On the basis of these results we have tentatively identified the factor as ergothioneine or some closely related compound.

Details of the mechanism of the enzyme-catalyzed exchange reaction and the significance of ergothioneine will be discussed in subsequent papers.

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RECEIVED JUNE 13, 1956

A NEW METHOD FOR THE PREPARATION OF BOROHYDRIDES

Sir:

The authors have succeeded in preparing a series of borohydrides by the general reaction of hydrolysis of magnesium diboride, MgB_2, with bases. The MgB_2 used in this study was prepared by direct combination of boron and magnesium at 950° in a closed system under an argon atmosphere. The MgB_2 (84%) dissolved in acid solutions, leaving only small amounts of acid insoluble borides (*e.g.*, MgB_4) as a residue; it reacted exothermically with water to give hydrogen, traces of boranes, a water soluble fraction and grey water insoluble solid. The latter consisted mostly of $Mg(OH)_2$ and magnesium borates; the dark brown water soluble fraction gave off large amounts of hydrogen when acidified.

Hydrolysis of MgB_2 in strong basic media gave similar results but in KOH and $(CH_3)_4NOH$ solutions, KBH_4 and $(CH_3)_4NBH_4$, respectively, were isolated from solution. For example, 23 grams of MgB_2, (82.4%) were digested for 8 to 12 hours in 250 ml. of 3*M* KOH. The reaction mixture was kept well stirred and cooled during the addition of the MgB_2 to the base and during the first few hours of the reaction. Thereafter the reaction ran smoothly at room temperature. The water soluble fraction was rapidly filtered and slowly evaporated under vacuum. Due to their relatively low solubility the first crystals were easily separated from the remainder of the solution by filtration. Analytical data showed that this product of the hydrolysis of MgB_2 and strong KOH was KBH_4 (B: Calcd. 20.06%; Found, 19.99%). Four moles of gas per mole of KBH_4 were evolved upon acidification, in agreement with the equation $KBH_4 + H^+ + 3H_2O \rightarrow H_3BO_3 + K^+ + 4H_2$. Our observed value of $a_0^{25°} = 6.7274 \pm 0.0003$ Å. is in complete agreement with the reported value for KBH_4 of $a_0^{25} = 6.7274$ Å.[1] A 13% conversion of boron to borohydride was obtained, as determined by the amount of hydrogen evolved upon acidification of the solution. Other crystals which formed in the solution were found by analysis to be a potassium borate of the formula $KBO_2 \cdot 1\ 1/4\ H_2O$. The powder diffraction pattern shows principal lines having "d" values of 5.5m, 3.78m, 2.97s, 2.73m, 2.48m, 2.25s, 1.85m and 1.60m.

The hydrolysis of MgB_2 was carried out with other bases with comparable results—*e.g.*, 7.7 g. of MgB_2 reacted in 85 ml. of 4M $(CH_3)_4NOH$, was filtered, and the filtrate evaporated slowly in vacuum. The first crystalline product to separate from the solution was $(CH_3)_4NBH_4$. A powder diffraction pattern of the crystals showed a tetragonal lattice with $a_0 = 7.29$, $c_0 = 5.696$ and $c/a = 0.719$.

The experimental results show that one can produce any borohydride from the general reaction of hydrolysis of MgB_2 in a strong basic medium. The borohydride can be isolated from solution if it is stable in the basic medium at room temperature and less soluble than its borate, also present in solution. In any event one has a simple means available for the preparation of laboratory quantities of a basic solution of most borohydrides.

(1) S. C. Abrahams and J. Kalnais, *J. Chem. Phys.*, **22**, 434 (1954).

