μmoles per ml.) did not replace ADP. ATP (at 5 μmoles per ml.) was completely inactive, unless added together with hexokinase, glucose, and $MgCl_2$, which generated ADP from the ATP. These observations suggested that ADP was acting as a phosphate acceptor for a phosphorylation accompanying the conversion of FIG to glycine.

A partially purified fraction of *C. cylindrosporum* was prepared by acetone fractionation. Using this preparation, the conversion of FIG to glycine and formic acid is accompanied by the formation of an equivalent amount of ATP (Table III). The reaction may be summarized by the equation: FIG + ADP + P_i → glycine + HCOOH + NH_3 + ATP. This reaction with the purified fraction, which had not been treated with Dowex, is not stimulated by the addition of N-5-formyltetrahydrofolic acid (Leucovorin); however, the amount of ATP formed was doubled by the addition of a boiled extract of *C. acidi-urici*, which may contain the true coenzyme form of folic acid.[11] The activity of the purified fraction is completely dependent on the addition of ADP.

TABLE III

STOICHIOMETRY IN FORMIMINOGLYCINE DEGRADATION

The incubation mixture contained 250 μmoles of potassium phosphate, *p*H 7.0, 25 μmoles of ADP (Sigma, sodium salt), 2.5 mg. of hexokinase (Pabst), 125 μmoles of glucose, 50 μmoles of $MgCl_2$, 10 μmoles of ferrous sulfate, 50 μmoles of 2-mercaptoethanol, 1.0 ml. of a boiled extract of *C. acidi-urici* (see Table I), 50 μmoles of FIG, 0.5 ml. of an acetone precipitate of an extract of *C. cylindrosporum* containing 3.0 mg. of protein in a total volume of 5.0 ml. The mixture was incubated at 37° for the time indicated.

Time min.	FIG[a] utilized μmoles/ml.	Glycine[b] formed, μmoles/ml.	HCOOH[c] formed, μmoles/ml.	ATP[d] formed, μmoles/ml.	NH_3[e] formed, μmole/ml.
15	3.9	4.0	4.3	4.0	..
30	5.6	5.0	6.7	5.8	..
60	8.1	7.8	9.6	8.2	7.9

[a] Determined by a colorimetric procedure described elsewhere.[2] [b] Determined by a modification[2] of the method of Alexander, *et al.*[8] The values have been corrected for the blank which contained 3.4 μmoles per ml. of glycine. [c] Determined manometrically with formic hydrogenlyase.[9] The values have been corrected for the blank which contained 3.0 μmoles per ml. of formic acid. [d] ATP was determined as the G-6-P formed through the action of hexokinase. G-6-P was measured using *Zwischenferment* prepared by the method of Kornberg.[10] These values have been corrected for the G-6-P formed in a control tube from which FIG was omitted. The amount formed in this control was approximately 2 to 3 μmoles per ml. and was due to the presence of adenylate kinase. [e] Determined by nesslerization after adsorption on XE 64 K^+ and elution with 0.2 *N* NaOH.

The steps leading to the formation of FIG from xanthine which have been previously demonstrated[2,12,13] appear to be hydrolytic in nature, and thus are not likely to provide energy for the growth of the organism. The present reaction, which produces ATP, provides such a source of energy. While the detailed mechanism of this reaction is not known, formylglycine is not an intermediate since it is not converted to glycine by these extracts. Subsequent steps in the conversion of glycine to acetic acid may also provide energy for growth, and the mechanism of these reactions is under investigation.[14]

(8) B. Alexander, G. Landwehr and A. M. Seligman, *J. Biol. Chem.*, **160**, 51 (1945).

(9) H. Gest, Phosphorus Symposium, Vol. II. The Johns Hopkins Press, Baltimore, 1952, p. 522.

(10) A. Kornberg, *J. Biol. Chem.*, **182**, 805 (1950).

(11) B. E. Wright, THIS JOURNAL, **77**, 3030 (1955).

(12) J. C. Rabinowitz, *J. Biol. Chem.*, **218**, 175 (1956).

(13) J. C. Rabinowitz and W. E. Pricer, Jr., *ibid.*, **218**, 189 (1956).

(14) Isotope experiments[3] indicate qualitative differences with the conversion of glycine to acetate reported by T. C. Stadtman in *Clostridium sticklandi* (personal communication).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
UNITED STATES PUBLIC HEALTH SERVICE
BETHESDA, MARYLAND
JESSE C. RABINOWITZ
W. E. PRICER, JR.

