there are 3 or 4 residues rigidly linked together in each segment. It is clear that even "melted" collagen is a rather rigid material on a molecular scale.[10]

Since this communication was first submitted, we have had the opportunity to discuss it with Professor P. J. Flory. He points out that the entropy change calculated by equation (1) is an over-all entropy change which included an integral entropy of mixing with solvent.

(10) Support of this research by the U. S. Office of Naval Research is gratefully acknowledged. Reproduction in whole or in part by or for the United States Government is permitted.

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ENZYMATIC SYNTHESIS OF S-METHYLCYSTEINE

Sir:

S-Methylcysteine, the lower homolog of methionine, has been found to be enzymatically synthesized in an extract of yeast. The synthesis utilizes methyl mercaptan and L-serine

$$CH_3SH + CH_2OHCHNH_2COOH \longrightarrow CH_3SCH_2CHNH_2COOH + H_2O$$

This reaction was discovered in the course of experiments designed to determine the metabolic role of another enzymatic process involving methyl mercaptan.[1]

Roberts, *et al.*, have shown that S-methylcysteine can be utilized by bacteria in isotope competition experiments and by an organic sulfur-requiring mutant of *E. coli* in a manner which suggests that it may be an intermediate in the microbial biosynthesis of cysteine.[2] It has also been considered as a possible precursor of S-methylcysteine sulfoxide, a major constituent of the nonprotein nitrogen fraction of certain plant tissues,[3] and its natural occurrence in plants was recently established.[4] Schlenk and Tillotson have reported[5] S-methyladenosine to be formed from CH_3SH in yeast, and it is possible that this occurs with the reaction reported here as an intermediate step.

Incubation of CH_3SH and serine with a partially purified enzyme yielded a product which, upon purification on a cation exchange column, moved on paper chromatograms in four solvent systems just as authentic S-methylcysteine does (Table I). After treatment with H_2O_2 the product, when chromatographed on paper, behaved as the mixture of diastereo-isomers of S-methylcysteine sulfoxide which is obtained by treating S-methylcysteine with H_2O_2[3] (Table I). The enzymatic product develops a color in the nitroprusside test for methionine[6] which has a somewhat different absorption spectrum from that developed by methionine but which is identical to that found with S-methylcysteine.

TABLE I

The 8-fold purified enzyme was prepared from a yeast extract[7] by precipitating it as a protamine complex from dilute neutral solution after removing an inactive precipitate at *p*H 5.0. The incubation mixture contained the enzyme preparation (600 mg. of protein), 50.0 millimoles of triethanolamine chloride buffer (*p*H 7.5), 40.0 millimoles of DL-serine, and 3.0 millimoles of CH_3SH in a final volume of 1.0 liter. The mixture was incubated at 24° for 2.5 hours. After deproteinization the product, 1.1 millimoles, was purified by chromatographing on a column of Dowex 50 (H^+ form) and precipitating from water by addition of ethanol. A portion of the reaction product was oxidized with H_2O_2 as indicated by Morris and Thompson for the preparation of S-methylcysteine sulfoxide from S-methylcysteine.[8] Oxidized and unoxidized samples were then chromatographed on Whatman paper No. 1 in the following solvents: A, *n*-butanol–acetic acid–H_2O, 200:30:75; B, 2,4-lutidine–2,4,6-collidine–H_2O–diethylamine, 100:100:100; C, methanol–H_2O–pyridine, 80:20:4; and D, phenol–H_2O, 80:20. The compounds were located on the chromatograms with ninhydrin.

	R_f			
Solvent	SMC[a]	Product	Oxidized SMC	Oxidized Product
A	0.12	0.12	0.025	0.025
B	.41	.40	.23	.23
C	.56	.57	.43, .35	.47, .38
D	.72	.70	.65	.63

[a] SMC = S-methylcysteine.

The extent of the enzymatic reaction may be determined by use of the nitroprusside test, or by measuring the incorporation of $CH_3S^{35}H$ into a heat-stable, non-volatile product. Using these tests the enzyme was found to be specific for L-serine and completely inactive with D-serine, L-threonine, and L-alanine. It was also inactive with DL-homoserine, which suggests that methionine is not formed in an analogous reaction. C_2H_5SH reacts in the system at about 60% of the CH_3SH rate.[8] No cysteine or cystine was found when H_2S was substituted for CH_3SH in the incubation mixture.

The enzyme was completely inactivated by incubation at 60° for 10 minutes. The ability of a crude yeast extract to form S-methylcysteine deteriorated 70% when stored at 0° for several days, and was completely restored by addition of pyridoxal phosphate. Pyridoxal phosphate-requiring preparations were rapidly inactivated by dialysis and other purification procedures in contrast to fresh preparations which were more stable.

Though this reaction is not measurably reversible it is inhibited considerably by the product, S-methylcysteine. The inhibition was partially relieved by a relatively high concentration of serine which

(1) S. Black and N. G. Wright, *J. Biol. Chem.*, **221**, 171 (1956).

(2) R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton and R. J. Britten, "Studies of Biosynthesis in Escherichia Coli," Carnegie Institution of Washington, Washington, D. C., 1955, p. 318.

(3) C. J. Morris and J. F. Thompson, THIS JOURNAL, **78**, 1605 (1956).

(4) J. F. Thompson, C. J. Morris and R. M. Zacharius, *Nature*, **178**, 593 (1956).