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THE ENZYMATIC SYNTHESIS OF N^{10}-FORMYLTETRAHYDROFOLIC ACID AND ITS ROLE IN ATP FORMATION DURING FORMIMINOGLYCINE DEGRADATION

Sir:

Extracts of *Clostridium acidi-urici* and *Clostridium cylindrosporum* degrade purines to formiminoglycine ($NH{=}CH{-}NH{-}CH_2{-}COOH$)[1] in a series of hydrolytic reactions.[2] The further metabolism of FIG by a purified enzyme preparation requires ADP, P_i and a cofactor present in boiled extracts, and leads to the formation of ATP as shown by reaction (1).[3]

$$\text{FIG} + \text{ADP} + P_i \longrightarrow \text{glycine} + \text{HCOOH} + NH_3 + \text{ATP} \quad (1)$$

The activity of the boiled extract is completely replaced by 10-formyl-THF or THF, and evidence has now been adduced for the following steps in the over-all reaction (1)

$$\text{FIG} + \text{THF} \longrightarrow \text{glycine} + NH_3 + \text{10-formyl-THF} \quad (2)$$

$$\text{10-formyl-THF} + \text{ADP} + P_i \rightleftharpoons \text{HCOOH} + \text{THF} + \text{ATP} \quad (3)$$

With substrate amounts of 10-formyl-THF, FIG is not required for the formation of ATP (Table I) [equation (3)]. As in the over-all reaction (1), ATP cannot be demonstrated unless hexokinase, glucose and $MgCl_2$ are added as a trapping system. In the absence of the trapping system, the equilibrium lies far to the left and formic acid and THF are readily converted to 10-formyl-THF in the presence of ATP.[4]

(1) Abbreviations used are: FIG, formiminoglycine; THF, tetrahydrofolic acid; 10-formyl-THF, N^{10}-formyltetrahydrofolic acid; 5-formyl-THF, N^5-formyltetrahydrofolic acid (leucovorin or citrovorum factor); 5,10-formyll-THF, the cyclic N^5-N^{10}-imidazolinium derivative of 5-formyl-THF (anhydroleucovorin or anhydrocitrovorum factor).

(2) J. C. Rabinowitz and W. E. Pricer, Jr., *J. Biol. Chem.*, **218**, 189 (1956), and earlier references cited therein; J. C. Rabinowitz and W. E. Pricer, Jr., *ibid.*, in press.

(3) J. C. Rabinowitz and W. E. Pricer, Jr., THIS JOURNAL, **78**, 1513 (1956).

(4) The formation of 10-formyl-THF from HCOOH, ATP and THF with a purified pigeon liver extract has been described by Greenberg, *et al.*[7]

TABLE I

UTILIZATION OF 10-FORMYL-THF FOR ATP FORMATION

The incubation mixture contained 50 μmoles of K maleate buffer, *p*H 7.0, 100 μ moles of K phosphate buffer, *p*H 7.0, 1 μmole of ADP (Sigma, sodium salt), 4.0 μmoles of 10-formyl-THF,[a] 0.5 μmole of ferrous sulfate, 2.5 μmoles of 2-mercaptoethanol, 3.0 mg. of hexokinase (Pabst), 20 μmoles of $MgCl_2$, 100 μmoles of glucose, 1.0 mg. of an acetone precipitate of an extract of *C. cylindrosporum* in a total volume of 1.5 ml. The tubes were flushed with helium, stoppered and incubated at 37°. The reaction was stopped by the addition of 0.5 ml. of 4% perchloric acid.

Time, min.	10-Formyl-THF utilized,[b] μmoles	ATP formed,[c] μmoles
5	0.90	0.97
15	1.35	1.42

[a] 10-Formyl-THF was prepared from 5-formyl-THF under conditions similar to those described by May, *et al.*[5] 65 mg. of 5-formyl-THF (leucovorin), 25 mg. of ascorbic acid, 0.5 ml. of 1 *N* HCl and 4.5 ml. of water were incubated in an evacuated tube overnight at room temperature. 5,10-Formyl!-THF thus formed was converted to 10-formyl-THF by adjusting the mixture to *p*H 7.0 with KOH. Both compounds are diastereoisomeric mixtures,[6] and if only one form is biologically active, the maximum amount of 10-formyl-THF which could be utilized would be 2.0 μmoles. Leucovorin was a gift of Dr. H. P. Broquist, Lederle Laboratories. [b] 10-formyl-THF was determined as 5,10-formyl!-THF within 5 min. after acidification of the incubation mixture, using a molar extinction coefficient of 22,000 at 350 mμ for the conversion of THF to 5,10-formyl!-THF.[7] [c] Measured as the glucose 6-P formed by the action of hexokinase.[8] The added ADP acts catalytically since it is regenerated by the action of hexokinase. The deproteinized sample was treated with 0.2 volume of a 30% suspension of acid washed Norite to remove the folic acid derivatives.

