

β -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 α position of serine (via α -deuterioglycine⁶), which would significantly change these ratios, is unlikely, since glycine is a poor source of methyl groups,^{2,3} and the α -hydrogen atoms of glycine⁷ and serine⁸ 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 β -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 D₂O. This makes it likely that the D is predominantly attached to the β -carbon atom of L-serine in only one of two possible configurations. If the unlabeled hydrogen is selectively eliminated⁹ by enzymatic oxidation, the C¹⁴/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 N¹⁰-formylfolic¹⁰ or N⁵-formyl-5,6,7,8-tetrahydrofolic acid¹¹⁻¹³ ("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 β -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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(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 dimer derivative obtained from carbon-3 (following oxidation of serine with NaIO₄) 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 β -C¹⁴/ β -D/N¹⁵ ratios to be the same as in the compound fed. The α -D was labilized, being only 1/3 as high as the β -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).

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CRYSTALLINE CITROVORUM FACTOR FROM LIVER

Sir:

Subsequent to our observation that the citrovorum factor in liver (I) differed from a synthetic compound¹ resulting from the formylation and reduction of petroylglutamic acid² (II), we have been able to isolate citrovorum 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 pH 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 Al₂O₃ 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 units³ per γ . However, the barium salt of the synthetic compound (II) was calculated to contain 115 units per γ 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% NH₃ showed a maximum at 286 m μ ($T = 35.3\%$) and a minimum at 243 m μ ($T = 77.9\%$). 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)

\AA .	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

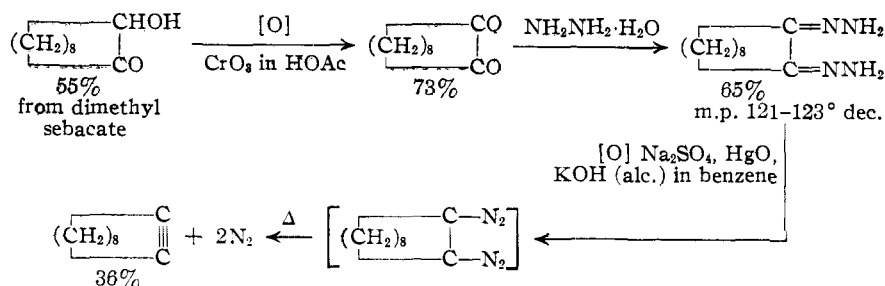
Sir:

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. Hultskamp and A. Rouve, *ibid.*, **31**, 543 (1948).

by Dominin³ in his presumed synthesis of cyclooctyne always seems to have afforded mixtures of cy-



clac acetylenes and isomeric allenes when applied to the nine- and ten-membered carbon rings. Thus, for example, dehydrohalogenation of 1-chlorocyclononene or dehalogenation of 1-bromo-2-chlorocyclodecene gave a mixture of C₉H₁₄ and C₁₀H₁₆ hydrocarbons, respectively, which upon ozonolysis yielded a mixture of suberic and azelaic acids or a mixture of azelaic and sebacic acids. Further, the infrared spectrum of the C₁₀H₁₆ hydrocarbon mixture showed a very weak absorption at 4.55 μ (C≡C stretching) and a much stronger absorption at 5.16 μ (C=C=C stretching).

However, we have obtained the cyclic C₉ and C₁₀ acetylenes employing a modification of a method developed by Curtius for synthesizing diaryl acetylenes.⁴ The method as applied to the nine- and ten-membered carbocycles is illustrated by the synthesis of cyclodecyne from sebacoin.

The cyclic acetylenes (C₉ and C₁₀) as first isolated were found to contain small amounts of carbonyl compounds and saturated hydrocarbons but they were not contaminated with allenes or other unsaturated hydrocarbons. Final purification of the two acetylenes was achieved by chromatography through silica gel. Their purification was followed by means of infrared absorption spectra, refractive index measurements, and by quantitative catalytic hydrogenation.