(5) F. Schlenk and J. A. Tillotson, *Federation Proc.*, **13**, 290 (1954).

(6) E. A. Csonka and C. A. Denton, *J. Biol. Chem.*, **163**, 329 (1946).

(7) S. Black and N. G. Wright, *J. Biol. Chem.*, **213**, 27 (1955).

(8) S-Ethylcysteine was used as an analytical standard in this test.

allowed the product to accumulate in quantities up to 2.0 micromoles/ml.

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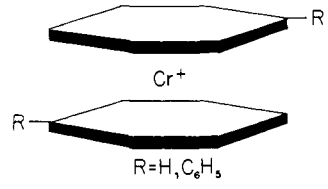
SUBSTITUTED AROMATIC-CHROMIUM COMPLEXES

Sir:

First in 1954[1] and later in 1955[2] we proposed the general bis-arene structure I for aromatic-chromium compounds on the basis of results obtained with lithium aluminum hydride and deuteride reductions,[3] in explanation of the numerous anomalies



I

reported earlier by Fr. Hein, *et al.*,[3] in the course of their work with these substances, and as a result of other theoretical considerations.[4] As a further step in the development of this chemistry we have succeeded in isolating the *predicted* water-soluble *bis-benzene-chromium* (I) cation, previously unreported from the reaction between phenylmagnesium bromide and chromyl chloride *or* chromic trichloride, as a major product. Reduction of its *tetraphenylboron salt* II [Calcd. for $C_{36}H_{32}BCr$: C, 81.98; H, 6.11. Found: C, 82.06; H, 6.27] or *picrate*, explosion p. 138° [Calcd. for $C_{18}H_{14}O_7N_2Cr$: C, 49.55; H, 3.23; N, 9.63. Found: C, 49.30; H, 3.36; N, 9.36] with hypophosphorous acid yielded *bis-benzenechromium*, m.p. 282–284°, whose physical, chemical and spectral properties[5] are the same as those exhibited by "di-benzol-chrom" prepared from benzene.[6] More fundamentally, we have now discovered the presence of a Grignard reaction intermediate whose usefulness in preparing a wide range of bis-arene-chromium complexes will be apparent.

Formation of the bis-arene-chromium cation clearly involves both oxidation–reduction and coordination-complexing steps. The former may be written as

$$2C_6H_5MgBr + CrCl_3 \longrightarrow (C_6H_5)_2 + Cr^{I}Cl + MgBr_2 + MgCl_2$$

and the latter as

$$2C_6H_5MgBr + Cr^{I}Cl \longrightarrow \underset{A}{[C_6H_5MgBr]_2Cr^{I}Cl}$$

or as

$$C_6H_5MgBr + CrCl_3 \longrightarrow C_6H_5CrCl_2 + MgBrCl \quad (a)$$

$$2\,C_6H_5CrCl_2 + Cr^{I}Cl \longrightarrow \underset{B}{[C_6H_5CrCl_2]_2Cr^{I}Cl} \quad (b)$$

A simple test for the presence of an intermediate of either type A or B was made by carbonating the reaction mixture at −10° and then hydrolyzing the product with aqueous alkali. A water-soluble sodium salt of the product complex was then precipitated by tetraphenylboron ion as a less soluble yellow salt whose infrared spectrum was quite similar to that of II but which also contained very strong bands at 6.24 and 7.3 μ (carboxylate ion). This salt was further purified by precipitation from dilute, aqueous solution with barium ion, and this complex salt, likewise yellow, gave the same absorption bands. The introduction of carbon dioxide into the aromatic complex was confirmed by isotopic hydrolysis of the Grignard reaction intermediate with deuterium oxide. The tetraphenylboron salt of this product, after filtration, washing with water and recrystallization from acetone, exhibited the characteristic aromatic C–D band at 4.42 μ.[7] The existence of this intermediate also permits a reasonable explanation for the observed formation of benzene-diphenyl- and bis-diphenylchromium cations in this Grignard reaction.[1–3] Details of this work together with the results of the investigation of the manifold ramifications will be reported shortly.

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(1) Abstr., 126th Meeting, Amer. Chem. Soc., p. 29-0, Sept. 14 1954, New York, N. Y.; *Angew. Chem.*, **67**, 282 (1955).

(2) *Yale Sci. Mag.*, **29**, 14 (1955); *Handbook, XIVth International Congress of Pure and Applied Chemistry*, p. 262, July 23, 1955, Zürich; and in lectures presented at Munich, Heidelberg and Tübingen, June–July, 1955.

(3) *Cf.* F. A. Cotton, *Chem. Rev.*, **55**, 551 (1955).

(4) Professor L. Onsager first proposed this structure during the progress of the original experimental work being done by Dr. M. Tsutsui at Yale University.

(5) Nuclear magnetic resonance studies on this and related complexes are being made by Dr. L. N. Mulay together with Professor E. G. Rochow at Harvard University and will be published separately.

(6) E. O. Fischer and W. Hafner, *Z. anorg. Chem.*, **286**, 146 (1956).

(7) *Cf.* C. R. Bailey, R. R. Gordon, J. B. Hale, N. Herzfeld, C. K. Ingold and H. G. Poole, *J. Chem. Soc.*, 299 (1946).

(8) Monsanto Research Fellow ,1956–57.