Synthesis of S-(5′-*Deoxyadenosine*-5′)-*homocysteine, a Product from Enzymic Methylations involving "Active Methionine."*

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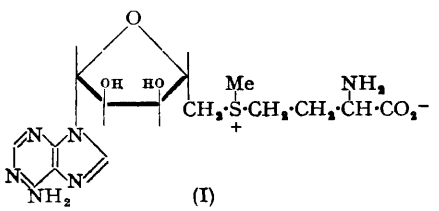
S-(5′-Deoxyadenosine-5′)-homocysteine (II) has been synthesised by reaction of 2′ : 3′-*O*-*iso*propylidene-5′-*O*-toluene-*p*-sulphonyladenosine (IV) and the disodium salt of homocysteine, followed by removal of the *iso*-propylidene group with acid. It is identical with a product formed from "active methionine" during enzymic methylations.

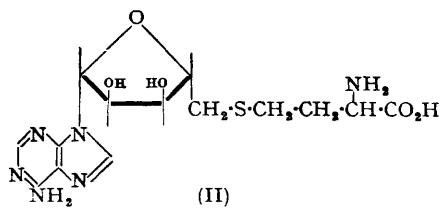
Methylation of the product (II) gives "active methionine" in good yield.

The course of enzymic methylations, in which the methyl group of methionine is transferred to certain substrates, has been clarified considerably by Cantoni (*J. Biol. Chem.*, 1951, **189**, 203; 1951, **189**, 745; *J. Amer. Chem. Soc.*, 1952, **74**, 2942; "Phosphorus Metabolism," 1952, Vol. II, 129). The sequence of events is illustrated here in the typical case of the formation of creatine from guanidinoacetic acid. Methionine reacts with adenosine triphosphate (ATP) in the presence of an enzyme to give "active methionine" and three mols. of inorganic phosphate. "Active methionine" then donates a methyl group to guanidinoacetic acid in the presence of a second enzyme, with the formation of creatine. Until recently, however, the fate of the "active methionine" molecule was uncertain.

The formulation of "active methionine" as the sulphonium compound (I) (Cantoni, *J. Amer. Chem. Soc.*, 1952, **74**, 2942; *J. Biol. Chem.*, 1953, **204**, 403) has been confirmed by further degradation studies (Baddiley, Cantoni, and Jamieson, *J.*, 1953, 2662) and by a total synthesis (Baddiley and Jamieson, *J.*, 1954, 4280). Consequently, it has been suggested that the primary product resulting from the transmethylation reactions involving "active methionine" should be *S*-(5′-deoxyadenosine-5′)-homocysteine (II) (Woolley, *Nature*, 1953, **171**, **323**; Baddiley, Cantoni, and Jamieson, *loc. cit.*; Challenger, *Endeavour*, 1953, **12**, 173). This has now been confirmed by the isolation of this primary product from an enzymic system in which "active methionine" effects the methylation of guanidinoacetic acid (Cantoni and Scarano, *J. Amer. Chem. Soc.*, 1954, **76**, 4744) and by its identification with a sample of synthetic *S*-(5′-deoxyadenosine-5′)-homocysteine prepared by the methods described here.



(I)



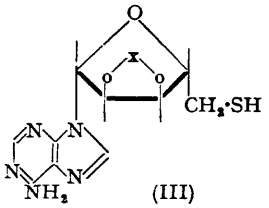
(II)

In a possible route for the synthesis of (II) we envisaged the preparation of 5′-deoxy-5′-mercapto-2′ : 3′-*O*-*iso*propylideneadenosine (III) and subsequent reaction of this with α-amino-γ-bromobutyric acid, followed by hydrolysis of the *iso*propylidene residue. The mercapto-derivative (III) of adenosine was prepared in rather low yield by heating together 2′ : 3′-*O*-*iso*propylidene-5′-*O*-toluene-*p*-sulphonyladenosine and potassium thiolacetate under conditions similar to those adopted by Chapman and Owen (*J.*, 1950, 579) for the conversion of toluene-*p*-sulphonyl esters into esters of thiolacetic acid. The acetyl group was removed readily by the action of methanolic ammonia.

