Synthesis of "Active Methionine."

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S-(5′-Deoxyadenosine-5′)-methionine (I) has been synthesised and shown to be chemically indistinguishable from the natural transmethylation intermediate "active methionine."

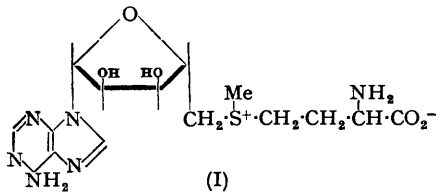
DL-α-Amino-γ-butyrolactone (II) was converted into DL-α-amino-γ-bromobutyric acid hydrobromide (III) which reacted with 5′-deoxy-5′-methylthioadenosine (IV) to give the sulphonium compound (I). The product possessed about half of the activity of "active methionine" in enzyme tests. This is the maximum activity expected from a synthesis which starts from racemic α-amino-γ-butyrolactone.

IN certain biological methylation processes adenosine triphosphate reacts enzymically with methionine to form an intermediate substance which is generally known as "active methionine" (Cantoni, *J. Biol. Chem.*, 1951, **189**, 745). The structure (I), proposed tentatively by Cantoni for this intermediate (*J. Amer. Chem. Soc.*, 1952, **74**, 2942; *J. Biol. Chem.*, 1953, **204**, 403), has been substantiated by a further study of its chemical and physical properties (Baddiley, Cantoni, and Jamieson, *J.*, 1953, 2662). A synthesis of *S*-(5′-deoxyadenosine-5′)-DL-methionine (I) described in this paper fully confirms the structure of this important nucleoside.

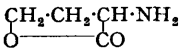
The most satisfactory general method for the unequivocal synthesis of asymmetrically substituted sulphonium compounds involves reaction between a sulphide and an alkyl halide. Three routes may be envisaged for the synthesis of (I) according to this general method. The first would require the condensation of methionine with a derivative of a 5′-deoxy-5′-halogenoadenosine. Adenosine derivatives of this type are not known and, by analogy with the corresponding toluene-*p*-sulphonyl compounds, would be very unstable, readily rearranging to *cyclo*nucleosides (Clark, Todd, and Zussman, *J.*, 1951, 2952). On the other hand this route should be applicable to the inosine and uridine series, where *cyclo*nucleoside formation has not been observed. Consequently, model experiments were carried out on 2′ : 3′-*O*-*iso*propylidene-5′-*O*-toluene-*p*-sulphonylinosine (Levene and Tipson, *J. Biol. Chem.*, 1935, **111**, 313) and 5′-deoxy-5′-iodo-2′ : 3′-*O*-*iso*propylideneuridine (*idem*, *ibid.*, 1934, **106**, 113). When these substances were treated with methionine under a variety of conditions they were usually recovered substantially unchanged and no evidence of sulphonium salt formation was obtained.

A second, more promising, route involves the formation of *S*-(5′-deoxyadenosine-5′)-homocysteine and subsequent methylation of this with methyl iodide. This approach is still under investigation and will be discussed fully in a later paper. The third route envisages reaction between 5′-deoxy-5′-methylthioadenosine ("adenine thiomethyl pento-

side") (IV) and a suitable derivative of an amino-halogeno-acid. 5'-Deoxy-5'-methylthioadenosine (IV) has been synthesised independently and almost simultaneously in three different laboratories (Baddiley, *J.*, 1951, 1348; Satoh and Makino, *Nature*, 1951, **167**, 238;

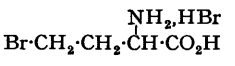


(I)

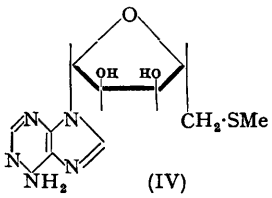


(II)

Weygand and Trauth, *Ber.*, 1951, **84**, 633) but the method of synthesis was similar in each case and the overall yield was very low. This was probably due in part to the formation of a *cyclo*nucleoside during the synthesis. During the course of the work described here the overall yield from 2' : 3'-*O*-*iso*propylideneadenosine has been increased to about 40%. This was achieved by the modifications given in the Experimental section and in particular by the use of a sulphonic acid-type ion-exchange resin for the isolation of the final product. The good overall yield on this three-stage synthesis disposes of suggestions (Clark, Todd, and Zussman, *loc. cit.*; Tipson, *Adv. Carbohydrate Chem.*, 1953, **8**, 107) that it might be unreliable as a proof of the structure of "adenine thiomethyl pentoside." At the same time, it should be emphasised that the original structural proof (Baddiley, *loc. cit.*) was quite independent of this synthesis.

