Syntheses of Adenosinesulfuric Acids

By Fujio EGAMI and Noriko TAKAHASHI

(Received July 22, 1955)

As is well known, adenosinephosphoric acids play important roles in metabolism as such or as components of the coferments such as DPN, TPN, FAD, CoA etc. In 1953[1], I. Yamashina and one of us (Egami), considering the fact that the substances having sulfuric acids group in place of phosphoric acid group in these metabolites might compete with the latter in metabolism or in biochemical reactions in vitro, tried to synthesize adenosinesulfuric acids. In fact they have found in the preliminary experiments that adenosinemonosulfate (AMS) competes with DPN in the dehydrogenation of ethanol by alcohol dehydrogenase. However, AMS used in the experiments was not a chemical individual, but a mixture of adenoshine-5'-monosulfate and adenosine-3'- or/and 2'-monosulfate. It is quite clear that in order to carry out quantitative studies concerning the effects of adenosinesulfates on metabolism or on biochemical reactions, adenosinesulfate with a definite constitution must be used. So we tried to separate adenosinesulfates by ion-exchange resins. The present paper describes the separation of adenosinesulfates and especially the preparation of adenosine-5'-monosulfate.

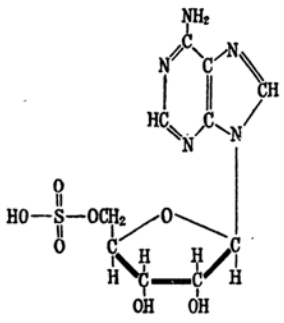

Fig. 1. Adenosine-5'-monosulfuric acid.

Experimental

1. Syntheses of Calcium Adenosinesulfates.—Chlorosulphonic acid in dry chloroform was added slowly to adenosine solution in dry pyridine at 0°C with vigorous stirring and with strict prevention of moisture. After standing overnight at room temperature, the upper solvent layer was removed by decantation, and the remaining syrupy material was dissolved in water, then neutralized with calcium carbonate. After removal of calcium sulfate and the excess of calcium carbonate, the filtrate was evaporated in vaccuo (40°C/15mmHg) until it became syrupy. A crude calcium salt was obtained by addition of alcohol. As will be shown later, the crude product was composed of unchanged adenosine and a mixture of calcium adenosinemono-, di- and trisulfate. Experiments were carried out under several conditions. It was found that the proportion of each component varied according to the amount of chlorosulphonic acid used and to the reaction temperature. Two examples, which will be described later, were carried out with the following conditions:

(Example 1) 2 g. adenosine in 100 ml. pyridine, 1.3 ml. chlorosulphonic acid in 4 ml. chloroform, reaction temperature 0°C.

(Example 2) 2.5 g. adenosine in 130 ml. pyridine. 3 ml. chlorosulphonic acid in 7 ml. chloroform, reaction temperature 13~15°C.

2. Analysis of the Crude Product.—A method for the quantitative separation of AMP, ADP and ATP by ion-exchange chromatography has been devised by Cohn and Carter[2]. By slight modification of their method, the resolution of the mixture of adenosinemono-, di- and trisulfuric acid (AMS, ADS, ATS) and unchanged adenosine into the individual components has been achieved successfully. The aqueous solution of crude product was passed through a column of an anion exchanger, Dowex-1. Unreacted adenosine was removed by washing with water, but the other acidic components remained on the column. Subsequent elution of the adsorbed column, successively with 1) 0.01N hydrochloric acid (AMS), 2) 0.2 or 0.5N hydrochloric acid (ADS), and 3) 1N hydrochloric acid (ATS), gave each component completely in separate fractions. In spite of the three possible structures for AMS, the largest part (100-95%) of effluents with 0.01N hydrochloric acid was composed of only one sharp peak, which was later proved to be adenosine-5'-monosulfuric acid. The other minor components, observed in a few cases, were too small in quantity to be served for analysis and remained unidentified.

