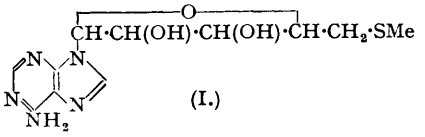
300. *Adenine 5′-Deoxy-5′-methylthiopentoside (Adenine Thiomethyl Pentoside): A Proof of Structure and Synthesis.*

By J. Baddiley.

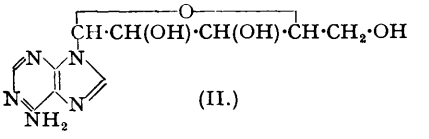
5′-Deoxy-5′-methylthioinosine (V) has been synthesised from 2′ : 3′-*iso*-propylidene 5′-toluene-*p*-sulphonyl inosine (III) by replacement of the toluene-*p*-sulphonyl by a methylthio-group, followed by hydrolysis of the *iso*propylidene residue. Its identity with hypoxanthine deoxymethylthio-pentoside, obtained by deamination of natural "adenine thiomethyl pentoside," was shown. This establishes the structure of the latter as 9-(5′-deoxy-5′-methylthio-β-D-ribofuranosyl)adenine, a synthesis of which is also reported.

The nucleoside often known as "adenine thiomethyl pentoside" or "adenyl thiomethyl pentose" was first isolated from yeast by Mandel and Dunham (*J. Biol. Chem.*, 1912, **11**, 85) and later by Suzuki (*J. Tokio Chem. Soc.*, 1914, **34**, 1134), Levene (*J. Biol. Chem.*, 1924, **59**, 465), Euler and Myrbäck (*Z. physiol. Chem.*, 1928, **177**, 237), and others. The early workers failed to detect the presence of sulphur in their materials, and the substance was formulated as an adenine hexoside. Not until 1924 did Suziki, Ohdake, and Mori (*Biochem. Z.*, 1924, **154**, 278) establish the correct formula, $C_{11}H_{15}O_3N_5S$. The sulphur atom was located in the carbohydrate moiety since acid hydrolysis of the nucleoside gave adenine and a sugar containing sulphur. Levene and Sobotka (*J. Biol. Chem.*, 1925, **65**, 551) and Sobotka (*J. Biol. Chem.*, 1926, **69**, 267) considered that this sugar was probably a ketose containing one methylthio- or methoxy-group, but it was recognised later as an aldose by Wendt (*Z. physiol. Chem.*, 1942, **272**, 152). Periodate titration of both the free sugar and the thiopentitol obtained from it on reduction and application of the Böeseken test for *cis*-hydroxyl groups strongly suggested that the sugar was 5-deoxy-5-methylthioribose (Satoh and Makino, *Nature*, 1950, **165**, 769). Evidence in support of this formulation has been obtained by Weygand, Trauth, and Löwenfeld (*Chem. Ber.*, 1950, **83**, 563), who showed that the osazone of the natural sugar was identical with that of synthetic 5-deoxy-5-methylthio-D-arabinose. As the free sugars themselves were not identical this was evidence that the natural one was 5-deoxy-5-methylthio-D-ribose. The position of attachment of the thio-sugar in the nucleoside was assigned to $N_{(9)}$ (as in I) by Falconer and Gulland on the basis of spectroscopic evidence (*J.*, 1937, 1912). The configuration about the glycosidic centre had not been established.

A synthesis of this substance seemed highly desirable, not only to confirm the structure (I) and determine the configuration about the glycosidic linkage, but also to make this interesting but inaccessible nucleoside more readily available for biological study. Its known pharmacological effects (Kuhn and Henkel, *Z. physiol. Chem.*, 1941, **269**, 41; Ewing and Schlenk, *J. Pharmacol.*



(I.)



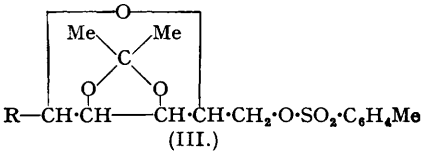
(II.)

