AN IMPROVED SYNTHESIS OF S-ADENOSYL-L-HOMOCYSTEINE AND RELATED COMPOUNDS*

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5'-Chloro-5'-deoxy-2',3'-O-isopropylideneadenosine reacts with disodium salts of L-homocysteine, L-cysteine or 3-mercaptopropanoic acid in liquid ammonia to afford 2',3'-O-isopropylidene derivatives which are easily desalted by chromatography on octadecyl-silica column. On acid treatment, the high purity preparations of S-adenosyl-L-homocysteine, S-adenosyl-L-cysteine, and 5'-carboxyethylthio-5'-deoxyadenosine are obtained in respectable yields.

The recently increasing interest in S-adenosyl-L-homocysteine (SAH) follows from the understanding of its implication in the regulatory system of biological methylations[1,2]. Therefore, suitable synthetic procedures for the preparation of SAH, 5'-substituted 5'-deoxy-5'-thioadenosines and their analogues are urgently needed. In addition to the methods which utilize enzymatically catalyzed reactions[3,4] several chemical procedures have been hitherto described for this purpose. All the recent approaches consist in a replacement of a suitable leaving group at the $C_{(5')}$-position by a sulfur nucleophile. Depending upon the character and possible subsequent reactions of the nucleophile, such a reaction can be performed in an aqueous solution[5-7], dimethylformamide or perhaps most advantageously in anhydrous liquid ammonia[8]. However, the need for excess nucleophile, as well as the inevitable formation of inorganic salts during the reaction, substantiate the main obstacles in the isolation of pure reaction products which are easily soluble in water and possess an ionic character. The purification problem is particularly important for these compounds since they are generally aimed at the biological and/or medicinal application.

This paper describes an improved procedure for the synthesis of SAH and some of its analogues which is based on the above principles but subordinates the strategy to the requirements of the isolation techniques. For the separation of salts and other low-molecular ionic materials from SAH and its derivatives we have chosen chromatography on an octadecyl-silica column as the most promissing technique. This separation is a simple procedure which does not involve acid or alkaline conditions, it has

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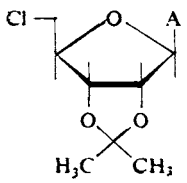
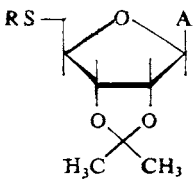
a high capacity and is not time-consuming. Since unprotected purine nucleoside derivatives would not be sufficiently retained by the column, it is necessary to increase the hydrophobicity by introducing a suitable substituent. The 2',3'-O-isopropylidene group at the adenosine moiety was found satisfactory for this purpose. Thus, the synthesis starts from the 5'-chloro-5'-deoxyadenosine[9] which is converted into its 2',3'-O-isopropylidene derivative *I* (*cf.*[10]). This compound reacts with disodium salt of L-homocysteine (L-cysteine, 3-mercaptopropanoic acid) generated *in situ* from the corresponding disulfide in boiling anhydrous ammonia[7,8]. Since the nucleophilic displacement reaction could be accompanied by a β-elimination to a ,,4',5'-dehydroadenosine" derivative, it is important to remove the solvent rapidly and at low temperature on evaporation *in vacuo*. The residual material must be rapidly transferred into a neutral aqueous solution and the traces of the above by-product removed by chloroform extraction. The reaction mixture is then separated on a octadecyl-silica column pre-equilibrated with water. Water elution removes virtually all inorganic salts, whereas the product *II* can be obtained by stepwise elution with increasing concentration of methanol (or other polar solvent) in water.

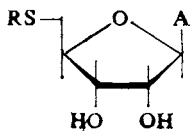
The obtained protected compounds *II* are treated with dilute sulfuric acid the excess of which is removed by exact neutralisation with barium hydroxide solution. The resulting products are passed through a cation exchanger in an ammonium cycle; on evaporation and crystallization from water, S-adenosyl-L-homocysteine (*IIIa*), S-adenosyl-L-cysteine (*IIIb*), and 5'-(2-carboxyethylthio)-5'-deoxyadenosine are obtained in very high purity. In particular, the compounds are free of amino acids, thio acids, and the products therefrom.



I



II



III

a, R = —$CH_2CH_2CH(NH_2)COOH$
b, R = —$CH_2CH(NH_2)COOH$
c, R = —CH_2CH_2COOH

A ... adenin-9-yl residue

The above isolation procedure can be modified by using a linear gradient of increasing polarity; also, the isopropylidene group can be replaced by more hydrophobic equivalents (cyclohexylidene, benzylidene, *etc.*) in those cases, where the

retention of isopropylidene derivatives would not suffice for effective desalting of the crude mixture.

