$\nu_{max.}^{Nujol}$ 1734, 1688, 1664, 1600 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.75. Found: C, 75.22; H, 7.74) was obtained.

Alkaline rearrangement[12] of III afforded a 17β-hydroxyestrane-3,6-dione (IX), m.p. 145–146°, $[\alpha]_D$ −14° (methanol), $\nu_{max.}^{Nujol}$ 3290, 1715, 1704 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 75.12; H, 9.23).

From the third fraction (20% acetone) was obtained 1.1 g. of 11α-hydroxy-19-nortestosterone (IV), m.p. 167–168°,[13] $[\alpha]_D$ −46° (chloroform), $\lambda_{max.}^{alcohol}$ 242 mμ (15,475), $\nu_{max.}^{Nujol}$ 3345, 1650, 1610 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 74.37; H, 8.95). On acetylation it gave a diacetate (X), m.p. 190.5–191.5°, $[\alpha]_D$ −39.6° (chloroform). Oxidation afforded 19-noradrenosterone (XI), m.p. 213.5–215°, $[\alpha]_D$ +145° (methanol), $\lambda_{max.}^{alcohol}$ 240 mμ (14,600), $\nu_{max.}^{Nujol}$ 1732, 1698, 1665, 1612 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.75. Found: C, 75.09; H, 7.81).

The structure assignment for 11α-hydroxy-19-nortestosterone is based upon (1) molecular rotation data, (2) infrared, ultraviolet and chemical properties of IV and its derivatives, and (3) a consideration of the microbiological data obtained from fermentation of a large number of steroids with *Rhizopus nigricans*.[14]

(12) E. Ellis and V. A. Petrow, *J. Chem. Soc.*, 1078 (1939).

(13) A higher melting polymorph, m.p. 185–187°, was subsequently obtained.

(14) Since the preparation of this M.S., F. B. Colton (U. S. Patent 2,729,654) has described the preparation of epimeric 10-hydroxy-19-nortestosterone *via* the epoxide derived from 17β-hydroxy-5(10)-estren-3-one. Although distinguishing constants for the 10–17 diols are not given, one of the 10-hydroxy-10,17-ketones he describes corresponds reasonably well with that derived from the 10,17-diol produced microbiologically.

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ATP[1] FORMATION ACCOMPANYING FORMIMINOGLYCINE UTILIZATION

Sir:

Formiminoglycine (FIG) is formed from 4-aminoimidazole or xanthine by extracts of *Clostridium cylindrosporum*.[2] Washed cell suspensions of *Clostridum acidi-urici* convert FIG to acetic acid and carbon dioxide.[3] Extracts of this organism or of *C. cylindrosporum* have now been obtained which carry out the partial reaction in which FIG is converted to glycine, formic acid, and ammonia. When the extracts of *C. acidi-urici* are treated with Dowex-1 chloride, the activity of the enzyme is dependent on the addition of a boiled extract of the organism; this can be replaced by a number of folic acid derivatives (Table I). Sagers, *et al.*,[7] using a similar preparation, have also reported the activation of this reaction by tetrahydrofolic acid.

TABLE I

STIMULATION OF FORMIMINOGLYCINE DEGRADATION BY PTERIDINE DERIVATIVES

Compound	Pteridine,[a] μmoles/ml.	Activity[b]
No addition	0	1.6
Boiled extract[c]	0.024	3.8
	.24	8.4
Folic acid	.4	3.0
Tetrahydrofolic acid[d]	.9	5.5
Teropterin[e]	.3	6.2
N-10-Formylfolic acid[f]	.14	2.9
N-5-Formyltetrahydrofolic acid[g]	.06	7.1
Diglutamyl-N-10-formylfolic acid[h]	.04	6.8

[a] Calculated from the molar extinction coefficient at the absorption maximum in 0.1 *N* KOH. This was assumed to be 26,000 at 260 mμ for the boiled extract, which on this basis contained 2.4 μmoles per ml. [b] μMoles of FIG utilized in 20 min. at 37° in a system containing 10 μmoles of FIG, 50 μmoles of potassiun phosphate, *p*H 7.0, 0.5 μmole of Na_2S, 2 μmoles of ferrous sulfate, 0.4 ml. of enzyme, and the additions shown, in 1 ml. FIG was determined colorimetrically as described elsewhere.[2] The enzyme was an alumina-ground extract of *C. acidi-urici* treated with Dowex-1 chloride at 0° for 15 min. This preparation contained 21 mg. of protein per ml. [c] Prepared by heating 2.5 g. of lyophilized cells of *C. acidiurici* in 50 ml. of 0.01 *M* KPO_4, *p*H 7, 0.02 *M* cysteine in a boiling water-bath for 5 min. [d] Prepared by Dr. T. Miles from purified folic acid by catalytic reduction.[4] [e] Gift of Dr. H. P. Broquist, Lederle Laboratories, purified by Dr. B. E. Wright. [f] A sample obtained from the Lederle Laboratories and purified as previously described.[5] This was provided by Dr. M. Silverman. [g] A gift of Leucovorin from Dr. H. P. Broquist. [h] A sample isolated from *C. cylindrosporum* by Dr. B. E. Wright.[6]

Dowex treated extracts prepared from lyophilized cells of *C. cylindrosporum* which had been stored for over 2 years at −10° show an additional requirement for ADP and orthophosphate (Table II). UDP, CDP, IDP, and GDP (tested at 2

TABLE II

REQUIREMENTS OF FORMIMINOGLYCINE UTILIZATION

	FIG utilized, μmoles/ml.
Complete system[a]	6.1
Omit N-5-formyltetrahydrofolic acid	1.9
Omit ADP	1.7
Omit P_i[b]	1.6

[a] The complete system contained, per ml., 50 μmoles of potassium phosphate, *p*H 7.0, 10 μmoles of FIG, 1 μmole of ferrous sulfate, 0.5 μmole of 2-mercaptoethanol, 0.2 μmole of N-5-formyltetrahydrofolic acid, 5 μmoles of ADP, an extract of lyophilized cells of *C. cylindrosporum* equivalent to 6 mg. of protein, prepared in maleate buffer and treated with Dowex-1 chloride. Tubes were incubated at 37° for 30 min. [b] The phosphate was replaced by 25 μmoles of maleate buffer, *p*H 6.8, which showed no inhibition.

(1) The following abbreviations have been used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; CDP, cytidine diphosphate; IDP, inosine diphosphate; GDP, guanosine diphosphate; UDP, uridine diphosphate; G-6-P, glucose 6-phosphate.

(2) J. C. Rabinowitz and W. E. Pricer, Jr., in preparation.

(3) J. C. Rabinowitz and W. E. Pricer, Jr., *Federation Proc.*, **15**, in press (1956).

(4) H. P. Broquist, M. J. Fahrenbach, J. A. Brockman, Jr., E. L. R. Stokstad and T. H. Jukes, THIS JOURNAL, **73**, 3535 (1951).

(5) M. Silverman, J. C. Keresztesy and G. J. Koval, *J. Biol. Chem.*, **211**, 53 (1954).

(6) B. E. Wright, *ibid.*, in press.

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

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, C. 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**, 3930 (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[2] indicate qualitative differences with the conversion of glycine to acetate reported by T. C. Stadtman in *Clostridium sticklandi* (personal communication).

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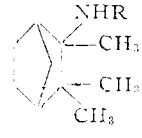
THE REACTION OF CAMPHENE WITH HYDROGEN CYANIDE

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 *rearranged* 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).