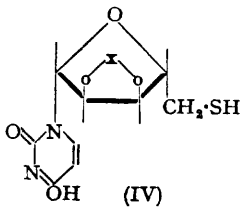
In view of the difficulties encountered in the preparation of useful amounts of the thiol (III), experiments were performed on uridine derivatives. One of the main side reactions in the adenosine series, *cyclo*nucleoside formation, does not occur readily with uridine derivatives. 2′ : 3′-*O*-*iso*Propylidene-5′-*O*-toluene-*p*-sulphonyluridine reacted smoothly

with potassium thiolacetate and the resulting thiolacetate was deacetylated to the thiol (IV), in 88% overall yield. However, attempts to prepare a mixed sulphide from (IV) and α-amino-γ-bromobutyric acid hydrobromide or γ-bromo-α-formamidobutyric acid were unsuccessful.

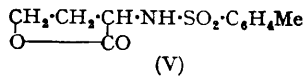
It is possible that the acidic nature of uridine and its derivatives was responsible in part for our failures in this route. This is supported by the observation that 5′-deoxy-5′-mercapto-2′ : 3′-*O*-*iso*propylideneadenosine (III), when heated with methyl γ-bromo-α-formamidobutyrate and then subjected to cautious acid and alkaline hydrolysis, yielded traces of a product with the properties expected for *S*-(5′-deoxyadenosine-5′)-homocysteine (II).



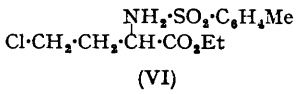
(III)



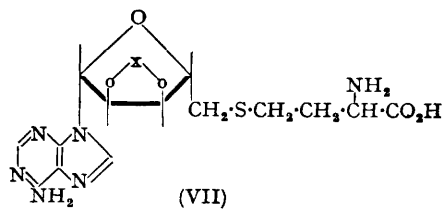
(IV)



(V)



(VI)



(VII)

X = CMe_2.

The low yield and multiplicity of products in the above synthesis might have arisen in the attempted removal of the formyl group. Toluene-*p*-sulphonyl groups, on the other hand, can be removed readily from amino-compounds with sodium in liquid ammonia. A modified synthesis adopting this feature was attempted. α-Toluene-*p*-sulphonamido-γ-butyrolactone (V) was converted into ethyl γ-chloro-α-toluene-*p*-sulphonamidobutyrate (VI) by the action of dry hydrogen chloride in ethanol. This reacted readily with sodium ethyl sulphide and then yielded ethionine on hydrolysis. The chloro-ester (VI) was heated in dimethylformamide with the sodium derivative of the thiol (III); the resulting ester was hydrolysed with dilute alkali and then treated with sodium in liquid ammonia. Paper chromatography of the product indicated that it was a mixture. However, one spot corresponded with that given by *S*-(5-deoxy-2′ : 3′-*O*-*iso*propylideneadenosine-5′)-homocysteine (VII) (see below). One of the contaminants was *S*-(5′-deoxyadenosine-5′)-homocysteine (II) itself, which presumably arose through partial loss of the *iso*propylidene group during isolation. The *iso*propylidene derivative (VII) was partly converted into the desired compound (II) by acid hydrolysis but the final product was a rather complex mixture which was not examined further.

A much more satisfactory synthesis starts from homocysteine. The disodium derivative of homocysteine, prepared in liquid ammonia, was allowed to react with 2′ : 3′-*O*-*iso*propylidene-5′-toluene-*p*-sulphonyladenosine either in liquid ammonia at a low temperature or in dimethylformamide at 100°, the product (VII) being obtained as a white solid after removal of toluene-*p*-sulphonate and sodium ions on ion-exchange resins. The *iso*propylidene group in (VII) was rather resistant towards acid hydrolysis. Paper chromatography showed that, under conditions which usually effect complete hydrolysis of *iso*propylidene derivatives of adenosine, not more than about 50% removal had occurred from (VII). More prolonged reaction, however, led to complete hydrolysis and a good yield of crystalline *S*-(5′-deoxyadenosine-5′)-homocysteine.