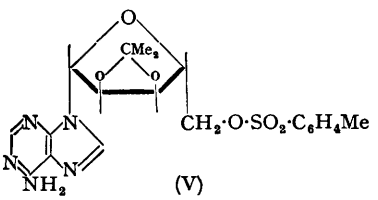


(III)

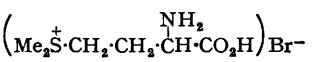


(IV)

An intermediate in the preparation of the methylthio-nucleoside is 2' : 3'-*O*-*iso*propylidene-5'-*O*-toluene-*p*-sulphonyladenosine (V) which is obtained by the action of toluene-*p*-sulphonyl chloride on 2' : 3'-*O*-*iso*propylideneadenosine in pyridine. There appears to be some confusion about the nature of the products of this reaction (cf. Tipson, *loc. cit.*). The *ON*-ditoluene-*p*-sulphonyl derivative is only formed in small amounts under the conditions described in this and earlier publications (Baddiley, *loc. cit.*; Clark, Todd, and Zussman, *loc. cit.*). The main product is the 5'-*O*-toluene-*p*-sulphonyl compound, which is rather unstable, readily yielding the *cyclo*nucleoside on standing. However, it is clear that, in view of the good yield of 5'-deoxy-5'-methylthioadenosine which can be obtained from it, the mono-compound is sufficiently stable to be of value in synthetic work.



(V)



(VI)

The required bromo-amino-acid, α-amino-γ-bromobutyric acid hydrobromide (III), was prepared in excellent yield by heating together the hydrobromide of α-amino-γ-butyrolactone (II) (Livak, Britton, VanderWeele, and Murray, *J. Amer. Chem. Soc.*, 1945, **67**, 2218) and hydrogen bromide in acetic acid. The structure of (III) was confirmed by its reaction with sodium ethyl sulphide in dimethylformamide, whereupon ethionine was obtained in 68% yield. DL-Methionine was prepared similarly from (III) and sodium methyl sulphide. The yield in this case was 60%. On hydrolysis in boiling water the bromo-acid gave α-amino-γ-butyrolactone. The bromo-amino-acid was converted into

the readily crystallisable γ-bromo-α-formamidobutyric acid by reaction with formic acid in the presence of acetic anhydride. The bromo-amino-acid and its *N*-formyl derivative evolved hydrogen bromide at the melting point : this reaction, characteristic for γ-bromo-acids, presumably yielded the respective γ-lactones.

The suitability of α-amino-γ-bromobutyric acid hydrobromide (III) for the preparation of sulphonium compounds was demonstrated by its reaction with dimethyl sulphide. Optimum conditions were not determined but it was shown that methionine methylsulphonium bromide (VI) was produced, although in rather low yield.

The choice of conditions for the conversion of the bromo-acid into " active methionine " is very limited in view of the great instability of the latter, particularly at elevated temperatures. Consequently, when the DL-bromo-compound (III) was allowed to react with 5′-deoxy-5′-methylthioadenosine in a mixture of formic and acetic acids (cf. Toennies and Kolb, *ibid.*, 1945, **67**, 849) much unchanged nucleoside could be recovered, even after several weeks at room temperature. However, paper chromatography of the reaction mixture in butanol–acetic acid–water indicated that, in addition to considerable amounts of starting materials, a substance was present which did not move from the origin. This was eluted from the paper and shown to be indistinguishable from " active methionine " on paper chromatography and electrophoresis. It absorbed ultra-violet light at 260 mμ and gave positive periodate-Schiff and ninhydrin reactions (Baddiley, Cantoni, and Jamieson, *loc. cit.*). Furthermore, it was readily converted into 5′-deoxy-5′-methylthioadenosine by heating of its aqueous solution. A preliminary account of this synthesis has been published (Baddiley and Jamieson, *Chem. and Ind.*, 1954, 375).