EXPERIMENTAL

If not stated otherwise, the solutions were evaporated at 40°C/2 kPa. Melting points were estimated on a Kofler apparatus and are uncorrected. Ultraviolet spectra were recorded on a Specord UV/VIS spectrometer in aqueous solutions. The elution of the columns was continually followed by the Uvicord apparatus (L.K.B., Sweden). Paper chromatography was made using paper Whatman No 1, in the system (S1) 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), paper electrophoresis on the same paper at 40 V/cm (1 h) in 0·1 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5; thin-layer chromatography was performed on Silufol UV 235 silica sheets (Kavalier, Czechoslovakia) in the system (S2) chloroform–methanol (9 : 1). Octadecyl-silica (20 μ) was purchased from Laboratory Equipment, Prague (Czechoslovakia).

5-Chloro-5′-deoxyadenosine

Thionyl chloride (15 ml) was added dropwise under stirring and icecooling to hexamethylphosphoramide (100 ml). After 15 min stirring at 0°C, adenosine (10 g) was added and the mixture stirred under exclusion of moisture for 2 h at 0°C and then at room temperature overnight. The resulting opalescent solution was poured into ice-water (1 litre), left standing for 2 h and filtered through Celite. The extract was extracted with chloroform (5 × 100 ml) and the aqueous solution passed through Dowex 50X8 (H^+) column (600 ml). The column was eluted (5 ml/min) with water until the UV-absorption and conductivity of the eluate dropped. The resin was suspended in water (1 litre) and concentrated aqueous ammonia was added dropwise under stirring to reach a constant value of pH 9·0—9·1. The slurry was filtered and washed with boiling water (total, 3 l) and the filtrate and washings were combined and concentrated *in vacuo* to approx. 150 ml. After standing overnight at 0°C, the mixture was filtered, the product washed with water, ethanol and ether and dried *in vacuo* over phosphorus pentoxide. The filtrate was concentrated to approx. 50 ml affording a second crop which was processed analogously. Total yield 9·4 g (88%), m.p. 188—190°C (decomp.).

5′-Chloro-5′-deoxy-2′,3-O-isopropylideneadenosine (*I*)

A mixture of 5′-chloro-5′-deoxyadenosine (9·4 g), acetone (90 ml), 2,2-dimethoxypropane (60 ml), dimethylformamide (90 ml) and 6 mol l^{-1} hydrogen chloride in dimethylformamide (9 ml) was stirred in a closed flask overnight at room temperature and neutralized with triethylamine. The suspension was filtered, the precipitated salt washed with acetone (25 ml) and the filtrate evaporated at 40°C/13 Pa. The residue in chloroform (50 ml) was applied onto a short column (250 ml) of silica packed in chloroform and eluted with the same solvent. The product-containing fractions were combined, evaporated *in vacuo* and crystallized from ethyl acetate (light petroleum added to turbidity). Yield 8·6 g (80%) decomp. < 180°C. For $C_{13}H_{16}ClN_5O_3$ (325·8) calculated: 47·93% C, 4·95% H, 10·88% Cl, 21·50% N; found: 48·08% C, 4·95% H, 10·71% Cl, 21·53% N; R_F 0·46 (S2).

S-(2′,3′-O-Isopropylideneadenosyl)-L-homocysteine (*IIa*)

Ammonia (250 ml) was distilled from sodium onto L-homocystine (4·1 g, 16 mmol) in a 500 ml flask equipped with glass magnetic stirring rod and dry ice reflux condenser with soda lime

protecting tube. Metallic sodium cuttings were gradually added under stirring and cooling to −50°C until blue colour persisted. The mixture was decolourized by addition of acetic acid and compound *Ia* (10·4 g, 32 mmol) was added in one portion. The mixture was stirred without cooling under reflux for 5 h and the solvent was rapidly removed by evacuation of the stirred mixture under occasional heating on a water-bath (50°C). Water (300 ml) was added to the residue and the mixture immediately neutralized with 2 mol l^{-1} hydrochloric acid to pH 6—7. The solution was extracted with chloroform (three 50 ml portions), the aqueous phase made alkaline by ammonia to pH 9 and applied (5 ml/min) onto a column (500 ml) of octadecyl-silica. The column was washed (10 ml/min) with water (5 l), 10% aqueous methanol (2 l), and 20% aqueous methanol. The latter elution was continued till the UV-absorption ceased, the relevant fraction evaporated *in vacuo*, codistilled with ethanol (50 ml) and filtered from ethanol (50 ml) to remove traces of silica. The filtrate was added dropwise into ether (300 ml), the precipitate filtered, washed with ether and dried *in vacuo* to afford compound *IIa* (9·9 g, 73%), m.p. 218 to 219°C, R_F 0·54 (S1). For $C_{17}H_{24}N_6O_5S$ (424·5) calculated: 48·10% C, 5·70% H, 19·80% N, 7·55% S; found: 48·16% C, 5·82% H, 19·88% N, 7·59% S.