Exper. Therap., 1943, **79**, 164) and probable biological activity (Nakahara, Inugai, Ugami, and Nagata, *Sci. Jap. Inst. Phys. Chem. Res.*, 1945, **42**, 153) are worthy of further study as is the suggestion that it may participate in biological methylation processes (Lipmann, *Adv. Enzymol.*, 1941, **1**, 99).

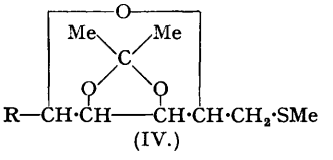
If it is assumed that "adenine thiomethyl pentoside," like other naturally occurring nucleosides, is a β-glycoside, then it should be possible to synthesise it from adenosine (II) by substitution of the primary hydroxyl at position 5′ by a methylthio-group inasmuch as the structure of adenosine is fully established as 9-β-D-ribofuranosyladenine (*inter al.*, Davoll, Lythgoe, and Todd, *J.*, 1946, 833; 1948, 967; Lythgoe, Smith, and Todd, *J.*, 1947, 355). The envisaged synthesis involved the introduction of a toluene-*p*-sulphonyl group in the 5′-position in 2′ : 3′-*iso*propylidene adenosine, followed by its replacement by methylthio- and hydrolysis of the *iso*propylidene residue. Considerable difficulty was experienced, however, in the preparation of the necessary toluene-*p*-sulphonate in a pure state. Reaction between toluene-*p*-sulphonyl chloride and 2′ : 3′-*iso*propylidene adenosine under various conditions gave syrupy products from which some unchanged *iso*propylidene compound was usually isolated. A small and variable amount of a crystalline material, m. p. 283°, was obtained and its analysis agreed with that expected for a monotoluene-*p*-sulphonate but insufficient was available for structural investigation. Later, the author was informed by Professor A. R. Todd that similar observations have been made in Cambridge.

The difficulties encountered probably arose through the reactivity of the 6-amino-group in the purine ring; consequently, attention was directed to a proof of the structure of "adenine thiomethyl pentoside" in another way.

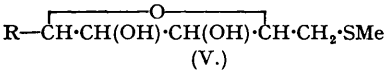
A toluene-*p*-sulphonate of *iso*propylidene inosine was prepared by Levene and Tipson (*J. Biol. Chem.*, 1935, **111**, 313), which contained the toluene-*p*-sulphonyl residue at position 5′ since it could be substituted by an iodine atom when heated with sodium iodide in acetone (cf. Oldham and Rutherford, *J. Amer. Chem. Soc.*, 1932, **54**, 366), and is thus 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl inosine (III).



(III.)



(IV.)



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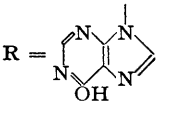

Raymond (*J. Biol. Chem.*, 1934, **107**, 85) effected substitution of a toluene-*p*-sulphonyl group by alkylthio in the sugar series by heating toluene-*p*-sulphonates and sodium or potassium derivatives of thiols in acetone at 100°. Reaction between (III) and potassium methyl sulphide under similar conditions was complicated by the formation of potassium salts which were insoluble in acetone, but this was avoided by the use of dimethylformamide as solvent; a further advantage of this solvent was that the process could be carried out at atmospheric pressure on a steam-bath. By this method (III) gave an excellent yield of 2′ : 3′-*iso*propylidene 5′-deoxy-5′-methylthioinosine (IV). Hydrolytic removal of the *iso*propylidene residue proceeded smoothly in a mixture of acetic and N-sulphuric acids to give 5′-deoxy-5′-methylthioinosine, m. p. 220—221°.

