tions result in a simple method for generating diazo compounds *in situ* and studying their decomposition by cationoid and carbenoid processes. Reaction of camphor tosylhydrazone and sodium methoxide in diethylene glycol at 140–180° thus gives camphene (55%) and tricyclene (45%) in near-quantitative yield; decomposition of the hydrazone by sodium methoxide in diethyl Carbitol gives the hydrocarbons (~100% yield) in proportions > 49:1.[3] The effects of solvents on such processes are also indicated by reaction of 2-methylpropanal tosylhydrazone and sodium methoxide in diethylene glycol to give 2-methylpropene (65%), *cis*-2-butene (4%), *trans*-2-butene (8%), 1-butene (10%), and methylcyclopropane (12%) in 30% yield, whereas in diethyl Carbitol or hexadecane, 2-methylpropene (62, 64%) and methylcyclopropane (37, 36%) are formed in 80 and 78% yields.[4]

The initial reaction of 2-methylpropanal tosylhydrazone (and other tosylhydrazones) is formation of its salt and methanol; thermal decomposition of this salt in diethyl Carbitol or hexadecane gave 2-methylpropene (61, 62%) and methylcyclopropane (39–37%) in composition essentially identical with that from the hydrazone and sodium methoxide in aprotic solvents. It is suggested that salts of tosylhydrazones decompose to diazo compounds; the diazo compounds undergo (1) proton transfer from donor solvents and cationic decomposition of the Wagner–Meerwein type involving hydrogen and carbon-skeleton rearrangement and (2) carbenic decomposition in aprotic solvents to give olefins by hydrogen migration and *cyclopropanes by intramolecular insertion*. Additional evidence for the carbenic processes is derived from the observations that diazo compounds are detectable in the aprotic reaction products and that thermal decomposition of 1-diazo-2-methylpropane yields 2-methylpropene (67%) and methylcyclopropane (33%).

The carbenoid decomposition of other tosylhydrazones in sodium methoxide-diethyl Carbitol has been investigated.[5] The hydrocarbons and their per cent. compositions as obtained from these tosylhydrazones are: (1) propanal; propene (90%), cyclopropane (10%); (2) butanal; 1-butene (92%), methylcyclopropane (4.6%), *trans*-2-butene (2.3%), *cis*-2-butene (1.2%); (3) 2,2-dimethylpropanal; 1,1-dimethylcyclopropane (92%), 2-methyl-2-butene (7%), 2-methyl-1-butene (1%); (4) 2-butanone; *trans*-2-butene (67%), *cis*-2-butene (28%), 1-butene (5%), methylcyclopropane (0.5%); and (5) 3,3-dimethyl-2-butanone[6] (pinacolone); 3,3-dimethyl-1-butene (52%), 1,1,2-trimethylcyclopropane (47%). It is concluded that (1) rearrangements in carbenoid decomposition of diazo compounds involving hydrogen migration[7a] occur more readily than do carbon-skeleton rearrangements, (2) carbenoid decomposition of diazo compounds results in extensive intramolecular cyclization to give cyclopropanes,[7b] and (3) the secondary carbenes presumably formed as reaction intermediates are more selective in their decomposition than are their primary analogs.

We wish to acknowledge the assistance of Drs. R. R. Hopkins and I. J. Oita, Whiting Research Laboratories, Standard Oil Company (Ind.).

(3) For related reactions see H. Meerwein and K. v. Emster, *Chem. Ber.*, **53**, 1815 (1920); W. Hückel and F. Nerdel, *Ann.*, **528**, 57 (1957).

(4) The yields of hydrocarbons from proton-donor solvents are considerably smaller than that in aprotic solvents.

(5) Cationic decomposition is not totally suppressed in these systems because methanol is formed.

(6) Decomposition in diethylene glycol gave 3,3-dimethyl-1-butene (16%), 2,3-dimethyl-2-butene (16%), 2,3-dimethyl-1-butene (56%), 1,1,2-trimethylcyclopropane (11%). The fact that the decomposition of tosylhydrazones of 2-methylpropanal and 3,3-dimethyl-2-butanone in diethylene glycol yields cyclopropanes indicates that carbenoid decomposition occurs competitively with cationoid processes.

(7) (a) F. O. Rice and A. L. Glasebrook, THIS JOURNAL, **56**, 741 (1934) report that diazoethane decomposes to ethylene and nitrogen. (b) For related reactions of methylene see W. von E. Doering, R. G. Buttery, R. G. Laughlin and N. Chaudhuri, *ibid.*, **78**, 3224 (1956).