The synthetic substance was compared by paper chromatography with material isolated by Cantoni and Scarano (*loc. cit.*) from an enzyme system in which " active

methionine " had effected methylation of guanidinoacetic acid. Both substances had the same R_F in several solvent systems, absorbed ultra-violet light at 260 mμ, and gave positive reactions on the paper with the periodate–Schiff, ninhydrin, and iodoplatinate spray reagents. We conclude that the two substances are identical, and hence that the primary enzymic demethylation product formed from " active methionine " is correctly represented as (II). The subsequent fate of this product in biological systems is uncertain but it is hoped that the solution of this problem may be facilitated by the availability of synthetic *S*-(5′-deoxyadenosine-5′)-homocysteine.

Methylation of *S*-(5′-deoxyadenosine-5′)-homocysteine with methyl iodide gave an excellent yield of " active methionine." This not only confirms the structure of the synthetic material but also provides a convenient route for the preparation of quantities of " active methionine."

Experimental

5′-Deoxy-5′-mercapto-2′ : 3′-O-iso*propylideneadenosine* (III).—A solution of *2′ : 3′-O-iso*propylidene-*5′-O*-toluene-*p*-sulphonyladenosine (5·2 g.) (cf. Baddiley and Jamieson, *loc. cit.*) and dry potassium thiolacetate (6 g.) in alcohol–acetone (50 c.c.; 1 : 1) was refluxed for 3 hr. Solid was filtered off and washed with acetone, then the filtrate and washings were evaporated to dryness *in vacuo*. The residue was dissolved in chloroform and washed with a little water, and solvent was removed *in vacuo*. The impure acetyl derivative was dissolved in methanol (50 c.c.), and the solution was saturated at room temperature with dry ammonia. After 10 min. the solvent was evaporated and the partly crystalline residue was washed with a little cold alcohol. The *mercapto-compound* had m. p. 186—189° (Found: C, 48·0; H, 5·2; N, 21·7; S, 10·3. $C_{13}H_{17}O_3N_5S$ requires C, 48·2; H, 5·2; N, 21·6; S, 9·9%).

2′ : 3′-O-iso*Propylideneuridine*.—Levene and Tipson (*J. Biol. Chem.*, 1934, **106**, 113) report m. p. 159—160° for this substance. Our material, prepared in a similar manner, had m. p. 162°.

2′ : 3′-O-iso*Propylidene-5′-toluene*-p-*sulphonyluridine*.—This compound was obtained as a resin by Levene and Tipson (*loc. cit.*). It was found here that, after being boiled with ether, the toluene-*p*-sulphonyl derivative formed well-defined crystals (17 g. from 17 g. of the *iso*-propylidene compound). Recrystallised from acetone–ether containing a little light petroleum (40—60°) it had m. p. 150°.

5′-Deoxy-5′-mercapto-2′ : 3′-O-iso*propylideneuridine* (IV).—This was prepared from the above toluenesulphonyl derivative (4·4 g.) and potassium thiolacetate (2·2 g.) in acetone (100 c.c.) as described above for the adenosine compound. Fine needles of the *mercapto-compound* were formed during the treatment with methanolic ammonia. These were filtered off after concentration of the solution, then washed with ether. Recrystallised from methanol–ethanol (1 : 1) they had m. p. 200—215° (2·5 g., 88%) (Found: C, 48·0; H, 5·2; N, 9·7; S, 11·5. $C_{12}H_{16}O_5N_2S$ requires C, 47·4; H, 5·3; N, 9·4; S, 10·7%).

α-Toluene-p-*sulphonamido-γ-butyrolactone* (V).—Toluene-*p*-sulphonyl chloride (5 g.) was shaken with a suspension of α-amino-γ-butyrolactone hydrobromide (4·5 g.) in dry pyridine (50 c.c.) at room temperature overnight. Pyridine was removed *in vacuo*, a little water was added, and the mixture was extracted with 3 lots of chloroform. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated to dryness *in vacuo*. Recrystallisation of the residue from water gave white prisms (4·2 g.) of the *toluene*-p-*sulphonyl derivative*, m. p. 108—110° (Found: N, 5·4; S, 12·9. $C_{11}H_{13}O_4NS$ requires N, 5·7; S, 13·1%).

Ethyl γ-Chloro-α-toluene-p-*sulphonamidobutyrate* (VI).—A solution of the above toluene-*p*-sulphonyl derivative (1 g.) in alcohol (30 c.c.) was saturated with dry hydrogen chloride at room temperature and set aside overnight. Solvent was removed *in vacuo* and the residue was recrystallised from light petroleum. The *chloro-ester* (0·8 g.) had m. p. 65—67° (Found: C, 49·3; H, 5·8; N, 4·7; Cl, 10·4. $C_{13}H_{18}O_4NSCl$ requires C, 48·8; H, 5·6; N, 4·4; Cl, 10·9%).

