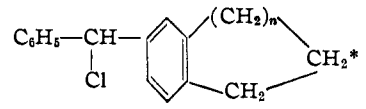
of the transition state by hyperconjugation (*i.e.*, no-bond resonance) has been most invoked to explain[1] this observation.

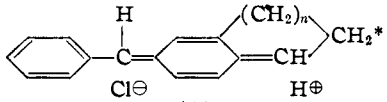
We have now measured solvolysis rates for compounds I, II and III at 0 and 25° in acetone–water (4:1) with the following results:



| Compound | ($k \times 10^5$ sec.$^{-1}$) 0° | 25° |
|---|---|---|
| I | 214 | 3996 |
| II | 147 | 2717 |
| III | 103 | 2066 |

I ($n$ = 1); II ($n$ = 2); III ($n$ = 3)

By analogy with the corresponding cyclic amines[2] and cyclic ketones[3] containing five-, six- and seven-membered rings, and in agreement with molecular models, the carbon atom marked C* lies at increasing distances from the plane of the benzenoid ring as one passes through the series I, II and III. Consequently, the energy required to form a quinoidal type transition state (represented by "A") must increase regularly in the order I < II < III.



(A)

In order to explain adequately these rate differences, it has become necessary to assume steric inhibition of hyperconjugation. We believe this to be the first experimental evidence in support of the concept.

Analytically pure samples of I, II and III, employed in this study, were prepared from highly purified crystalline alcohols using newly devised synthetic routes and special techniques which will be described in detail at a later date. It is now clear that the incorrect rate constants reported earlier[4] for compounds I and II resulted from erroneous analytical data.

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RECEIVED OCTOBER 9, 1951

(1) E. D. Hughes, C. K. Ingold and N. A. Taher, *J. Chem. Soc.*, 949 (1940).

(2) W. G. Brown and S. Fried, THIS JOURNAL, **65**, 1841 (1943).

(3) R. G. Kadesch, *ibid.*, **66**, 1207 (1944).

(4) R. T. Arnold, K. Murai and R. M. Dodson, *ibid.*, **72**, 4193 (1950).

(5) Du Pont Postdoctorate Fellow, 1949–1950.

## THE STRUCTURE OF $ZrMo_2$[1]

*Sir:*

The existence of an intermediate phase in the zirconium–molybdenum system having the composition $ZrMo_3$ and the A15 (beta-wolfram) structure has been reported.[2] We have prepared the alloys of compositions $ZrMo_2$ and $ZrMo_3$ by arc melting (using a technique which has been described elsewhere[2,3]) followed by heating for four hours at 1370° in an atmosphere of high-purity helium. Powder patterns were then taken, using radiation from a copper target filtered through nickel foil, and a camera of 22.92 cm. diameter. Inspection of the two patterns showed at once that they were identical except for a few weak lines, and that the common lines could readily be indexed on the basis of a face-centered cubic lattice with a parameter of 7.58 Å. Relative intensities were computed on the assumption that this face-centered cubic phase is $ZrMo_2$ with the C15 ($MgCu_2$) structure, taking into account the Lorentz, polarization, multiplicity, and structure factors. The calculated relative intensities were found to be in very good agreement with those estimated visually from the powder patterns.

We accordingly propose that the intermediate phase in the zirconium–molybdenum system has the ideal stoichiometric composition $ZrMo_2$ and the C15 crystal structure, and that there is no $ZrMo_3$ phase. $ZrMo_2$ thus has the same structure as that previously reported for $ZrW_2$.[3,4]

(1) This work was done under contract number DA-04-495-ORD-18 with the Army Ordnance Department, Washington, D. C.

(2) H. J. Wallbaum, *Naturwiss.*, **30**, 149 (1942).

(3) C. H. Schramm, P. Gordon and A. R. Kaufmann, *Trans. AIME*, **188**, 195 (1950).

(4) A. Claasen and W. G. Burgers, *Z. Kryst.*, (A) **86**, 100 (1933).