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ISOLATION OF CYTIDINE-5′-MONOPHOSPHO-N-ACETYLNEURAMINIC ACID[1]

Sir:

In conjunction with studies[2,3,4] on the metabolism and structure of the sialic acids, we have now isolated a new nucleotide, cytidine-5′-monophospho-N-acetylneuraminic acid, from *Escherichia coli* K-235, an organism which produces a polymer of NAN.[5,6]

The nucleotides from sonically disrupted cells were fractionated on Dowex-1,Cl^- resin using LiCl as eluting agent. A nucleotide, giving characteristic color reactions for sialic acid, was eluted slightly behind C5P, but before other nucleoside monophosphates. Paper chromatography of the material in this peak yielded two major components, C5P and C5P-NAN (R_{NAN} 0.36 and 0.61, respectively); free NAN was not detected.[7]

After elution from the paper, the C5P-NAN yielded these analyses (molar ratios): NAN, 0.97; cytidine, 1.00; organic phosphate, 1.01. The isolated C5P-NAN represented 6% of the total nucleotide adsorbed by the ion-exchange resin. Evidence that C5P-NAN was a single substance,[7] not a mixture of C5P and NAN, was obtained by paper chromatography in three solvent systems, paper electrophoresis at *p*H 5.0 and 7.7, and complete resistance to attack by NANaldolase[2] and rattlesnake venom 5′-nucleotidase. The ultraviolet-absorbing material on the paper chromato-

(1) The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies of The University of Michigan. This investigation was aided by a grant from the American Cancer Society and one from the National Institutes of Health (A-512).

(2) D. G. Comb and S. Roseman, THIS JOURNAL, **80**, 497 (1958).

(3) D. G. Comb and S. Roseman, *Biochim. et Biophys. Acta*, **29**, 653 (1958).

(4) S. Roseman and D. G. Comb, THIS JOURNAL, **80**, 3166 (1958); C. T. Spivak and S. Roseman, *ibid.*, **81**, 2403 (1959).

(5) G. T. Barry and W. F. Goebel, *Nature*, **179**, 206 (1957); G. T. Barry, *J. Exp. Med.*, **107**, 507 (1958).

(6) These abbreviations are used: NAN, N-acetylneuraminic acid; C5P, cytidine-5′-monophosphate; C5P-NAN, cytidine-5′-monophospho-N-acetylneuraminic acid; NANaldolase, N-acetylneuraminic acid aldolase.

(7) When stored in the dry state at −16°, C5P-NAN decomposed to NAN and C5P to the extent of 5 to 10% per day. Fresh samples of C5P-NAN exhibited trace spots of C5P and NAN on the chromatograms; these became more apparent each day the samples were stored.

grams and electrophoresis strips coincided exactly with the NAN positive spots.

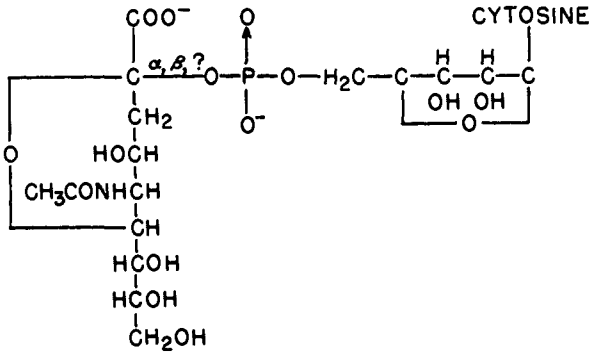
C5P-NAN is labile to acid; *e.g.*, 0.01 *N* HCl at 23° for 5 min. yielded 83% hydrolysis. After hydrolysis, the fragments were characterized as follows:

C5P by its absorption spectra, paper chromatography in three solvent systems,[8] stability of the phosphate ester to acid hydrolysis, rate of liberation of inorganic phosphate and cytidine on treatment with the 5′-nucleotidase, and resistance to purified phosphodiesterase.[9]

NAN was identified by paper chromatography and electrophoresis, its conversion to pyruvate and N-acetyl-D-mannosamine on incubation with purified NANaldolase, and the characteristic sialic acid color reactions with the resorcinol, direct Ehrlich, and thiobarbituric acid reagents.

