543. *Structural Observations on "Active Methionine."*

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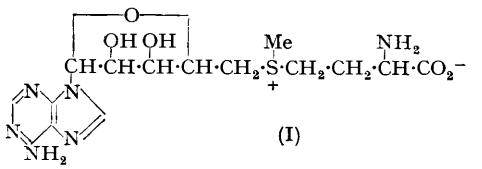
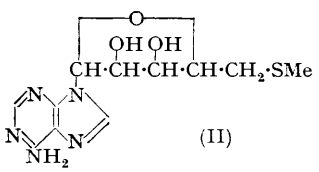
The intermediate substance, "active methionine," which participates in the enzymic transfer of a methyl group from methionine to a variety of substrates has been hydrolysed to 5′-deoxy-5′-methylthioadenosine. This and other evidence confirms its formulation as *S*-(5′-deoxyadenosine-5′)-methionine.

THE methyl group in methionine is readily transferred to other substances by enzymic processes in biological systems. In normal cells a stable dynamic state is achieved whereby continuous interchange of methyl groups occurs. However, such transmethylations involving methionine require the presence of adenosine triphosphate (ATP) (Borsook and Dubnoff, *J. Biol. Chem.*, 1947, **171**, 363; Cantoni, *ibid.*, 1951, **189**, 203; Cohen, *ibid.*, 1951, **193**, 851) and it has been shown recently that an intermediate substance, "active methionine" is formed (Cantoni, *ibid.*, 1951, **189**, 745). This unstable intermediate does not contain phosphorus and gives, after acid hydrolysis at 100°, adenine, homoserine, and an unidentified sulphur-containing compound (Cantoni, *J. Amer. Chem. Soc.*, 1952, **74**, 2942; *J. Biol. Chem.*, 1953, in the press). On the basis of this evidence "active methionine" has been formulated tentatively as *S*-(5′-deoxyadenosine-5′)-methionine (I).



(I)



(II)

Sulphonium compounds have hitherto been encountered only infrequently in Nature and, since the mechanism for the formation of (I) from ATP presents unusual features, further degradative evidence for the proposed structure was desirable. Sulphonium compounds decompose under a variety of conditions giving sulphides. The sulphides which could be formed theoretically from (I) are methionine, *S*-(5′-deoxyadenosine-5′)-homocysteine and 5′-deoxy-5′-methylthioadenosine ("adenine thiomethyl pentoside") (II). If (I) correctly represents "active methionine" it may be presumed that *S*-(5′-deoxyadenosine-5′)-homocysteine is formed biologically. However, the earlier observation that homoserine was produced during chemical hydrolysis suggested that the most vulnerable point of chemical attack on "active methionine" was the bond between the sulphur atom and the carbon chain of the amino-acid residue. Consequently, very cautious hydrolysis should yield 5′-deoxy-5′-methylthioadenosine (II). This has been shown to be the case. When a sample of "active methionine" was kept in the freeze-dried state at room temperature for a few weeks and then examined by paper chromatography the original spot had disappeared and a new spot was observed which absorbed ultra-violet light strongly and gave a negative ninhydrin reaction, and a positive aldehyde colour reaction after spraying of the paper with periodate according to the method of Buchanan, Dekker, and Long (*J.*, 1950, 3162). In all these respects it was indistinguishable from a synthetic sample of 5′-deoxy-5′-methylthioadenosine (Baddiley, *J.*, 1951, 1348; Weygand and Trauth, *Chem. Ber.*, 1951, **84**, 633; Satoh and Makino, *Nature*, 1951, **167**, 238). A larger sample of "active methionine" was heated at 100° in an acetate buffer at pH 7 whereupon crystalline 5′-deoxy-5′-methylthioadenosine was isolated. Small amounts of adenine were produced under these conditions but no adenosine or *S*-(5′-deoxyadenosine-5′)-homocysteine were observed.

The ready conversion of "active methionine" into (II) confirms formula (I) and establishes beyond doubt the biological origin of "adenine thiomethyl pentoside." Although the 5′-methylthio-compound was first isolated from yeast in 1912 (Mandel and Dunham, *J.*

Biol. Chem., 1912, **11**, 85), only recently has it been established that its formation by micro-organisms depends on a good supply of methionine in the medium (Weygand, Junk, and Leber, *Z. physiol. Chem.*, 1952, **291**, 191; Smith and Schlenk, *Arch. Biochem.*, 1952, **38**, 167). The suggestion (Weygand, Junk, and Leber, *loc. cit.*) that it may arise from "active methionine" is confirmed by our findings. Furthermore, its presence in extracts of biological origin (cf. Smith, Anderson, Overland, and Schlenk, *Arch. Biochem.*, 1953, **42**, 72) should probably be attributed to chemical, rather than enzymic, decomposition of "active methionine" in the course of the procedure employed for the isolation of the 5′-methylthio-compound.