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## THE SYNTHESIS OF METHYL GROUPS FROM SERINE AND ITS BEARING ON THE METABOLISM OF ONE-CARBON FRAGMENTS[1]

*Sir:*

Further investigations on the conversion of the β-carbon of serine to the methyl groups of choline[2] and thymine[3] have shown that both β-hydrogen atoms accompany the carbon in this process. Following the administration of 2,3-deuterio-3-$C^{14}$-$N^{15}$-L-serine[4] to rats the choline from the internal organs was degraded and the $C^{14}$ activity and D concentration[5] of the methyl groups determined. The data (Table I) show that the $C^{14}$ and D of the

TABLE I

THE UTILIZATION OF THE β-CARBON AND β-HYDROGEN ATOMS OF L-SERINE FOR THE SYNTHESIS OF METHYL GROUPS

| | Serine administered | | Choline methyl groups | | | |
|---|---|---|---|---|---|---|
| Expt. | β-$C^{14}$, c.p.m.[a] × $10^{-5}$ | β-Deuterium, atoms D per β-carbon[b] | $C^{14}$, c.p.m.[a] | D, atoms per methyl group | Dilution $C^{14}$ | Dilution D |
| 1[c] | 3.13 | 0.725 | 2970 | 0.0061[d] | 106 | 119 |
| 2[e] | 0.626 | .575 | 461 | .0041[f] | 136 | 140 |

[a] Counts per minute per dish of carbon at infinite thickness and under standard conditions. [b] Atom per cent. excess D in serine × $10^{-2}$ × 7/2. See footnote 8. [c] Fed 0.47 mM. per 100 g. of body weight per day for 2 days. [d] Calculated from D concentration in betaine derived from choline (unpublished method). [e] Fed 0.53 mM. per 100 g. body weight per day for 2 days. [f] Atom per cent. excess D in $[(CH_3)_3N]_2H_2PtCl_6$ × $10^{-2}$ × 10/3 (V. du Vigneaud, *et al.*, *J. Biol. Chem.*, **140**, 625 (1941)).

(1) This work was supported by a grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council, and in part, by a grant from the Lederle Laboratories Division of the American Cyanamid Company.

(2) A. Weissbach, D. Elwyn and D. B. Sprinson, THIS JOURNAL, **72** 3316 (1950).

(3) D. Elwyn and D. B. Sprinson, *ibid.*, **72**, 3317 (1950).

(4) D. Elwyn and D. B. Sprinson, *J. Biol. Chem.*, **184**, 465 (1950).

(5) J. Graff and D. Rittenberg, *in press*.

$\beta$-carbon of serine underwent approximately the same dilution.

The methyl group of the thymine, isolated in experiment 1, had an activity of 7360 c.p.m.[3] and 0.0126 atom D. This would indicate a dilution of 43 for the carbon and 57 for the D.

A contribution of D to the methyl groups from the $\alpha$ position of serine (via $\alpha$-deuterioglycine[6]), which would significantly change these ratios, is unlikely, since glycine is a poor source of methyl groups,[2,3] and the $\alpha$-hydrogen atoms of glycine[7] and serine[8] undergo extensive labilization *in vivo*.

These findings impose certain restrictions on hypotheses concerning the mechanism of transport of one-carbon units. In the synthesis of methyl groups from serine the $\beta$-carbon does not appear to go through the oxidation level of formate since that would result in loss of at least half of its D. It should be noted that the DL-serine, from which the L-serine was obtained, was synthesized by reduction of ethyl formylhippurate with Al–Hg in the presence of $D_2O$. This makes it likely that the D is predominantly attached to the $\beta$-carbon atom of L-serine in only one of two possible configurations. If the unlabeled hydrogen is selectively eliminated[9] by enzymatic oxidation, the $C^{14}/D$ ratio would remain unchanged even if conversion to formate had occurred.

Exclusion of formate would also exclude formyl derivatives of folic acid, such as $N^{10}$-formylfolic[10] or $N^5$-formyl-5,6,7,8-tetrahydrofolic acid[11–13] ("citrovorum factor," folinic acid-SF, leucovorin) as actual carriers of a one-carbon fragment in this process, unless they also serve as specific carriers of the $\beta$-hydrogens of serine. There is considerable evidence to show that folic acid is linked to the metabolic reactions of one-carbon units, such as the synthesis of the methyl groups of choline and thymine and the various reactions of formate. Subject to the indicated limitations, our results suggest, however, that if leucovorin is the biological form of folic acid, its function is other than that of carrier of these units. These considerations may be limited to the reactions studied. In the utilization of other precursors, and in the synthesis of other products (*e.g.*, purines) a different mechanism may be involved.

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RECEIVED AUGUST 7, 1951

(6) D. Shemin, *J. Biol. Chem.*, **162**, 297 (1946).