A Beckman ultraviolet spectrophotometer Model DU was used to detect and estimate adenosine derivatives in effluents, taking advantage of the characteristic ultraviolet adsorption at 260mμ.

Quantitative analysis of N and S content for each fraction, also served as a means of identification.

The results obtained with the products, synthe-

1) I. Yamashina and F. Egami, *J. Japan Biochem. Soc.*, 23, 281 (1953); F. Egami, *Medicina Revuo*, 2, 26 (1954).

2) W.E. Cohn and C.E. Carter, *J. Am. Chem. Soc.*, 72, 4273 (1950).

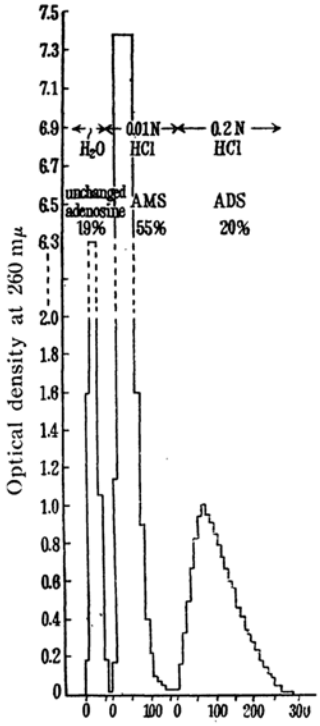

Fig. 2. (example 1). Ion-exchange separation of adenosinesulfates.
Exchanger: Dowex-1 (50~100 mesh), 0.8 $cm^2 \times 9$ cm. chloride form.
Flow rate: 1 ml./min./cm^2.
Test material: 30 mg.

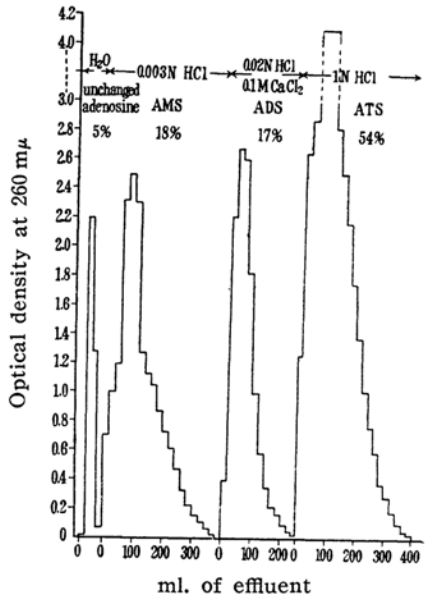

Fig. 3. (example 2). Ion-exchange separation of adenosinesulfates.
Exchanger: Dowex-1 (50~100 mesh), 1.77 $cm^2 \times 4$ cm. chloride form.
Flow rate: 0.5 ml./min./cm_2.
Test material: 60 mg.

sized under the conditions described above, was shown in Figs. 2 and 3, on which the experimental details for the analysis will be seen.

3. Preparation of Calcium and Barium Adenosinemonosulfate.—Two procedures were tried in order to obtain these components separately, one with hydrochloric acid as eluting solution, another with sulfuric acid. These were carried out as follows:

(A) Elution with Hydrochloric Acid.—Column of Dowex-1 (50~100 mesh) in the chloride form, 3.1 $cm^2 \times 20$ cm., flow rate 1 ml./min./cm^2 was used. 0.5 g. of the crude product in 100 ml. of water was passed through the column and the adsorbed materials were eluted with hydrochloric acid in a similar manner as used in the analytical procedure.

The pooled AMS fraction of the effluents (ca. 350 ml.) were neutralized with calcium carbonate and evaporated in vaccuo (40°C/15 mmHg) until it became syrupy. By addition of alcohol, the precipitate of Ca-AMS was obtained. The purification was achieved by reprecipitation with alcohol.

But, the similar procedure was not suitable for the preparation of di- and trisulfate in solid form, because of the high concentration of hydrochloric acid in ADS and ATS fractions, which brought about the formation of a large amount of calcium chloride interfering with the precipitation of Ca-ADS and Ca-ATS. However, this procedure was proved to be quite satisfactory for the analysis of these components, as described in the preceding paragraph.