RECEIVED MARCH 5, 1956

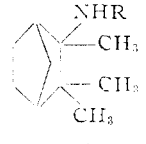
THE REACTION OF CAMPHENE WITH HYDROGEN CYANIDE

Sir:

On treating racemic camphene with hydrogen cyanide under strongly acidic conditions differing somewhat from those reported by Ritter,[1] we were surprised to obtain 3-formamidoisocamphane (I, m.p. 173–176°) instead of the expected N-formylisobornylamine. Use of excess cyanide and a reaction temperature below 5° resulted in a high yield of the new compound while alkaline hydrolysis of the mother liquors followed by careful isolation yielded only traces of isobornylamine.

Compound I is isomeric with the expected formylisobornylamine, *Anal.* Calcd. for $C_{11}H_{19}NO$: C, 72.86; 10.56; N, 7.73. Found: C, 73.06; H, 10.29; N, 7.42. It was found homogeneous (purity over 95%) by solubility analysis,[2] and was saponified in high yield to an amine (II), m.p. 175–176°, which was analyzed as the hydrochloride; *Anal.* Calcd. for $C_{10}H_{20}NCl$: C, 63.30; H, 10.63; N, 7.38. Found: C, 63.59; H, 10.79; N, 7.06. Comparison of the infrared spectra and other physical constants of both the amine hydrochloride and formyl derivative with the corresponding derivatives of authentic bornyl and isobornylamines revealed marked differences.

Oxidation of the amine with potassium permanganate resulted in a small amount of nitro compound, m.p. 198°; *Anal.* Calcd. for $C_{10}H_{17}NO_2$: C, 65.51; H, 9.14; N, 7.64. Found: C, 65.55; H, 9.16; N, 7.43, which could be hydrogenated back to starting material. With this indication of tertiary carbinamine structure,[3] II was tentatively identified as 3-aminoisocamphane, *i.e.*, the *unrearranged* structure resulting from addition of hydrogen cyanide to camphene.



I, R = CHO
II, R = H
III, R = CH_3

While racemic 3-aminoisocamphane is unreported, Hückel and Nerdel[4] prepared an optically active isomer from *d*-camphene hydrochloride by reaction with silver nitrite followed by sodium and

(1) J. J. Ritter and P. P. Minieri, THIS JOURNAL, **70**, 4045 (1948).

(2) T. J. Webb, *Anal. Chem.*, **20**, 100 (1948).

(3) N. Kornblum and R. J. Clutter, THIS JOURNAL, **76**, 4494 (1954).

(4) W. Hückel and F. Nerdel, *Ann.*, **528**, 57 (1937).

alcohol reduction of the nitro compound. Repetition of their work using racemic camphene gave nitro and amino compounds identical (infrared spectra and other physical constants) with those described above. In addition, lithium aluminum hydride reduction of I yields 3-methylaminoisocamphane (III), b.p. 72° (4 mm.), $n^{25}D$ 1.4881; hydrochloride, m.p. 243–246° dec., *Anal.* Calcd. for $C_{11}H_{22}NCl$: C, 64.83; H, 10.87; N, 6.88. Found: C, 64.54; H, 10.67; N, 6.90, identical with the product obtained in low yield by reaction of methylamine and camphene hydrochloride.

Isolation of 3-formamidoisocamphane from the acid-catalyzed reaction of hydrogen cyanide with camphene led us to re-examine this reaction with a series of simple nitriles. In every case the product was the N-acylisobornylamine to be expected from reaction with Wagner–Meerwein rearrangement, as reported for a few cases by Ritter and Minieri.[1]

The primary amine I shows a significant degree of ganglionic blocking action and this activity becomes pronounced in 3-methylaminoisocamphane (III). In animal experiments the ganglionic blocking properties of III, in terms of potency and specificity, compared favorably with the conventional bisquaternary ammonium drugs of the hexamethonium type. Notwithstanding its chemical dissimilarity, 3-methylaminoisocamphane appears to differ from these only in possessing an inherently longer duration of action and almost quantitative absorption following oral administration.[5]

We have assigned 3-methylaminoisocamphane the generic name mecamylamine, and it is currently undergoing extensive clinical trials.[6]

(5) We are indebted to our collaborators, Drs. K. H. Beyer and C. A. Stone of the Sharp & Dohme Division of Merck & Co., Inc., for the biological data.