10-Formyl-THF is formed from FIG and THF by this preparation [reaction (2)]. However, reaction (2) appears to consist of two steps and evidence has been obtained for the occurrence of an intermediate with properties similar to those of 5,10-formyl!-THF. Thus, at *p*H 7.0, a compound with an absorption maximum at 356 mμ is formed on incubation of FIG and THF with the enzyme (Table II). This compound is distinct from 10-formyl-THF or 5-formyl-THF which shows no absorption maximum in this region. The spectral characteristics of the enzymatic product resemble those of 5,10-formyl!-THF in that both show absorption maxima at 356 mμ at *p*H 7.0,[9] and at 350 mμ in acid solution. The enzymatic product behaves identically with 5,10-formyl!-THF on paper chromatography; this, however, does not exclude an intermediate which is converted to 5,10-formyl!-THF under the conditions of chromatography.

On further incubation with the enzyme preparation, the intermediate disappears completely and 10-formyl-THF is formed.[10] Synthetic 5,10-formyl!-THF prepared from *dl*-leucovorin is also degraded by the enzyme preparation (Table III). However, in this instance only one-half of the added formyl!-THF is converted to 10-formyl-THF[11] suggesting that only one optical isomer is active in this enzymatic reaction.

TABLE II

FORMATION OF AN INTERMEDIATE FROM THF AND FIG

Cuvettes contained 50 μmoles of K maleate buffer, *p*H 7.0, 10 μmoles of 2-mercaptoethanol and the additions indicated below in a volume of 3.0 ml. THF was omitted from the reference cell. Optical density (O.D.) changes were determined with the Cary Model 14 recording spectrophotometer. The rates are calculated from the values observed for a 30 second period, 30 seconds after the addition of the enzyme.

Enzyme γ	Additions FIG μmoles	THF[a] μmoles	Δ O.D.$_{350}$/min.
...	...	1.0	0.000
80	...	1.0	0.000
80	2.0	1.0	+0.235
...	20	0.5	0.000
25	20	.5	+0.190
50	20	.5	+0.360
100	20	.5	+0.780[b]

[a] Prepared by catalytic reduction of recrystallized folic acid, as described by L. Jaenicke and G. R. Greenberg (personal communication). The glacial acetic acid used as a solvent was removed by lyophilization. Solutions were prepared in 0.1 *M* 2-mercaptoethanol and were stored *in vacuo* at 0°. [b] The compound formed in the enzymatic reaction, as well as 5,10-formyl!-THF, were visualized as fluorescent spots after chromatography in 0.5 *M* formic acid (R_f = 0.28) and *n*-propanol:*n*-butanol:0.1 *M* HCl::2:1:1 (R_f = 0.07).

TABLE III

ENZYMATIC CONVERSION OF 5,10-FORMYL!-THF TO 10-FORMYL-THF

Cuvettes contained 50 μmoles of K maleate buffer, *p*H 7.0, 10 μmoles of 2-mercaptoethanol, 50 λ of 5,10-formyl!-THF solution giving a final optical density at 350 mμ of 1.73, the amount of enzyme shown and water to make 3.0 ml. 5-formyl-THF was converted to 5,10-formyl!-THF by treatment with 0.1 *N* HCl and 0.1 *M* 2-mercaptoethanol in an evacuated tube overnight. This acid solution was used directly in the reaction vessel. Optical density changes were determined in a Cary Model 14 recording spectrophotometer. The rates were calculated from the initial values observed over a 30-second period.