(7) D. B. Sprinson and D. Rittenberg, *ibid.*, **184**, 405 (1950).

(8) D analyses on the administered serine and formaldehyde dimedon derivative obtained from carbon-3 (following oxidation of serine with $NaIO_4$) showed that the D was equally distributed between carbons 2 and 3. A similar degradation of serine isolated from the internal organ proteins in exp. 1 showed the $\beta$-$C^{14}/\beta$-$D/N^{15}$ ratios to be the same as in the compound fed. The $\alpha$-D was labilized, being only $^1/_3$ as high as the $\beta$-D (*cf.* ref. 7).

(9) A. G. Ogston, *Nature*, **162**, 963 (1948).

(10) M. Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).

(11) J. A. Brockman, Jr., *et al.*, *ibid.*, **72**, 4325 (1950).

(12) (a) M. May, *et al.*, *ibid.*, **73**, 3067 (1951); (b) A. Pohland, *et al.*, *ibid.*, **73**, 3247 (1951).

(13) H. P. Broquist, *et al.*, *ibid.*, **73**, 3538 (1951).

(14) Life Insurance Medical Research: (a) Postdoctoral Fellow, 1950–1951; (b) Predoctoral Fellow, 1950–1951.

## CRYSTALLINE CITROVORUM FACTOR FROM LIVER

*Sir:*

Subsequent to our observation that the citrovorum factor in liver (I) differed from a synthetic compound[1] resulting from the formylation and reduction of petroylglutamic acid[2] (II), we have been able to isolate citrovorun factor as its crystalline barium salt from horse liver.

The method used involved the following fractionation steps: (1) autolysis of the ground liver, (2) adsorption on charcoal and elution therefrom, (3) precipitation and removal of water-acid insoluble materials, (4) extraction into butanol at *p*H 3, (5) precipitation of impurities in the aqueous ammoniacal extract of the butanol extract with methanol, (6) adsorption on Dowex 1 column and subsequent elution, (7) adsorption of active fraction on charcoal and subsequent elution, (8) adsorption on $Al_2O_3$ column from aqueous alcohol solution and elution therefrom, (9) fractional crystallization of the barium salt.

When assayed with *Leuconostoc citrovorum* the isolated crystalline barium salt of citrovorum factor (I) was found to contain 237 units[3] per $\gamma$. However, the barium salt of the synthetic compound (II) was calculated to contain 115 units per $\gamma$ based on the reported activity of the free acid of II.[1] Thus, the product which we have obtained from horse liver is approximately twice as active for *L. citrovorum* as is the synthetic compound II.

The crystalline barium salt (I) at a concentration of 10 mg./l. in 30% ethanol containing 0.03% $NH_3$ showed a maximum at 286 m$\mu$ ($T$ = 35.3%) and a minimum at 243 m$\mu$ ($T$ = 77.9%). The X-ray powder diffraction data (obtained by William C. White) are given in Table I.

TABLE I

INTERPLANAR SPACINGS OF CRYSTALLINE BARIUM SALT (I)

| Å. | 8.11 | 7.51[a] | 7.31[a] | 6.52[a] | 5.35 |
|---|---|---|---|---|---|
| | 5.06 | 4.70 | 4.45 | 4.01 | 3.50[a] |

[a] Denotes most intense lines.

(1) M. Silverman and J. C. Keresztesy, THIS JOURNAL, **73**, 1897 (1951).

(2) J. A. Brockman, B. Roth, H. P. Broquist, M. E. Hultquist, J. M. Smith, M. J. Fahrenbach, D. B. Cosulich, R. P. Parker, E. L. R. Stokstad and T. H. Jukes, *ibid.*, **72**, 4325 (1950).

(3) J. C. Keresztesy and M. Silverman, *J. Biol. Chem.*, **183**, 473 (1950).

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## MANY-MEMBERED CARBON RINGS. IV. SYNTHESIS OF CYCLONONYNE AND CYCLODECYNE

*Sir:*

We have found that the synthesis of many-membered carbon rings containing an acetylenic group using the methods employed by Ruzicka in preparing cyclopentadecyne and cycloheptadecyne,[1] by Stoll in obtaining cycloheptadecyne-10-one,[2] and

(1) L. Ruzicka, M. Hürbin and H. A. Boekenoogen, *Helv. Chim. Acta*, **16**, 498 (1933).

(2) M. Stoll, J. Hultkamp and A. Rouve, *ibid.*, **31**, 543 (1948).