(6) Under the Sharp & Dohme Division trade name Inversine ® Hydrochloride.

G. A. STEIN
M. SLETZINGER
H. ARNOLD
D. REINHOLD
W. GAINES
K. PFISTER, III

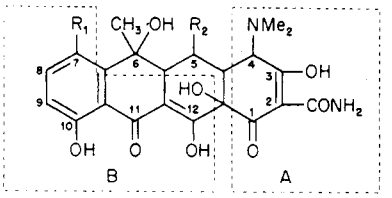
RESEARCH LABORATORIES
CHEMICAL DIVISION
MERCK & CO., INC.
RAHWAY, N. J.

RECEIVED FEBRUARY 2, 1956

EPITETRACYCLINE—THE CHEMICAL RELATIONSHIP BETWEEN TETRACYCLINE AND "QUATRIMYCIN"

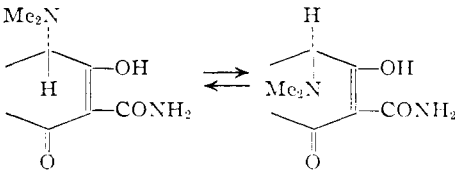
Sir:

In a recent communication[1] from another laboratory, it has been revealed that a reversible isomerization can occur in the antibiotics tetracycline, oxytetracycline and chlorotetracycline. The workers isolated an isomer from tetracycline[2] (I, R's = H) which they called "Quatrimycin." Previous to this report, we had also observed these phenomena and, in addition, had studied the chemical nature of the isomerization reaction. Our studies have established beyond reasonable doubt that racemization at C.4 carbon atom leads



I

to the equilibrium mixture previously described. Thus, the relationship between tetracycline and "Quatrimycin" (we have employed the term "epitetracycline" to describe this epimer—m.p. 170–171° (dec.), $(\alpha)^{25}D$ −339° (0.5% in methanol, 0.1 *N* in HCl), *Anal.* Calcd. for $C_{22}H_{24}N_2O_8$: C, 59.45; H, 5.44; N, 6.31. Found: C, 59.63; H, 5.52; N, 6.42) may be illustrated by expressions II and III—though no definite information on



II III

absolute configuration is available. A similar situation would apply in the case of oxytetracycline[3] (I, R = H, R_2 = OH) and chlorotetracycline[4] (I, R_1 = Cl, R_2 = H).

The evidence leading to this conclusion, taking the case of tetracycline as an example, is as follows: The ultraviolet absorption spectrum (*cf.* ref. 1 and Fig. 1) of epitetracycline (quatrimycin) differs significantly from that of tetracycline only in the region where the chromophoric group[5] A (see I) makes its contribution—absorption due solely to group B being essentially identical in each isomer. This relationship limits the site of isomerization to the relatively few atoms associated with group

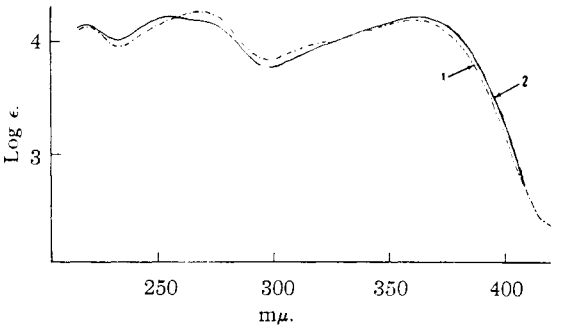


Fig. 1.—Ultraviolet absorption spectra in 0.01 *N* methanolic HCl: 1, · · · · · tetracycline; (2), ——— epitetracycline.

(1) Albert P. Doerschuk, Barbara A. Bitler and J. R. D. McCormick, THIS JOURNAL, **77**, 4687 (1955).

(2) Tetracyn is the registered trade-mark of Chas. Pfizer & Co., Inc., for the antibiotic tetracycline. Achromycin is the registered trademark of the Lederle Laboratories Division, American Cyanamid Company, for tetracycline.

(3) Terramycin is the registered trade-mark of Chas. Pfizer & Co., Inc., for the antibiotic oxytetracycline.

(4) Aureomycin is the registered trade-mark of the American Cyanamid Company for the antibiotic chlorotetracycline.

(5) *Cf.* C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **76**, 3568 (1954), and earlier papers for a discussion of the ultraviolet chromophores in the tetracycline series. It has been shown (R. B. Woodward, private communication) that the configuration of the C.12a center has a distinct effect on chromophore B.