Enzyme added, γ	ΔO.D.$_{350}$/min.
0	−0.005
12.5	−0.135
25	−0.200
50	−0.465
75	−0.630

Since 5,10-formyl!-THF is converted to 10-formyl-THF, it is tempting to postulate that 5,10-formyl!-THF occurs as an intermediate in the formation of 10-formyl-THF from FIG and THF,

(5) M. May, T. J. Bardos, F. L. Barger, M. Lansford, J. M. Ravel, G. L. Sutherland and W. Shive, THIS JOURNAL, **73**, 3067 (1951).

(6) D. B. Cosulich, J. M. Smith, Jr., and H. P. Broquist, *ibid.*, **74**, 4215 (1952).

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

(8) Evidence for the formation of 10-formyl-THF from formiminoglutamic acid and THF has been presented by A. Miller, *Fed. Proc.*, **15**, 316 (1956).

(9) 5,10-formyl!-THF is converted to 10-formyl-THF non-enzymatically at *p*H 7.0,[5] but at a slow rate under the conditions used (maleate buffer, *p*H 7.0, see Table III).

(10) The intermediate was formed as in Table II using 20 μmoles of FIG and 0.5 μmoles of THF. The O.D. at 350 mμ rose from 0.040 to 0.730 within 2 min. after the addition of 500 γ of the enzyme preparation. After 13 min., the peak had disappeared and the O.D. had decreased to 0.190. Within 5 min. after acidification with 1% perchloric acid and removal of the precipitated protein, an absorption maximum appeared at 350 mμ with an O.D. of 0.590. 5-formyl-THF is converted to 5,10-formyl!-THF under acid conditions at a much slower rate.[5]

(11) Within 2.5 min. after the addition of 500 γ of the enzyme preparation, the O.D. due to 5,10-formyl!-THF decreased from an initial value of 1.02 (0.96 in 1% perchloric acid) at 350 mμ to a constant value of 0.52, under conditions similar to those described in Table III. On deproteinization with 1% perchloric acid, the O.D. at 350 mμ was restored to 0.90 within 5 minutes.

and is, in fact, identical with the observed intermediate having an absorption maximum at 356 mμ. However, these results do not exclude other compounds, such as the hypothetical formiminotetrahydrofolic acid,[12] as intermediates which could give rise to 10-formyl-THF directly or by way of 5,10-formyl!-THF.

(12) R. D. Sagers, J. V. Beck, W. Gruber and I. C. Gunsalus, THIS JOURNAL, **78**, 694 (1956).

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RECEIVED JUNE 25, 1956

THE NUCLEAR MAGNETIC RESONANCE SPECTRA OF F^{19} IN METHYL- AND ETHYLFLUOROSILANES

Sir:

It has been suggested that the chemical shift of F^{19} atoms in binary fluorides has a direct relationship with the electronegativity of the atom bound to fluorine.[1] The extent of magnetic shielding of fluorine in the fluorosilanes is reported here. As would be expected the chemical shift differs from one compound to another.

Figure 1 shows the relative shifts of the methyl- and ethylfluorosilanes and silicon tetrafluoride in cycles per second in a magnetic field of approximately 9,989 gauss.[2] We used diethyldifluorosilane as reference substance. All the values given correspond to the pure liquids at room temperature. Extrapolation of values for an infinitely dilute solution in carbon tetrachloride from data obtained at various concentrations does not change the sequence of the shifts, nor does it affect the qualitative interpretation of the results. The probable error with the applied technique is within 2%.