Enzyme tests performed by Dr. G. L. Cantoni showed that the synthetic material possesses 40—50% of the activity of the natural substance, both in the methylation of nicotinamide (Cantoni, *J. Biol. Chem.*, 1951, **189**, 203) and in creatine synthesis. Since the α-amino-γ-butyrolactone hydrobromide used in this synthesis was racemic, this represents a high order of biological activity. From the chemical and biological properties of both " active methionine " and the synthetic product we conclude that " active methionine " is correctly represented by (I).

Experiments on the preparation of L-α-amino-γ-butyrolactone and its subsequent conversion into *S*-(5′-deoxyadenosine-5′)-L-methionine are in progress.

Experimental

5′-Deoxy-5′-methylthioadenosine (*Adenine Thiomethyl Pentoside*).—2′ : 3′-*O-iso*-Propylideneadenosine (4·0 g., dried at 110°/0·1 mm. for 2 hr.) was dissolved in dry pyridine (100 c.c.) by gentle warming. The solution was cooled to −5° and toluene-*p*-sulphonyl chloride (2·75 g.) in dry benzene (10 c.c.) was added in a gentle stream. The bright yellow solution was kept at room temperature overnight and water (10 c.c.) was added. Solvent was removed *in vacuo* below 45°. The residue was dissolved in chloroform and washed with ice-cold N-sulphuric acid, cold sodium hydrogen carbonate solution, and with water. The chloroform layer was dried (Na_2SO_4) and evaporated below 40° to a brittle resin. This was used in the subsequent reactions without delay.

Methanethiol (dried over $CaCl_2$) was passed into a solution of sodium (2·3 g.) in dry methanol (*ca.* 50 c.c.) until an increase of 5 g. was observed. A large volume of dry ether was added and the sodium salt was filtered off and dried in a desiccator over phosphoric oxide.

The resinous toluene-*p*-sulphonyl derivative was dissolved in freshly distilled dimethylformamide (*ca.* 50 c.c.) and sodium methyl sulphide (3·0 g.) (above) was added. The resulting solution was heated on a steam-bath for 2 hr., during which solid was deposited. A little water was added to the cooled mixture, and the product was extracted with 4 lots of chloroform. The combined dried chloroform extracts were evaporated *in vacuo* and the residue was dissolved in acetic acid (25 c.c.). 0·2N-Sulphuric acid (25 c.c.) was added and the solution was kept at room temp. for 20 hr. Sulphuric acid was removed by adding the calculated amount of barium hydroxide solution. Barium sulphate was removed by centrifugation, and washed with water, and the combined supernatant liquid and washings were evaporated to dryness *in vacuo*. The residue was dissolved in water containing a little alcohol and passed through a column of Amberlite IR-120 resin (H^+ form). After washing of the column with 20% alcohol the product was eluted with a large volume of ammonia (equal parts of water and ammonia of *d* 0·880). The

eluate was evaporated to small volume *in vacuo* and the crystalline nucleoside (1·8 g.) was filtered off. Recrystallised from water it had m. p. 207°, undepressed on mixing with an authentic sample of 5′-deoxy-5′-methylthioadenosine (Baddiley, *loc. cit.*).

DL-α-*Amino-γ-bromobutyric Acid Hydrobromide.*—A mixture of α-amino-γ-butyrolactone hydrobromide (2·0 g.) and acetic acid (20 c.c.) saturated with hydrogen bromide was heated in a sealed tube at 110° for 7 hr. with occasional rocking. The contents of the tube were evaporated to dryness *in vacuo* and the residue was recrystallised from acetic acid. The *bromo-acid hydrobromide* (2·3 g.) formed elongated prisms, m. p. 163—166° with evolution of hydrogen bromide (Found: C, 18·9; H, 3·4; N, 5·3; Br, 60·1. $C_4H_9O_2NBr_2$ requires C, 18·3; H, 3·4; N, 5·3; Br, 60·7%).