Ethionine.—Ethanethiol (0·26 g.) was added to a solution of sodium (0·09 g.) in alcohol (25 c.c.), and the solution was evaporated to about 10 c.c. Dimethylformamide (25 c.c.) was then added, followed by a solution of the above chloro-ester (0·63 g.) in dimethylformamide (25 c.c.), and the mixture was heated at 100° for 3 hr. Solvent was removed *in vacuo* and the residue was extracted with 3 lots of chloroform. Chloroform was evaporated and the residue was heated under reflux for 20 min. in N-sodium hydroxide (20 c.c.). The solution was then passed down a column of Amberlite IRC-50 resin (H^+ form) to remove sodium ions and concentrated *in vacuo* to a light oil. This was dissolved in liquid ammonia (20 c.c.), and

sodium was added until a blue colour persisted. Ammonia was allowed to evaporate and the resulting solid was dissolved in water and passed through a column of Amberlite IR-120 (ammonium form). After concentration the eluate was passed through a column of Amberlite IR-4B (hydroxyl form) to remove toluene-*p*-sulphonic acid. The eluate was evaporated to dryness and the crystalline product was washed with alcohol and recrystallised from aqueous alcohol. Ethionine (0·04 g.) had m. p. 235° (decomp.) and R_F 0·51 in butanol–acetic acid–water, indistinguishable from that of an authentic sample.

S-(5′-*Deoxyadenosine*-5′)-*homocysteine* (II).—*Method* 1. To a solution of γ-bromo-α-formamidobutyric acid (1·7 g.) (Baddiley and Jamieson, *loc. cit.*) in dry methanol was added an excess of freshly distilled ethereal diazomethane. Solvent was removed *in vacuo*, leaving a clear yellow oil. Sodium was added in small pieces to a solution of the mercaptoadenosine derivative (III) (0·6 g.) in liquid ammonia until the blue colour persisted. Ammonia was evaporated *in vacuo* and dry dimethylformamide (5 c.c.) was added followed by a solution of the above methyl ester of γ-bromo-α-formamidobutyric acid (0·45 g.) in dimethylformamide (10 c.c.). The mixture was heated for 3 hr. at 100° under nitrogen. Solvent was removed *in vacuo*, a little water was added, and the solution was extracted with 3 lots of chloroform. The aqueous layer was acidified and re-extracted with chloroform, then the combined organic extracts were evaporated to dryness *in vacuo*. The residue was heated under reflux in 0·1N-sodium hydroxide (5 c.c.) for 3 min. The resulting solution was passed through a column of Amberlite IR-120 resin (H^+ form), the column was washed well with water, and the products were eluted with a large volume of ammonia [equal parts of ammonia (*d* 0·88) and water]. The eluate was evaporated to dryness *in vacuo*. Paper chromatography of the product showed the presence of several components. One of these components had R_F 0·69 in butanol–acetic acid–water, absorbed ultra-violet light, and gave a positive test for a sulphide with the iodoplatinate spray and negative tests with ninhydrin and periodate–Schiff reagents. This was probably the formyl derivative of (VII). A faint spot, R_F 0·08, gave positive ninhydrin and periodate–Schiff tests and was probably the desired homocysteine derivative (II), arising by hydrolysis of the formyl derivative on the column. However, in view of the complex nature of the mixture, suitable conditions for the hydrolysis of the formyl derivative of (VII) to (II) were not determined.

Method 2. A solution of ethyl γ-chloro-α-toluene-*p*-sulphonamidobutyrate (VI) (0·9 g.) in dry dimethylformamide (50 c.c.) was added to the dry, freshly prepared sodium derivative of the mercaptoadenosine compound (III) (0·87 g.). The resulting solution was heated at 100° for 1 hr. during which some solid was deposited. Alkaline hydrolysis of the ester, removal of the toluene-*p*-sulphonyl group, and isolation of the product were carried out as described in the ethionine synthesis above. The expected product at this stage is the *iso*propylidene derivative (VII). In fact, the product was not homogeneous but one component, R_F 0·3 in butanol–acetic acid–water, had the properties expected for this substance. It absorbed ultra-violet light and gave positive reactions with ninhydrin and iodoplatinate spray reagents and a negative reaction with the periodate–Schiff reagent. It had the same R_F as the authentic *iso*propylidene compound (VII) prepared by the method described below. A small amount of *S*-(5′-deoxyadenosine-5′)-homocysteine (II) was also present, the amount being increased by acid hydrolysis as described in Method 3.