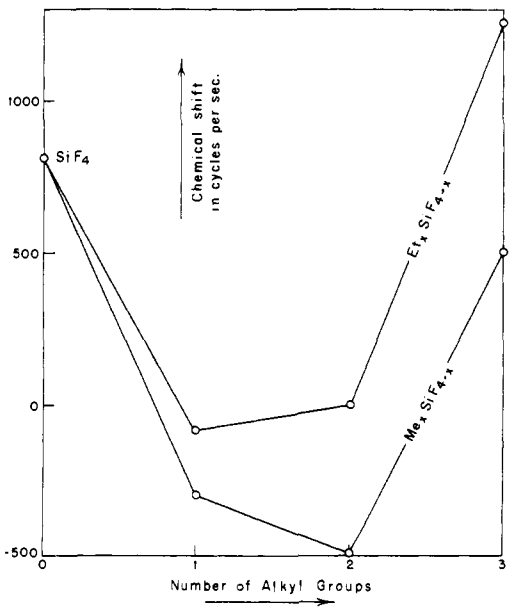

Fig. 1.

The values for silicon tetrafluoride were obtained from measurements at various concentrations in carbon tetrachloride and in *n*-hexane, and extrapolation of the value for the pure compound. For this particular measurement we believe that the maximum probable error does not exceed ±30 cycles per second.

The fluorine resonance of our reference substance is shifted 2,665 cycles per second toward higher fields from that of trifluoroacetic acid, and thus the F^{19} resonance of silicon tetrafluoride is 3,479 cycles per second higher than that of trifluoroacetic acid. (This would correspond to 0.554 gauss at 25.46 mc., which is the frequency used by Gutowsky and Hoffman[1] when they found 0.611.)

The shielding of the fluorine atoms in the fluorosilanes may well be controlled by a number of effects the most important of which are probably an inductive effect, and a π-bonding effect. Substitution of electron-releasing alkyl groups on silicon should result in an increase in shielding of the fluorine atoms. An opposing effect leading to a reduction in the fluoride shielding would be the occurrence of Si-F $d\pi$-$p\pi$-bonding.[3] From our present knowledge of chemical bonding it is not possible to quantitatively predict the extent of these two opposing effects. Certainly the electron-releasing effect would be increased by substituting ethyl groups for methyl.[4]

Extensive study of the proton resonance in methyl derivatives of silicon and the other group IV elements is now being conducted by Mr. A. L. Allred of this laboratory, and it is hoped that this will shed further light on the shifts of fluorine resonance noted above.

(1) H. S. Gutowsky and C. J. Hoffman, *Phys. Rev.*, **80**, 110 (1950); *J. Chem. Phys.*, **19**, 1295 (1951), and **20**, 200 (1952), who have measured a large number of fluorine compounds including silicon tetrafluoride.

(2) Measurements were made on a Varian N-M-R Spectrometer at 40 megacycles using a 5-mm. spinning sample tube containing a 1-mm. sealed capillary of reference substance as described by Aksel A. Bothner-By and Richard E. Glick, THIS JOURNAL, **78**, 1071 (1956).

(3) F. G. A. Stone and D. Seyferth, *J. Inorg. and Nuclear Chem.*, 1, 112 (1955).

(4) J. W. Baker, "Hyperconjugation," Oxford at the Clarendon Press, p. 5.

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RECEIVED JULY 11, 1956

CYCLOHEXADECA-1,3,9,11-TETRAYNE, A CYCLIC TETRA-ACETYLENE

Sir:

We have prepared the cyclic tetra-acetylene, cyclohexadeca-1,3,9,11-tetrayne (II), in one step from octa-1,7-diyne (Ia) by a novel cyclization reaction.

In the course of a general investigation into the oxidative coupling of terminal diacetylenes, the aerial oxidation of octa-1,7-diyne (Ia) in the presence of cuprous chloride and ammonium chloride was studied. When the reaction mixture in water was shaken in air at 20°, the ordinary dimer,[1] hexadeca-1,7,9,15-tetrayne (Ib) (m.p. 21–22°, b.p. 119–120° (0.1 mm.), $n^{23}D$ 1.5205, λ_{max} 226, 238 and 253 mμ, log ε, 700, 620 and 430 respectively, ν_{max} 3300 and 2235 cm.$^{-1}$, found: C, 91.32; H, 8.80)

(1) The terms "dimer" and "tetramer" are used to denote products derived respectively from two and four molecules of monomer, although their empirical formulas are of course not exact multiples of the monomer.