DL-*Ethionine.*—To a solution of sodium (0·46 g., 4 mols.) in methanol (25 c.c.) was added ethanethiol (2 c.c.) in dimethylformamide (50 c.c.), and the solution was evaporated to small volume *in vacuo*. Dimethylformamide (25 c.c.) was added, followed by a solution of the above hydrobromide (1·3 g., 1 mol.) in dimethylformamide (20 c.c.), and the resulting solution was heated at 100° for 2 hr. Solvent was removed *in vacuo* and the residue was dissolved in water then passed through a column of Amberlite IR-120 resin (ammonium form) to remove sodium ions. After concentration, the eluate was passed through a column of Amberlite IR-4B resin to remove bromide ions and evaporated to dryness. The crystalline residue was washed with warm alcohol, then acetone. Ethionine (0·55 g., 68%) formed colourless plates from aqueous alcohol. A sample run on paper in butanol–acetic acid–water (4 : 1 : 5) was homogeneous when examined by the ninhydrin spray and also by the spray for sulphides (Winegard, Toennies, and Block, *Science*, 1948, **108**, 506). It had R_F 0·53 and was indistinguishable from authentic ethionine (Found: C, 44·0; H, 7·8; N, 8·9; S, 20·1. Calc. for $C_6H_{13}O_2NS$: C, 44·1; H, 8·0; N, 8·6; S, 19·7%).

DL-*Methionine.*—Dry, gaseous methanethiol (Arndt, *Ber.*, 1921, **54**, 2238) was passed into a solution of sodium (0·46 g., 4 mols.) in methanol (25 c.c.) until an increase of 0·96 g. was obtained. Dimethylformamide (50 c.c.) was added and the solution was evaporated to small volume *in vacuo*. A further quantity (50 c.c.) of dimethylformamide was added, followed by a solution of α-amino-γ-bromobutyric acid hydrobromide (1·3 g., 1 mol.) in dimethylformamide (20 c.c.). The resulting solution was heated at 100° for 2 hr., then the product was isolated in a manner similar to that described for the ethionine synthesis. Methionine (0·43 g., 56%) was obtained as colourless plates from aqueous alcohol. A sample, when run on paper in butanol–acetic acid–water (4 : 1 : 5), was homogeneous with respect to the ninhydrin and sulphide spray reagents. It had R_F 0·55, identical with that of authentic methionine (Found: C, 39·8; H, 7·4; N, 9·4. Calc. for $C_5H_{11}O_2NS$: C, 40·0; H, 7·4; N, 9·4%).

DL-*γ-Bromo-α-formamidobutyric Acid.*—The above bromo-acid hydrobromide (5 g.) was dissolved in a mixture of formic acid (100 c.c.) and acetic acid (10 c.c.). Anhydrous sodium acetate (1·56 g.) was added, followed by acetic anhydride (15 c.c.), and the resulting solution was set aside at room temperature overnight. Solvent was removed by evaporation *in vacuo* and the crystalline residue was triturated with a little water. DL-*γ-Bromo-α-formamidobutyric acid* (3·75 g., 94%) had m.p. 142—143° after recrystallisation from alcohol. It had R_F 0·61 in butanol–acetic acid–water (4 : 1 : 5) (Found: C, 28·6; H, 3·6; N, 6·7; Br, 38·3. $C_5H_8O_3NBr$ requires C, 28·6; H, 3·8; N, 6·7; Br, 38·1%).

Hydrolysis of DL-α-*Amino-γ-bromobutyric Acid.*—A sample of the hydrobromide (112 mg.) was heated in water (2 c.c.) under reflux for 30 min. The solution was evaporated to small volume *in vacuo* and then evaporated to dryness over sodium hydroxide in a desiccator. White prisms of DL-α-amino-γ-butyrolactone hydrobromide (63 mg., 81%) were obtained, having m. p. 218—221°. The authentic DL-amino-lactone hydrobromide has m. p. 216—222°.

