exchange between eucarvone and deuteroethanol at room temperature (oximation conditions). Our findings indicate clearly that *three* hydrogens from eucarvone are replaceable by deuterium under these conditions.[3] Since eucarvone in the monocyclic form has only two readily replaceable hydrogens, and in view of the results mentioned above, the intervention of the bicyclic ketone VI and/or its anion seems probable.

If there is any of the bicyclic ketone VI normally in equilibrium with eucarvone, the amount is small (less than 1%) as determined from the ultraviolet spectrum of eucarvone, λ_{max} 302 mμ (log ϵ 3.82), only slight end absorption near 230 mμ.[4]

It seems likely that other transformation products of eucarvone (including some previously described) also possess the bicyclic nucleus. We are investigating some of these cases at present.

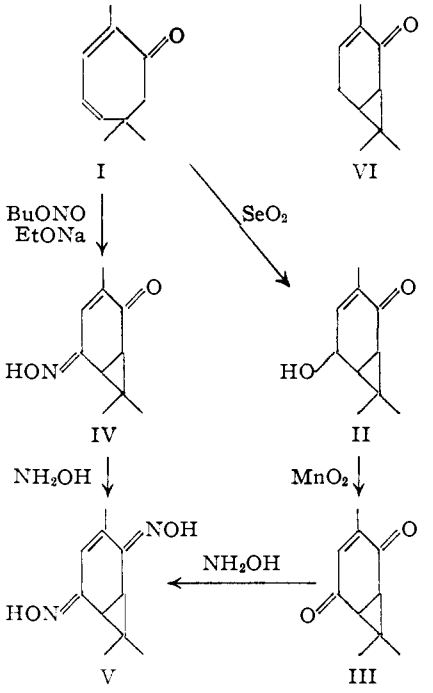

Fig. I.

(3) Determined by isolation of the eucarvone, combustion, and assay of the D_2O–H_2O mixture by the falling drop method: A. S. Keston, D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, **122**, 227 (1942).

(4) All ultraviolet spectra were determined in 95% ethanol.

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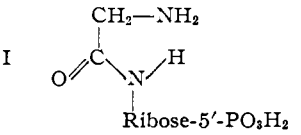
RECEIVED AUGUST 26, 1954

GLYCINE RIBOTIDE INTERMEDIATES IN THE *de novo* SYNTHESIS OF INOSINIC ACID[1]

Sir:

Pigeon liver extracts carry out the synthesis of two glycine-containing aliphatic ribotides which appear to be intermediates in the *de novo* synthesis of inosinic acid. Compound I has been tentatively assigned the following basic structure:



The second compound (II) appears to differ from I by the presence of a formyl group.

The synthesis of I by pigeon liver extracts, passed through a Dowex-1-chloride column and dialyzed, requires ATP[2], R-5-P, glycine and glutamine (Table I). This reaction is measured by the conversion of glycine-1-C^{14} to a radioactive compound which does not lose C^{14} when treated with ninhydrin. I is eluted from a Dowex-1-formate column with 0.05 *M* ammonium formate at *p*H 6.5.

TABLE I

REQUIREMENTS FOR THE SYNTHESIS OF I

Additions: 20 mg. of an extract of pigeon liver acetone powder treated with Dowex-1 chloride,[3] dialyzed *versus* 0.05 *M* K_2HPO_4 and lyophilized. 1.5 μM. ATP, 5 μM. Na phosphocreatine, 0.05 ml. 1:2 aqueous rabbit muscle extract dialyzed against water, 6.4 μM. $MgCl_2$, 5 μM. glycine-1-C^{14}, 5 μM. glutamine, 2.5 μM. K-ribose-5-phosphate, 30 μM. K_2HPO_4 (final quantity); vol. 0.67 ml.; time 20 min., 38°, air.

R-5-P		+	−	+	+
Glutamine		+	+	−	+
ATP[a]		+	+	+	−
μM. C^{14} glycine	Expt. 1	0.25	0.00	0.01	...
incorporated	Expt. 2	0.15	0.01	0.02	0.02

[a] Includes regenerating system of creatine phosphokinase (muscle extract) and phosphocreatine.

II is eluted from a Dowex-1-formate column with 0.05 *M* ammonium formate at *p*H 5.0. This compound possesses a characteristic acid-labile (0.1 *N* HCl, 100°, 15 min.) formyl group which allows its direct determination. II can be labeled by either C^{14} glycine or C^{14} formate, but not by $C^{14}O_2$. The requirements for the synthesis of II are: ATP, R-5-P, glycine, glutamine, formate and boiled extract of liver. The boiled extract can be completely replaced by leucovorin or by tetrahydrofolic acid.[3] Some of these requirements are shown in Table II.