A hypoxanthine deoxymethylthiopentoside, m. p. 220·5—221·5°, was prepared by Kuhn and

Henkel (*loc. cit.*) by treatment of " adenine thiomethyl pentoside " with nitrous acid, and the author is indebted to Professor F. Weygand who reports that the synthetic substance and this hypoxanthine derivative are identical as shown by melting points, mixed melting points, specific rotations, and behaviour on paper chromatography before and after hydrolysis. Final confirmation was obtained by hydrolysis to 5-deoxy-5-methylthio-D-ribose. Reduction of this with sodium amalgam (Suziki, Ohdake, and Mori, *Biochem. Z.*, 1925, **162**, 413) yielded 5-deoxy-5-methylthio-D-ribitol, identical with that obtained in a similar way from natural " adenine thiomethyl pentoside."

Since the starting material in this synthesis, namely, inosine, was obtained by deamination of adenosine and is, therefore, 9-β-D-ribofuranosylhypoxanthine, it follows that " adenine thiomethyl pentoside " is correctly described as 9-(5-deoxy-5-methylthio-β-D-ribofuranosyl)-adenine (I).

With the experience acquired in the above synthesis, the syrupy and certainly impure toluene-*p*-sulphonation product of 2′ : 3′-*iso*propylidene adenosine was treated as above. An acetone solution of the resulting syrup slowly deposited crystalline 5′-deoxy-5′-methylthioadenosine, identical with natural " adenine thiomethyl pentoside."

As the work described in this paper was nearing completion the author was informed by Professor Weygand of related but independent investigations in progress at Heidelberg. The German workers synthesised 5′-deoxy-5′-methylthioadenosine by a route similar to that outlined above and a preliminary account of these investigations has been published jointly (Baddiley, Trauth, and Weygand, *Nature*, 1951, **167**, 359). After this had been submitted for publication Satoh and Makino described briefly the synthesis of 2′ : 3′-*iso*propylidene 5′-deoxy-5′-methylthioadenosine which they isolated as its picrate and showed to be identical with the picrate of " *iso*propylidene adenine thiomethyl pentoside " prepared from the natural nucleoside (*Nature*, 1951, **167**, 238). Similarly, they showed that synthetic 2′ : 3′-*iso*propylidene 5′-deoxy-5′-methylthioinosine picrate was identical with the picrate of the corresponding substance obtained from " hypoxanthine thiomethyl pentoside."

During our work both 2′ : 3′-*iso*propylidene adenosine and 2′ : 3′-*iso*propylidene inosine have been prepared by simpler and more reliable methods than those described by Levene and Tipson (*loc. cit.*) (see below).

Experimental.

2′ : 3′-iso*Propylidene Adenosine*.—The following method is a modification of that described by Levene and Tipson (*loc. cit.*). To a filtered solution of pure, fused zinc chloride (55 g.) in acetone [550 c.c.; previously treated with a small amount of potassium permanganate at room temperature, distilled, dried (Na_2SO_4), and redistilled] was added adenosine (19 g.; dried for 5 hours at 110°/0·1 mm. over phosphoric oxide). After boiling under reflux with the exclusion of moisture for 5 hours the slightly cloudy solution was kept at room temperature for a further 12 hours. The volume was reduced to about a third by distillation under reduced pressure and the resulting slightly viscous solution poured into a warm (40°) solution of barium hydroxide (150 g. of octahydrate) in water (*ca.* 1 l.). The mixture was cooled at once to room temperature and carbon dioxide passed in vigorously until the liquid was no longer alkaline. Zinc and barium carbonates were filtered off and washed thoroughly with alternate lots of boiling methyl alcohol and water (*ca.* 700 c.c. of each) until the washings were free from carbohydrate. The filtrate and washings were refiltered if not clear, then evaporated at $<40°$ to about 500 c.c. During the evaporation 2′ : 3′-*iso*propylidene adenosine crystallised. After half an hour at room temperature the solid was filtered off, washed with water, and dried at 100°. A further small amount was isolated from the mother-liquors by evaporation to dryness at $<40°$, drying of the powdered residue at 100°, and extraction of it continuously for 5 hours with dry acetone. Evaporation of the acetone left a resin which was dissolved in a little water and made alkaline with ammonia. After storage a further amount of crystalline material was filtered off. The combined solids were dissolved in boiling 95% methyl alcohol (*ca.* 150 c.c.), and the solution was filtered and cooled. Pure 2′ : 3′-*iso*propylidene adenosine, m. p. 220°, was filtered off and dried. Evaporation of mother-liquors yielded a further small amount (total yield, 75—85%).