Methionine Methylsulphonium Bromide.—To a solution of α-amino-γ-bromobutyric acid hydrobromide (430 mg.) in formic acid (6 c.c.) and acetic acid (2 c.c.) was added dimethyl sulphide (450 mg., 4 mols.). The solution was set aside at room temperature overnight and solvent was evaporated *in vacuo*. A sample of the crystalline residue was examined by paper chromatography. In butanol–acetic acid–water a spot, R_F 0·1, was observed which was indistinguishable from that given by authentic methionine methylsulphonium iodide (Toennies and Kolb, *loc. cit.*). Both substances had R_F 0·41 in *n*-propanol–ammonia–water (6 : 3 : 1). On paper electrophoresis the sulphonium compound migrated towards the cathode at 0·7 cm. hr.$^{-1}$/v cm.$^{-1}$. However, the substance which had been synthesised from the bromo-acid was contaminated with a relatively large amount of starting material or its decomposition products. The yield of sulphonium compound was determined by precipitation as its phosphotungstate (Lavine and Floyd, *J. Biol. Chem.*, 1954, **207**, 97). The above residue (115 mg.) was dissolved in 30%

aqueous alcohol (1·5 c.c.) and to this was added a 25% aqueous solution of phosphotungstic acid (1 c.c.). The precipitate was centrifuged, suspended in alcohol, and re-centrifuged. The phosphotungstate (10 mg.) was dried over phosphoric oxide for 1·5 hr. at 80°/0·2 mm. Methionine methylsulphonium iodide (98 mg.), when treated similarly, gave 163 mg. of phosphotungstate.

S-(5′-*Deoxyadenosine*-5′)-DL-*methionine*.—To a solution of 5′-deoxy-5′-methylthioadenosine (100 mg.) in formic acid (1·5 c.c.) and acetic acid (0·5 c.c.) was added DL-α-amino-γ-bromobutyric acid hydrobromide (104 mg.). The resulting solution was set aside at room temperature in the dark for 5 days. Solvent was removed *in vacuo* at room temperature and the crystalline residue was dissolved in a little water and then applied to several paper chromatograms (Whatman No. 3) as a band at the origin. The chromatograms were developed in butanol–acetic acid–water (4 : 1 : 5) and the sulphonium compound remained at the origin. The yield (*ca.* 2%) was determined by eluting a known area of the ultra-violet-absorbing band at the origin and measuring the absorption at 260 mμ. The band gave ninhydrin and periodate-Schiff reactions, typical for " active methionine " (Baddiley, Cantoni, and Jamieson, *loc. cit.*).

A portion of the aqueous eluate was heated in a sealed tube at 120° for 15 min. and then evaporated to dryness in a desiccator. The residue was indistinguishable from authentic 5′-deoxy-5′-methylthioadenosine, R_F 0·71, when examined by paper chromatography in butanol–acetic acid–water (4 : 1 : 5). When examined by paper electrophoresis in the apparatus of Markham and Smith (*Nature*, 1951, **168**, 406) at pH 7 the rates of progression (cm. hr.$^{-1}$/v cm.$^{-1}$) towards the cathode were " active methionine " 0·45, *S*-(5′-deoxyadenosine-5′)-DL-methionine 0·43, and 5′-deoxy-5′-methylthioadenosine 0·25.

Enzymic Methylation (based on data supplied by Dr. G. L. Cantoni).—The synthetic sulphonium compounds were eluted from fresh chromatograms and the amount of material in the eluate was determined by ultra-violet spectroscopy. Creatine synthesis was measured by the method of Cantoni and Vignos (*J. Biol. Chem.*, in the press).

	μmol.	Creatine formed (μmol.)	Utilisation (%)
" Active methionine "	0·634	0·644	100
	0·317	0·336	100
Synthetic (I) from DL-bromo-acid	0·427	0·182	42·6
	0·213	0·106	49·5

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