The conversion of "active methionine" into (II) may be followed by the accompanying decrease in the Bial orcinol test for pentose, owing to the fact that "active methionine" behaves in this test in a manner similar to adenosine, whereas (II) yields only 31·6% and 46·5%, respectively, of the colour (mole for mole) after 40 and 80 min.' heating at 100°.

The behaviour of "active methionine" on paper chromatography and its reaction on the paper are also consistent with formula (I). It moves very slowly in predominantly organic solvent mixtures and rapidly in salt solutions. It absorbs ultra-violet light, and gives a positive ninhydrin reaction and a purple colour after oxidation with periodate and spraying of the paper with Schiff's reagent (Buchanan, Dekker, and Long, *loc. cit.*). The ready production of an aldehyde in this test indicates the absence of substituents at positions 2′ and 3′ in the adenosine residue.

The presence of a strong positive charge on "active methionine" is seen from its rapid movement towards the cathode on paper electrophoresis. This probably is the most conclusive evidence for the presence of a sulphonium grouping in the molecule.

Experimental

Chromatographic Examination of "Active Methionine" Concentrate.—Portions (0·005 c.c.) of an "active methionine" concentrate [containing *ca.* 100 micromoles/c.c. of "active methionine" (Cantoni, *J. Biol. Chem.*, 1951, **189**, 745)] were chromatographed on Whatman No. 4 filter paper with (*a*) ethanol–acetic acid–water (16 : 1 : 3), (*b*) 5% sodium dihydrogen phosphate solution, or (*c*) 5% ammonium sulphate solution covered with a surface layer of amyl alcohol.

R_F values varied within small limits owing to lack of temperature control, and those tabulated below for room temperature (*ca.* 20°) are averages for several runs. Adenine derivatives were detected by their fluorescence in ultra-violet light. The ninhydrin and periodate–Schiff reactions were used as diagnostic sprays. In addition to "active methionine" and a little 5′-deoxy-5′-methylthioadenosine, a trace of methionine was present in the concentrate.

	R_F		
	EtOH–AcOH–H_2O	5% Na_2HPO_4	5% $(NH_4)_2SO_4$
"Active methionine"	0·14	0·73	0·73
5′-Deoxy-5′-methylthioadenosine ...	0·57	0·58	0·58

Hydrolysis.—"Active methionine" concentrate (0·5 c.c.) was heated in a sealed tube for 48 hr. at 100°. Crystalline material was removed by filtration, then recrystallised twice from water; it had m. p. 205° (Kofler block), undepressed in admixture with a synthetic sample of 5′-deoxy-5′-methylthioadenosine. The two compounds were indistinguishable on chromatography in 5% sodium dihydrogen phosphate and in 5% ammonium sulphate solution.

Electrophoresis.—The cell employed was similar to that described by Markham and Smith (*Nature*, 1951, **168**, 406). Whatman No. 4 filter paper was soaked in an ammonium acetate buffer (pH 7), and the sample introduced at the centre of the strip. A potential of 200 v (D.C.) was applied for approx. 3 hr.; then the paper was examined in ultra-violet light. The spot observed for "active methionine" gave a positive ninhydrin test. The rates of progression towards the cathode were: "active methionine" 0·2; 5′-deoxy-5′-methylthioadenosine 0·07 cm. $hr.^{-1}/v$ $cm.^{-1}$.

Colorimetric Tests.—A solution (2 c.c.) containing the nucleoside was placed in a Pyrex test-tube. and an equal volume of the orcinol–ferric chloride–hydrochloric acid reagent was added (Mejbaum, *Z. physiol. Chem.*, 1939, **258**, 117). The colour which developed after 40 and

80 min.' heating in a boiling-water bath was measured at 660 mμ and related to the adenine content as determined by the absorption at 260 mμ (molar extinction coefficient: 16,000).

Compound	Adenine (micromole)	Pentose (micromole)			
		40 Min.*	80 Min.*		
	a	*b*	*c*	*c*/*b*	*b*/*a*
Adenosine	0·0234	0·0234	0·0230	0·98	1·0
Active methionine	0·0334	0·0360	0·0402	1·11	1·07
Active methionine (after hydrolysis at pH 6·4 and 100° for 4 hr.)	0·0427	0·0218	0·0263	1·21	0·51
Active methionine (after hydrolysis at pH 6·4 and 100° for 10 hr.)	0·0427	0·0173	0·0220	1·27	0·405
(II)	0·060	0·019	0·0278	1·46	0·316

* Heating time at 100°.

Quantitative chromatographic analysis of the solutions of "active methionine" used in this experiment (for technical details, see Cantoni, *J. Biol. Chem.*, 1953, in the press) confirmed the disappearance of "active methionine" on hydrolysis and concomitant formation of 5'-deoxy-5'-methylthioadenosine. Thus, before hydrolysis of the total adenine-containing compounds, 84% was identical with (I) and 16% migrated as (II). After 4 hr.' hydrolysis, the figures were 12% and 88%, respectively.

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