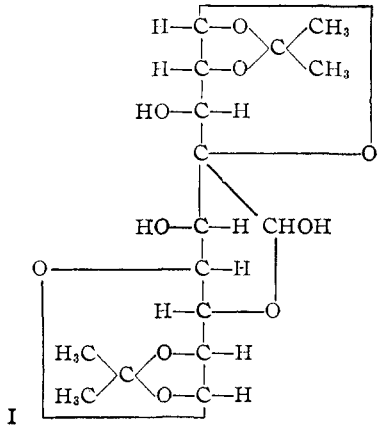
tion of suitable raw materials, branched-chain sugars containing from 8 to 14 carbon atoms may be prepared. A variety of sugar derivatives having from 4 to 7 carbon atoms is being investigated. The results are illustrated with 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose[7,8] (6 g. in 500 ml. of lime water), which gave a product, I, in 30% yield after 20 hr. at room temperature; compound I gradually decomposes above 235°; $[\alpha]^{24}D$ + 55.6° (*c* 1, water) at equilibrium; calculated molecular weight is 376 and a value of 374 was found in formamide. *Anal.* Calcd. for $C_{16}H_{24}O_{10}$: C, 51.1; H, 6.4. Found: C, 50.8; H, 6.5.



I

Structure I has been assigned to the product from the following: one mole of I reacted with only one mole of alkaline iodine; but after acid hydrolysis the resulting monobasic acid dialdehyde reacted with two more moles of hypoiodite per mole. Thus I is a branched-chain trialdehyde. I shows no carbonyl absorption in the infrared. On acetylation, only three acetyl groups are introduced. On reduction with sodium borohydride, followed by acetylation, four hydroxyl groups are substituted. I therefore has two free hydroxyl groups and a third in a hemiacetal ring. Mild acid readily removes one isopropylidene group from I. Partial oxidation of this product with periodate, followed by hydrolysis and separation of D-glucuronic acid, establishes the branching point to be at carbon atom 4 and, furthermore, establishes the configurations of carbon atoms 5 to 8. In forming I by an aldol condensation, no change in configuration of carbon atoms 1 to 3 of the starting material seems probable; hence those of carbon atoms 1 to 3 and 7 to 9 of the product are known. No evidence is yet available for assigning the configuration at carbon atom 4. A systematic name for the decose derivative is 9-aldo-4-*C*-formyl-1,2:8,9-di-*O*-isopropylidene-L-*xylo*-L-*altro*-(or L-*xylo*-L-*ido*)-nono-1,4:6,-9-difurano-4(1′),7-pyranose. This work will be described in detail in a forthcoming publication.

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(7) K. Iwadare, *Bull. Chem. Soc. Japan*, **16**, 40 (1941).

(8) R. Schaffer and H. S. Isbell, THIS JOURNAL, **79**, 3864 (1957), have characterized the crystalline product as a dimer, bis-(5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose)-3,5′:5′,5-cyclic acetal.

TERNARY OXIDES OF TETRAVALENT MOLYBDENUM

Sir:

In two recent publications[1,2] in THIS JOURNAL McCarroll, Katz and Ward present the results of work on the characterization and the crystal structure of ternary oxides of tetravalent molybdenum of the type $A_2Mo_3O_8$ (A = Mg^{+2}, Zn^{+2}, Co^{+2}, etc.). In the second of these publications[2] the authors state that "no ternary oxides of tetravalent molybdenum were known until Scholder, Klemm and Brixner[3,4] reported the preparation of the compounds $BaMoO_3$, $SrMoO_3$, $CaMoO_3$ and $MgMoO_3$." As the formulation of this statement is rather categorical, it should be pointed out that it is not entirely correct.

Seventy years ago Muthmann[5] in a paper on lower molybdenum oxides made a special section[6] devoted to "Verbindungen des Molybdändioxyds mit Basen" in which he describes the preparation, properties and composition of two compounds of exactly the type discussed by McCarroll, *et al.*, *viz.*, $Zn_2Mo_3O_8$ and $Mg_2Mo_3O_8$. Muthmann's paper is cited by Gmelin.[7]

(1) W. H. McCarroll, R. Ward and L. Katz, THIS JOURNAL, **78**, 2910 (1956).