2′ : 3′-iso*Propylidene Inosine*.—This was prepared from inosine (9 g.) as described above but with the following modification. The aqueous solution remaining after removal of zinc and barium carbonate was evaporated to dryness at $<40°$ and the dried residue extracted continuously with dry acetone for 5 hours. After evaporation of the acetone, the residue was dissolved in 90% methyl alcohol (200 c.c.) and made alkaline with ammonia, and a small amount of zinc removed by saturation with hydrogen sulphide, followed by filtration through silica. The filtrate was evaporated to dryness under reduced pressure and the residue recrystallised from methyl alcohol. 2′ : 3′-*iso*Propylidene inosine (5 g.) was obtained as fine needles, m. p. 267°, $[\alpha]_D^{20}$ −66·9° (*c*, 0·761 in methyl alcohol). Levene and Tipson (*loc. cit.*) record m. p. 240—245° and $[\alpha]_D^{24}$ −69·2° (*c*, 0·578 in methyl alcohol), but the discrepancy in m. p. is probably explained by the failure of these authors to remove zinc salts from their material.

Reaction between 2′ : 3′-iso*Propylidene Adenosine and Toluene*-p-*sulphonyl Chloride*.—A solution of toluene-*p*-sulphonyl chloride (0·68 g.) in dry benzene (2 c.c.) was added dropwise to a solution of *iso*propylidene adenosine (1·0 g.; dried for 1 hour at 120°/0·1 mm.) in anhydrous pyridine (100 c.c.),

cooled to −10°. After 3 days at room temperature water (10 c.c.) was added and the solvent removed under reduced pressure. The residue was dissolved in chloroform (50 c.c.) and washed successively with small amounts of ice-cold dilute sulphuric acid, sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The resulting resin was dissolved in alcohol–chloroform (1 : 1), and the solution passed through a short column of alumina. After the column had been washed with alcohol until free from carbohydrate, eluate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in a little alcohol and made just cloudy with *cyclo*hexane. Crystals of unchanged *iso*propylidene adenosine usually appeared at this point but occasionally these were mixed with another crystalline substance. The two compounds were separated by fractional crystallisation from alcohol–*cyclo*hexane. The *toluene*-p-*sulphonyl* derivative, m. p. 283° (decomp.), was recrystallised from alcohol–*cyclo*hexane (Found : C, 51·9; H, 4·9; N, 15·3; S, 6·9. $C_{20}H_{23}O_6N_5S$ requires C, 52·0; H, 5·0; N, 15·2; S, 6·9%).

2′ : 3′-iso*Propylidene 5′-Deoxy-5′-methylthioinosine*.—To a solution of 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl inosine (1·0 g.) in anhydrous dimethylformamide (15 c.c.) was added dry potassium methyl sulphide (1 g.; prepared from a solution of methanethiol in ether and powdered potassium). Suspended solid dissolved on warming. The solution was heated on a steam-bath with exclusion of moisture for 2 hours, cooled in ice, diluted with ice-water (30 c.c.), and neutralised with cold dilute sulphuric acid. The precipitated solid was dissolved in chloroform, and the chloroform solution separated, washed with a little water, and evaporated to dryness under reduced pressure. The crystalline residue was recrystallised from chloroform–ether. The *methylthio*-derivative (0·65 g., 90%) crystallised in prisms, m. p. 234°, $[\alpha]_D^{20}$ −16·5° (*c*, 3·29 in chloroform) (Found : C, 49·8; H, 5·4; N, 16·0; S, 9·3. $C_{14}H_{18}O_4N_4S$ requires C, 49·7; H, 5·3; N, 16·5; S, 9·5%).