(B) Elution with Sulfuric Acid.—Column of Dowex-1 (50~100 mesh) in the hydroxide form, 3.1 $cm^2 \times 31$ cm., flow rate 1 ml./min./cm^2 was used. 2 g. of the crude product in 100 ml. of water was served as starting material. Considerable amount of unreacted adenosine was adsorbed in this case, which was removed as a first fraction by elution with 0.03N sulfuric acid. Continuing elution with the same acid gave AMS as a sharply distinct fraction.

But, the other components did not show any difinite peaks with 0.5N, 1N, 2N, and even with 6N sulfuric acid. Therefore, application of this method to the elution of ADS and ATS was not satisfactory.

The pooled AMS fraction of the effluents (ca. 900 ml.) was neutralized with excess barium carbonate. By concentrating the filtrate in vaccuo (40°C/15 mmHg), Ba-AMS was obtained in colorless crystalline form. It was recrystallized from water.

4. Nature of Ba-AMS.—Crystalline Ba-AMS was found to contain 2 molecules of water which was lost by heating at 100°C in vaccuo over conc. sulfuric acid. The analytical results coincided with the formula $C_{10}H_{12}O_7N_5S$ Ba/2. Found: N, 16.5; S, 8.12; Ba, 16.4; Calcd.; N, 16.8; S, 7.73; Ba, 16.5%.

For the decision among possible positions 5′, 3′, and 2′ as the point of attachment of the sulfuric acid radical to ribose, the oxidation of Ba-AMS

with metaperiodate was examined. One mole of Ba-AMS consumed 0.94 moles of the oxidant. This indicates that the sulfuric acid radical is attached to the 5′-position of the ribose.

Spectrographic Data:[3] Measurements were made with a Beckman ultraviolet spectrophotometer Model DU. Comparisons of the ultraviolet absorption spectra from 220 mμ to 300 mμ of 0.1N hydrochloric acid solution of adenine, adenosine and Ba-AMS were shown in Fig. 4. It was clear from Fig. 4 that adenosine and Ba-AMS had a peak at 257mμ. But the maximum molecular extinction coefficient of AMS resembled more closely that of adenine than that of adenosine.

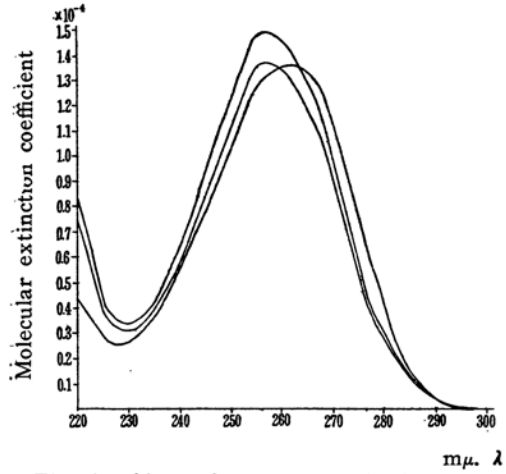

Fig. 4. Absorption spectra of adenosine (higher peak at 257 mμ), AMS (lower peak at 257 mμ) and adenine (peak at 262 mμ) respectively.

3) J.M. Gulland and E.R. Holiday, *J. Chem. Soc.*, 1936, 765.

Summary

Syntheses of adenosinesulfuric acids were achieved by sulfation of adenosine with chlorosulphonic acid.

The crude product was a mixture of AMS, ADS and ATS, and the proportion of each component varied according to the amount of chlorosulphonic acid used and to the reaction temperature.

Quantitative separation of AMS, ADS and ATS has been achieved successfully by ion-exchange chromatography.

Barium adenosine-5′-monosulfate was obtained in crystalline form, and the ultraviolet absorption spectra were measured.

Department of Chemistry, Nagoya University, Nagoya