S-(2′,3′-O-Isopropylideneadenosyl)-L-cysteine (*IIb*)

The reaction was performed analogously as given for compound *IIa*, with L-cystine (1·443 g, 6 mmol) and compound *I* (3·25 g, 10 mmol) in 100 ml liquid ammonia. The product was eluted from the modified silica column by 20% aqueous methanol and isolated as given for compound *IIa*. Yield 2·80 g (68·5%) compound *IIb*, m.p. 216—217°C, R_F 0·54 (S1). For $C_{16}H_{22}N_6O_5S$ (410·4) calculated: 46·82% C, 5·40% H, 20·48% N, 7·81% S; found: 47·06% C, 5·43% H, 20·70% N, 7·68% S.

S-Adenosyl-L-homocysteine (*IIIa*)

A solution of compound *IIa* (8·5 g, 20 mmol) in 0·1 mol l^{-1} sulfuric acid (400 ml) was incubated overnight at 40°C and neutralized with cold saturated barium hydroxide solution to pH 7·0 ± 0·1. After warming-up to 60—70°C the suspension was filtered over Celite, washed with warm (60°C) water (100 ml) and the filtrate concentrated *in vacuo* to approx. 50 ml. This solution was applied onto an Amberlite IRC 50 (NH_4^+) column (100 ml) and the product eluted with water. Its UV-absorbing eluate was evaporated *in vacuo*, the residue codistilled twice with water (20 ml each) and finally crystallized from water (100 ml) to afford pure compound *IIIa*. Yield 7·1 g (92·5%), m.p. 212°C (literature[7] gives m.p. 212°C); R_F 0·29 (S1), E_{Up} 0·23 (electrophoretic mobility related to uridine 3′-phosphate). $[\alpha]_D^{20}$ +38·0° (*c* 0·5, 1 mol l^{-1} hydrochloric acid); literature[7] gives $[\alpha]_D^{20}$ +37·5° (*c* 1, 0·2 mol l^{-1} hydrochloric acid). UV Spectrum (pH 2; 7; 12): λ_{max} 260 nm, ε_{max} 14 400. For $C_{14}H_{20}N_6O_5S$ (384·4) calculated: 43·74% C, 5·24% H, 21·86% N, 8·43% S; found: 43·80% C, 5·32% H, 21·64% N, 8·16% S. The product does not contain any additional UV-absorbing, ninhydrine-positive or sulfur-containing contaminants. Elution time, 162—165 min, colour yield 60·4%.

S-Adenosyl-L-cysteine (*IIIb*)

A solution of compound *IIb* (2·05 g, 5 mmol) in 0·1 mol l^{-1} sulfuric acid (100 ml) was treated and worked-up analogously as given for compound *IIIa*. Crystallization from water afforded *IIIb* (1·4 g, 75·5% yield), m.p. 227—228°C, R_F 0·27 (S1), E_{Up} 0·23, $[\alpha]_D^{20}$ +16·9° (*c* 0·5, 1 mol l^{-1} hydrochloric acid), UV spectrum (pH 2): λ_{max} 259 nm, ε_{max} 14 500. For $C_{13}H_{18}N_6O_5S$ (370·4) calculated: 42·15% C, 4·90% H, 22·69% N, 8·65% S; found: 42·10% C, 4·67% H, 22·87% N, 8·46% S. The product is free of ninhydrine-positive or sulfur-containing impurities.

5′-(2-Carboxyethylthio)-5′-deoxyadenosine (*IIIc*), Lithium Salt

3-Mercaptopropanoic acid (2·12 g, 20 mmol) was added under stirring into a suspension of sodium hydride (1·06 g, 44 mmol) in dimethylformamide (100 ml). After stirring for 1 h at room temperature under exclusion of moisture, compound *I* (2·6 g, 8 mmol) was added and the mixture stirred at 100°C for 5 h. After evaporation at 50°C/13 Pa the residue in water (200 ml) was neutralized by addition of Dowex 50X8 (H^+), made alkaline with triethylamine, filtered and the filtrate applied onto a column (200 ml) of octadecyl-silica and washed with water (5 ml : : min). After elution of the salts the product appeared in a considerably retained aqueous fraction (as followed by the UV-detection). The fraction was concentrated *in vacuo* to give an amorphous residue of compound *IIc* which was treated with dilute sulfuric acid as given for compound *IIIa*. The filtrate after removal of barium sulfate was concentrated to approx. 20 ml and applied onto a column (50 ml) of Dowex 50X8 (Li^+) which was then washed with water. The UV-absorbing eluate was evaporated, the residue codistilled with ethanol (2 × 20 ml) and finally precipitated from ethanol with ether. Yield 1·9 g (65·8%), R_F 0·43 (S1), E_{Up} 0·42, UV-spectrum (pH 2; 7; 12): λ_{max} 259·5 nm, ε_{max} 14 100. For $C_{13}H_{16}LiN_5O_5S$ (361·3) calculated: 19·39% N, 8·87% S; found: 20·05% N, 8·74% S. No additional sulfur-containing contaminants were detected in this material.

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