5′-*Deoxy-5′-methylthioinosine*.—A solution of the *iso*propylidene compound (IV) (0·2 g.) in acetic acid (10 c.c.) and N-sulphuric acid (10 c.c.) was kept at room temperature for 48 hours. The sulphuric acid was neutralised with the calculated amount of barium hydroxide solution, barium sulphate was removed by centrifugation and washed with water, and the supernatant liquid and washings were evaporated to dryness at <40°. The residue was dissolved in water (25 c.c.), filtered through Supercel silica, and evaporated to dryness again at <40°. The crystalline product (0·16 g., 92%) was recrystallised from a small amount of hot water. 5′-*Deoxy-5′-methylthioinosine* crystallised as fine needles, m. p. 220—221° (Found : C, 44·3; H, 4·6; N, 18·5; S, 10·6. $C_{11}H_{14}O_4N_4S$ requires C, 44·3; H, 4·7; N, 18·8; S, 10·7%).

(Based on a report by Professor F. Weygand.) The above compound and " hypoxanthine thiomethyl pentoside," after recrystallisation from water and drying ($CaCl_2$), had m. p. 221—222°, undepressed on admixture. Microscopic examination of samples showed the presence in each of prisms and fine needles, the melting points and specific rotations of which are tabulated. The mixed m. p. of samples

	$[\alpha]_D^{20\cdot1}$ (*c*, 0·17 in pyridine).	Recryst. from water; m. p. (Köfler block).	Recryst. from wet butanol; m. p. (Köfler block).
5′-Deoxy-5′-methylthioinosine	−24·1° ± 2°	Prisms 223—224°	223—224°
		Needles 226·5°	226·5°
" Hypoxanthine thiomethyl pentoside "	−22·5° ± 2°	Prisms 224°	222—223°
		Needles 227°	226·5°

recrystallised from water was 227° and that of samples recrystallised from wet butanol was 225—226°. Hydrolysis of the synthetic substance with 5% sulphuric acid and reduction with sodium amalgam (Suziki, Ohdake, and Mori, *loc. cit.*) gave 5-deoxy-5-methylthio-D-ribitol, m. p. 118—119° undepressed when mixed with the deoxymethylthiopentitol, m. p. 118—119°, obtained from " adenine thiomethyl pentoside."

5′-*Deoxy-5′-methylthioadenosine*.—The resin obtained by toluene-*p*-sulphonation of *iso*propylidene adenosine (4·0 g.) by the above-mentioned method was dissolved in anhydrous dimethylformamide (*ca.* 30 c.c.), and dry potassium methyl sulphide (2·0 g.) was added. The mixture was heated at 100° for 2 hours, cooled, and poured into water, and the crude product extracted with three lots of chloroform. Evaporation of the chloroform solution left a reddish-brown resin. This was dissolved in acetic acid (25 c.c.) and N-sulphuric acid (25 c.c.) added. After 2 days at room temperature the acid was neutralised with the calculated amount of barium hydroxide solution, and barium sulphate removed by centrifugation. The precipitate was washed twice with water, and the combined supernatant liquids were evaporated to dryness under reduced pressure. The resulting syrup was dissolved in a little acetone, adjusted to pH 7 with ammonia, and set aside at room temperature. After some time crystals appeared and during 3—4 weeks small crops were removed by filtration. The combined solids (*ca.* 400 mg.) were recrystallised from water and had m. p. 205°, unaltered by recrystallisation from water or wet butanol (Found : C, 44·5; H, 5·0; N, 23·6. Calc. for $C_{11}H_{15}O_3N_5S$: C, 44·4; H, 5·1; N, 23·5%).

Comparison of the synthetic nucleoside with " adenine thiomethyl pentoside " from yeast was carried out by Professor Weygand. The synthetic substance melted at 212° (Köfler block) and was undepressed when mixed with a sample of the natural nucleoside, m. p. 212—213°. On paper chromatography in butanol–water the substances were indistinguishable (R_F 0·58).

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