The available data suggest that the carbonyl group of NAN is bound to the 5′-phosphate group of cytidine by a glycosidic bond. Treatment of C5P-NAN with hydroxylamine[10] yielded no hydroxamate. Further, the keto function was resistant to reduction with sodium borohydride and to oxidation with hypoiodite which was not the case in a mixture of NAN and CMP.

The data summarized above suggest a tentative structure for C5P-NAN (Fig. 1).



(8) Two of the solvent systems easily separated deoxycytidine-5′-monophosphate from C5P; in addition, the unknown CMP spots yielded positive reactions to the periodate spray reagent.

(9) The crude rattlesnake venom used for the 5′-nucleotidase showed no activity at comparable concentrations against the 2′- and 3′-nucleoside monophosphates. The phosphodiesterase had been purified free of 5′-nucleotidase and was a gift of Mr. Ronald Somerville, Department of Biological Chemistry, The University of Michigan.

(10) M. E. Jones, S. Black, R. M. Flynn and F. Lipmann, *Biochim. et Biophys. Acta*, **12**, 141 (1953).

(11) U. S. Public Health Service Medical Student Trainee at the Rackham Arthritis Research Unit (U.S.P.H.S. 2A-5026; training grant).

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RECEIVED SEPTEMBER 11, 1959

ACTIVATION BY ELECTRON TRANSFER—INDUCED CIS–TRANS ISOMERISM[1]

Sir:

In an earlier note[2] evidence was presented for the activation of a bridging group for ester hydrolysis by electron transfer. In a similar series of experiments in which the reactants were methylmaleatopentamminocobaltic ion and Cr^{++} or V^{++}, we find that not only does ester hydrolysis occur during electron transfer in the presence of H^+, but there is also activation of the ligand for isomerization to the fumarate.

On the basis of the following observations we feel justified in concluding that this activation of the ligand resulting in isomerization occurs only as a result of electron transfer.

The importance of H^+ is shown by a series of experiments using Cr^{++} as reductant.[3] The resulting solutions were passed through a cation exchange resin, eluted first with 0.15 *M* $HClO_4$ to remove monopositive ions, then with 1 *M* to remove dipositive ions. The relative proportions of the two ions are shown in the table: the ratio (+2 ion/+1 ion) varies linearly with the H^+ concentration. After the reaction, the organic ligand is complexed with Cr(III). The maleate is present as a monopositive ion, while any fumarate will be present in solution as a dipositive ion, since the fumarate cannot chelate. In solutions of lower acidity, the fumarate complex does lose a proton and can be eluted as a monopositive ion.

TABLE I

$[H^+]$, *M*	+1 ion	+2 ion	Ratio +2 ion/+1 ion
0.5	16	84	5.25
0.2	35	65	1.85
0.045	90	10	0.11

Using V^{++} as reductant, similar proportions of fumaric and maleic acids are obtained: these proportions were determined by extraction of the reaction mixture with ether and infrared examination of the organic material. The amount of fumaric acid produced increases with increasing H^+ concentration (in 0.5 *M* $HClO_4$, 85% of the total organic acid was extracted in the first three hours and was found to be almost pure fumaric acid: the rest was maleic).

In an experiment with V^{++} repeated in 99.5% D_2O containing 0.6 *M* $HClO_4$, the fumaric acid was separated from the maleic by selective extraction. Infrared examination of the two acids showed the presence of C-D bonds at 2200, 2090 and 940 cm.$^{-1}$ in the fumaric but not in the maleic acid; this we believe indicates that there cannot be exchange between D^+ (or H^+) and maleato complex. It is known from the preparative method that the maleato complex does not isomerize in acid solution in the absence of reductant. Furthermore, a similar experiment with fumaratopentamminocobaltic ion with V^{++} in D_2O produced fumaric acid containing no C–D bonds.[4] Thus we conclude that the ligand is activated for isomerization during the electron transfer process in the presence of H^+. As a minimum, the exchange results show that in the reaction path which leads to fumaric acid, a new C–H bond is formed when maleate absorbs the electron—a proton is necessarily lost again when the electron passes to the Co(III).

(1) This work is supported by the Atomic Energy Commission under Contract AT(11-1)-378.

(2) R. T. M. Fraser, D. K. Sebera and H. Taube, THIS JOURNAL, **81**, 2906 (1959).

(3) In all experiments, Co(III) and reductant were 0.010 *M*.

(4) D. K. Sebera, Ph.D. Dissertation, University of Chicago.