Method 3. Sodium (0·46 g.) was added to a solution of homocysteine (1·36 g.) in liquid ammonia (50 c.c.), and then a solution of freshly prepared 2′ : 3′-*O*-*iso*propylidene-5′-*O*-toluene-*p*-sulphonyladenosine (from 4·6 g. of 2′ : 3′-*O*-*iso*propylideneadenosine) in liquid ammonia (50 c.c.). Moisture was excluded by a sodium hydroxide tube and solvent was allowed to evaporate. The residue was dissolved in water and unchanged homocysteine (0·1 g.) was filtered off. The filtrate was passed through a column of Amberlite IR-120 (ammonium form) to remove sodium ions, and the eluate was concentrated *in vacuo* and passed through a column of Amberlite IR-4B (hydroxyl form) to remove toluene-*p*-sulphonate ions. The eluate from the second column was evaporated to dryness *in vacuo*, leaving a solid residue of *S*-(5′-*deoxy*-2′ : 3′-O-iso*propylideneadenosine*-5′)-*homocysteine* (VII), R_F 0·3 in butanol–acetic acid–water, R_F 0·61 in *n*-propanol–ammonia–water. This substance absorbed ultra-violet light and gave positive reactions with ninhydrin and iodoplatinate spray reagents and a negative periodate–Schiff reaction. The only detectable impurity was a small amount of homocysteine. The *iso*propylidene residue was removed by hydrolysis in N-sulphuric acid (16 c.c.) at room temperature for 36 hr. Sulphuric acid was removed by addition of the calculated amount of barium hydroxide solution, and barium sulphate was then removed by filtration through Supercel silica and was washed well with water. The combined filtrate and washings were evaporated

to dryness *in vacuo* and the residue was recrystallised from aqueous alcohol. *S*-(5′-Deoxyadenosine-5′)-homocysteine (II) (520 mg.) formed small small prisms, m. p. 190—193° (decomp.) raised by several recrystallisations to 204° (decomp.). It gave a *picrate*, m. p. 170° (decomp.) (Found: C, 37·6; H, 3·9; N, 20·4; S, 4·6. $C_{20}H_{23}O_{12}N_9S,H_2O$ requires C, 38·0; H, 3·9; N, 19·9; S, 5·1%). The synthetic and the natural material were indistinguishable in R_F and chemical reactions on paper. In butanol–acetic acid–water both had R_F 0·08, and in *n*-propanol–ammonia–water R_F 0·38.

S-(5′-*Deoxyadenosine*-5′)-*methionine* (*Active Methionine*) (I).—The above derivative (II) (186 mg.) was dissolved in acetic acid (2 c.c.), and methyl iodide (0·5 c.c.) was added. The tube was sealed and kept at room temperature in the dark for 6 days. Solvent was removed at room temperature *in vacuo* and the resulting gum was dissolved in 0·1N-hydrochloric acid (3 c.c.). To this solution was added a saturated aqueous solution of Reinecke salt (adjusted to pH 2 with hydrochloric acid) until precipitation was complete. The precipitated salt (*ca.* 350 mg.) was shaken in dry acetone with silver sulphate (0·5 g.) at room temperature for 24 hr. Solid material was removed by filtration and washed with warm methanol. The combined filtrates were evaporated to dryness *in vacuo*. Chromatography of the residue in butanol–acetic acid–water showed the presence of a single ultra-violet-absorbing spot which did not move from the origin. This was indistinguishable from natural active methionine when examined by the spray reagents described by Baddiley and Jamieson (*loc. cit.*). A sample was heated in water at 90° for 2 hr. and re-examined by paper chromatography: like natural active methionine, it was converted in this way into 5′-deoxy-5′-methylthioadenosine, R_F 0·70 in butanol–acetic acid–water.

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