from Lipoic Anhydride and AMP. To a solution of 1.0 g of II in 9 ml of acetonitrile was added 0.8 g of dicyclohexyl-carbodiimide dissolved in 6 ml of acetonitrile. The mixture was stirred for 2 h at 20°, filtered, concentrated to 5 ml, and the unstable II anhydride was used directly in the preparation of I.

To a solution of 0.5 g of AMP in 15 ml of 30% aqueous pyridine was added II anhydride in 5 ml of acetonitrile at -2° . The reaction mixture was stirred for 1 h at -2 to -5° , diluted with 10 ml of cold water, washed with diethyl ether (twice in 15 ml), filtered, and extracted with cold chloroform (twice in 15 ml, then once in 10 ml). The gelatinous mass which separated was filtered off and washed with ice water (5 ml), absolute al-cohol (twice in 5 ml), and ether (10 ml). After drying in the vacuum desiccator over calcium chloride there was obtained 0.3 g (39%) of product, Rf 0.63. Found, %: C 40.11; H 5.1; S 12.3. C₁₈H₂₆N₅O₈PS₂. Calculated%: C 40.37; H 4.89; S 11.97.

<u>5'-(Lipoyl)adenylate (I) from Lipoic Acid (II) and AMP</u>. To a mixture of 0.310 g of II and 0.150 g of triethylamine in 15 ml of tetrahydrofuran was added dropwise with stirring 0.160 g of ethyl chloroformate in 2.0 ml of tetrahydrofuran at -5° . After 15 min, a cooled solution of 0.5 g of AMP in 10 ml of 30% aqueous pyridine was added. The reaction mixture was stirred for 1 h at -5 to -7° , filtered, diluted with 10 ml of water, and extracted with diethyl ether (twice in 10 ml), then chloroform (three times in 15 ml). The solid which separated on extraction was filtered off and washed quickly with ice water (10 ml), absolute alcohol (10 ml), and absolute ether (10 ml). Drying over calcium chloride in the vacuum desiccator gave 0.25 g (32.5%), R_f 0.63. Found, %: C 39.8; H 5.05; S 12.4. C₁₈H₂₆N₅₀₈PS₂. Calculated, %: C 40.37; H 4.89; S 11.97.

LITERATURE CITED

- 1. L. J. Reed, F. R. Leach, and M. Koike, J. Biol. Chem., 232, 123 (1958).
- 2. H. Nawa, W. F. Brady, M. Koike et al., J. Am. Chem. Soc., 82, 896 (1960).
- 3. K. Daigo and L. J. Reed, J. Am. Chem. Soc., 84, 666 (1962).
- 4. M. E. Semendyaeva, Z. S. Alekberova, and O. A. Ponomareva, Sov. Med., No. 2, 114 (1964).
- 5. A. Colarusso, in: Atti del Simposito Internazionale su L'acido Tioctico, (1955), p. 197.
- 6. F. Rausch, ibid., p. 177.
- 7. A. W. D. Avison, J. Chem. Soc., 732 (1955).