(2) W. H. McCarroll, L. Katz and R. Ward, *ibid.*, **79**, 5410 (1957).

(3) R. Scholder and W. Klemm, *Angew. Chem.*, **66**, 467 (1954).

(4) R. Scholder and L. Brixner, *Z. Naturforsch.*, **10b**, 178 (1955).

(5) W. Muthmann, *Ann. Chem. Liebigs*, **238**, 108 (1887).

(6) Ref. 5, pp. 134–137.

(7) "Gmelins Handbuch der anorganischen Chemie," System-Nr. 53: Molybdän, 8 Aufl., Verlag Chemie G. m. b. H., Berlin, 1935, pp. 288 and 298.

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RECEIVED DECEMBER 2, 1957

THE MECHANISM OF FORMATE ACTIVATION[1]

Sir:

The formation of N^{10}-formyltetrahydrofolic acid[2] ($f^{10}FH_4$) from formate, ATP and FH_4 according to equation 1 was first observed with pigeon liver preparations,[3,4] and later encountered during a study of formiminoglycine degradation by extracts of *Clostridium cylindrosporum*.[5] The mechanism of

$$HCOOH + ATP + FH_4 \longrightarrow f^{10}FH_4 + ADP + P_i \quad (1)$$

this reaction has now been investigated with an enzyme, the formate activating enzyme (also

(1) This investigation was supported by the Atomic Energy Commission (contract No. AT(45-1)-173), the Institutional Grant to the University of Washington by the American Cancer Society, the Life Insurance Medical Research Fund and the United States Public Health Service (Grant No. CY-3310).

(2) The following abbreviations will be used: FH_4, 5,6,7,8-tetrahydrofolic acid; $f^{10}FH_4$, N^{10}-formyltetrahydrofolic acid; $f^{5-10}FH_4$, N^5,N^{10}-methenyltetrahydrofolic acid; FH_4-P, a phosphorylated derivative of tetrahydrofolic acid (position of the phosphate group not specified); DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide; ATP and ADP, adenosine tri- and di-phosphates; Pi, inorganic phosphate; TRIS, tris-(hydroxymethyl)-aminomethane.

(3) G. R. Greenberg, *Federation Proc.*, **13**, 745 (1954).

(4) G. R. Greenberg, L. Jaenicke and M. Silverman, *Biochim. et. Biophys. Acta*, **17**, 589 (1955).

(5) J. C. Rabinowitz and W. E. Pricer, Jr., THIS JOURNAL, **78**, 4176 (1956).

called tetrahydrofolate formylase[6]), obtained from *Micrococcus aerogenes*.[7] A 200-fold purification has been achieved by protamine precipitation, calcium phosphate gel adsorption, fractionation with ammonium sulfate and precipitation with $ZnCl_2$. The reaction requires Mg^{++} or Mn^{++} ion and a reducing agent.

The stoichiometry of equation 1 has been established with the purified enzyme by estimating ATP and ADP on Dowex-1 columns, and P_i, colorimetrically. $f^{10}FH_4$ was determined spectrophotometrically after conversion to $f^{5-10}FH_4$, and also by coupling with the hydroxymethyl tetrahydrofolic dehydrogenase.[8] Since no $f^{5-10}FH_4$ appears during reaction 1 and the preparation is free from cyclohydrolase,[9] it is evident that $f^{10}FH_4$ is the reaction product. In a typical experiment to determine the stoichiometry of equation 1, the following values were obtained: ATP = -2.71 μmoles, ADP = $+2.68$ μmoles, $f^{10}FH_4$ = $+2.80$ μmoles, and P_i = $+3.22$ μmoles.

Evidence has been obtained that reaction 1 consists of 2 steps, although both reactions appear to be carried out by the same enzyme

$$ATP - FH_4 \longrightarrow FH_4\text{-}P + ADP \quad (2)$$

$$FH_4\text{-}P + HCOOH \longrightarrow f^{10}FH_4 + P_i \quad (3)$$

Reaction 2 may be followed spectrophotometrically by estimating the ADP formed with pyruvic kinase and lactic dehydrogenase according to equations 4 and 5

$$ADP + \text{phosphoenolpyruvate} \longrightarrow \text{pyruvate} + ATP \quad (4)$$

$$\text{pyruvate} + DPNH + H^+ \longrightarrow \text{lactate} + DPN^+ \quad (5)$$

A component study of ADP formation from ATP and FH_4 is shown in Table I. During the interaction of ATP and FH_4, no *inorganic* phosphate is released.