Cyclodecyne was purified by chromatographing through silica gel: b.p. 203–204° (740 mm.), *n*²⁰_D 1.4903, *d*²⁰₄ 0.8975 (Anal. Calcd. for C₁₀H₁₆: C, 88.16; H, 11.84. Found: C, 88.30, 88.06; H, 11.78, 11.98). Quantitative reduction in acetic acid using Adams catalyst required 99–100% of two molar equivalents of hydrogen. Ozonolysis gave only sebacic acid (37%), m.p. 126–129° after recrystallization and the mixed melting point with an authentic specimen of sebacic acid (m.p. 129–131°) was 126–129°. The ozonolysis product was also characterized as sebacic acid by conversion to the bis-*p*-toluidide, m.p. 198–200° (cor.). Hydration of the acetylene with sulfuric acid and mercuric sulfate in acetic acid gave cyclodecanone, isolated as its semicarbazone, m.p. 203.5–205.5° (cor.). A mixed melting point with an authentic specimen showed no depression. The infrared spectrum of the unsaturated hydrocarbon (Fig. 1) showed characteristic absorption for C≡C at 4.53 μ.

Cyclononyne was purified by chromatographing

(3) N. A. Dominin, *J. Gen. Chem. (U.S.S.R.)*, **3**, 851 (1938); *Chem. Abs.*, **33**, 1282 (1939).

(4) T. Curtius, *Ber.*, **22**, 2161 (1889); T. Curtius and K. Thun, *J. prakt. Chem.*, [2] **44**, 168 (1891).

through silica gel: micro b.p. 177–178° (740 mm.), *n*²⁰_D 1.4891, *d*²⁰₄ 0.8979. (Anal. Calcd. for C₉H₁₄: C, 88.45; H, 11.55. Found: C, 88.24; H, 11.64). Quantitative reduction in acetic acid using Adams catalyst required 102% of two molar equivalents of hydrogen. Hydration with sulfuric acid and mercuric sulfate in acetic acid gave cyclononone, identified as its semicarbazone (m.p. 179–180°). Mixed melting

point with an authentic specimen showed no depression. Infrared spectrum of cyclononyne (Fig. 1) showed characteristic absorption for C≡C at 4.54 μ.

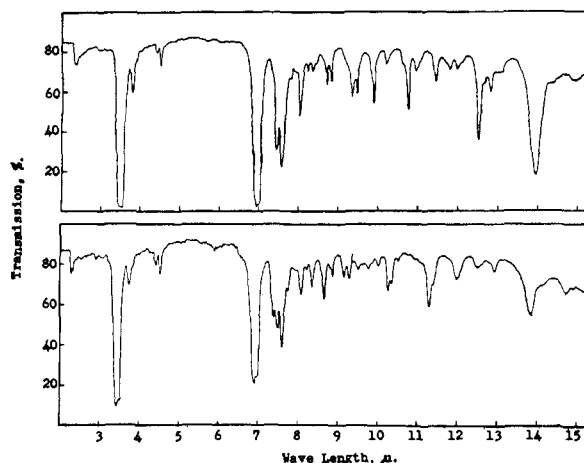


Fig. 1.—Upper curve infrared spectrum of cyclodecyne; lower curve infrared spectrum of cyclononyne.

Further details concerning these two cyclic acetylenes and the related cyclic *cis*- and *trans*-olefins will be presented as soon as possible in regular articles.

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A NEW TOTAL SYNTHESIS OF ESTRONE

Sir:

We wish to announce a novel and relatively short total synthesis of estrone in which the rings are formed in the sequence A, C, B, D starting from anisole (as ring A).¹ The scheme is stereospecific, except for one step which at the present stage of development, nevertheless, proceeds so as to give the correct stereoisomer as the only crystallizable product in 36% yield.

The tribasic acid I (R = H) was produced as previously described,² by reduction of the product

(1) This method was conceived prior to 1946 as an alternative to the approach of Johnson, Jones and Schneider (ref. 2); see also the M.S. thesis of W. P. Schneider, University of Wisconsin, 1946. A similar approach has been suggested independently by J. Dutta and D. K. Banerjee, *Science and Culture*, **13**, 408 (1947).

(2) W. S. Johnson, A. R. Jones and W. P. Schneider, *THIS JOURNAL*, **72**, 2395 (1950); see also D. I. Turner, *ibid.*, **73**, 1284 (1951).