If P^{32}-labeled ATP is incubated with the enzyme and FH_4, a radioactive, fluorescent compound may be isolated from the reaction mixture by chromatography on Dowex-1 columns, by paper ionophoresis, or by paper chromatography (in 70% ethanol–30% water containing 0.1% mercaptoethanol, the R_f for P_i, ATP and the radioactive, fluorescent compound are 0.01, 0.06 and 0.16, respectively[10]).

(6) G. R. Greenberg and L. Jaenicke in "The Chemistry and Biology of Purines" edited by G. E. W. Wolstenholme and C. O'Connor, Little, Brown and Company, Boston, Mass., 1957, pp. 204–232.

(7) H. R. Whiteley, *J. Bacteriol.*, **63**, 163 (1952).

(8) Y. Hatefi, M. J., Osborn, L. D. Kay and F. M. Huennekens, *J. Biol. Chem.*, **227**, 637 (1957).

(9) J. C. Rabinowitz and W. E. Pricer, Jr., THIS JOURNAL, **78**, 5702 (1956).

(10) Greenberg and Jaenicke[6] have likewise obtained chromatographic evidence for the occurrence of a fluorescent P^{32}-labeled compound during reaction 1, and have suggested the possibility of a two-step mechanism as outlined in equations 2 and 3.

TABLE I

FORMATION OF ADP FROM FH_4 AND ATP

The complete reaction mixture contained: 10 μM cysteine, 2 μM $MgCl_2$, 50 μM TRIS buffer at *p*H 7.0, 100 μM KCl, 100 μM NaF, 0.06 μM FH_4, 1.0 μM ATP, 1.5 μM phosphoenolpyruvate, 0.5 μM DPNH, 3 γ crystalline lactic dehydrogenase containing pyruvic kinase, and 60 γ formate activating enzyme in a total volume of 1.0 ml. The decrease in optical density at 340 mμ was measured over a 9-minute period.

Component omitted	DPNH disappearance, $\mu M \times 10^2$
None	9.27
ATP	0.65
FH_4	.65
Formate activating enzyme	.16
Mg^{++}	.81
Phosphoenolpyruvate	.97
Lactic dehydrogenase and pyruvic kinase	.16

Although the radioactive fluorescent compound is labile to air oxidation, it has been possible to elute the material from paper chromatograms and, upon incubation with formate and enzyme (but no ATP), to produce $f^{10}FH_4$.

Direct spectroscopic evidence for reaction 2 has also been obtained. The absorption spectrum of FH_4 is altered, *i.e.*, λ_{max} remains at 298,[11] but ϵ decreases by *ca.* 15% only after ATP and the enzyme have been added. Upon addition of formate, the spectrum then shifts to that of $f^{10}FH_4$.

The above evidence suggests that the first step in formate activation is the ATP-dependent phosphorylation of FH_4. The mechanism therefore resembles that proposed for succinate activation where S-phosphoryl Coenzyme A[12] is the intermediate, rather than that of fatty acid activation where an acyl adenylate[13] has been postulated. The finding that $f^{10}FH_4$ is the primary product of the reaction suggests that the postulated intermediate, FH_4-P, is the N^{10}-phosphoryl derivative of FH_4. The chemical synthesis and characterization of phosphorylated derivatives of FH_4 is in progress.

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RECEIVED OCTOBER 14, 1957

(11) M. J. Osborn, E. N. Vercamer, P. T. Talbert and F. M. Huennekens, THIS JOURNAL, **79**, 6565 (1957).

(12) R. A. Smith, I. F. Frank and I. C. Gunsalus, *Federation Proc.*, **16**, 251 (1957).

(13) P. Berg, THIS JOURNAL, **77**, 3163 (1955).

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