TABLE II

REQUIREMENTS FOR THE SYNTHESIS OF II

Conditions as in Table I plus the following: 0.2 mg. Ca leucovorin or tetrahydrofolic acid (neutralized), 2.5 μM. C^{14} Na-formate and non-radioactive glycine; time 30 min.

Experiment	1			2			
FAH_4	+	−	−	−	−	−	−
CF	−	+	−	+	+	+	+
R-5-P	+	+	+	+	−	+	+
Glutamine	+	+	+	+	+	−	+
Glycine	+	+	+	+	+	+	−
Hydrolyzable C^{14}-formyl, μm.	0.35	0.37	0.016	0.49	0.12	0.08	0.04

Under conditions for synthesis of II, small quantities of I are found by chromatographic anal-

(1) This investigation was aided by grants from the U. S. Public Health Service, National Institutes of Health, The Elisabeth Severance Prentiss Foundation and Eli Lilly and Company.

(2) Abbreviations: adenosine triphosphate, ATP; D-ribose-5-phosphate, R-5-P; leucovorin (*dl* form of citrovorum factor), CF; tetrahydrofolic acid, FAH_4.

(3) G. R. Greenberg, THIS JOURNAL, **76**, 1458 (1954).

ysis, but in the absence of the cofactor and of formate only I accumulates. These results suggest that I is a precursor of II and that a reduced folic acid derivative is required for the introduction of carbon 8 as well as carbon 2[3,4] of the purine ring. It is not known whether previously described ribose-phosphate compounds[5,6,7] are involved in this system.

I and II can each be separated on the ion exchange column into two compounds (Ia and Ib, IIa and IIb) which appear to be isomers, the nature of which is unknown. The radioactive carbon of I or II previously labeled by glycine-1-C^{14} is readily incorporated into inosinic acid in the presence of large pools of unlabeled glycine under conditions for *de novo* synthesis.[8] Both I and II behave on ion exchange columns and by paper chromatography and electrophoresis as ribotides. I is converted by formylation[9] under mild conditions to a compound which behaves identically with II on the Dowex-1-formate column. Analyses of partially purified preparations of I and II provide evidence that each contains R-5-P, amido and glycine moieties. The organic phosphate to pentose[10] ratio of I was 1.16[11] to 1.00. On acid hydrolysis of II[12] the only orcinol-reacting compound found corresponded exactly on an ion exchange column[12] to R-5-P. All of the phosphate of II showed an acid stability equivalent to that of adenosine-5-phosphate. Dilute acid hydrolysis of II produced approximately one mole of reducing sugar[13] per mole of pentose. The acid-labile nitrogen (1 *N* HCl, 30 min., 100°) to pentose ratio of I was 1.5, of IIa 1.17 and IIb 1.10. Since compound I labeled by glycine-1-C^{14} does not lose radioactivity by treatment with ninhydrin at *p*H 5.5 but does so after mild acid hydrolysis, glycine is presumed to be present as an amide. After acid hydrolysis, I and II yielded glycine, analyzed qualitatively by paper chromatography and quantitatively[14] by the formaldehyde produced in the ninhydrin reaction. Between 1 and 1.5 moles of glycine per mole of pentose was found. Total N to pentose ratios of 1.75 and 2.30 shown by IIa and IIb, respectively, are in accord with the suggested basic structure. I and II exhibited absorption beginning only below 240 mμ. It should be emphasized that the exact identity of I and II is not known. Whether the formyl group is on the amino or amido nitrogen has not been established.

The isolation of these glycine amide ribotides suggests that the formation of an aliphatic ribotide may be the primary step in purine synthesis.[15]

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RECEIVED SEPTEMBER 2, 1954

(4) J. M. Buchanan and M. P. Schulman, *J. Biol. Chem.*, **202**, 241 (1953).

(5) W. J. Williams and J. M. Buchanan, *ibid.*, **203**, 583 (1953).

(6) M. Saffran and E. Scarano, *Nature*, **174**, 959 (1953).

(7) A. Kornberg, I. Lieberman and E. S. Simms, THIS JOURNAL, **76**, 2027 (1954).

(8) G. R. Greenberg, *Federation Proc.*, **10**, 192 (1951).

(9) E. H. Flynn, *et al*, THIS JOURNAL, **73**, 1979 (1951).

(10) Except as noted all the analytical data presented below are on the same partially purified preparations of I and II. Pentose was determined by the orcinol method (W. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939), heating 45 min. and using adenosine-5′-phosphate as a standard).

(11) The lowest phosphate:pentose ratio obtained in a preparation of II was 1.8 but because of the apparent chemical formylation of I to yield II, the isolation of only R-5-P from II, and the chromatographic behavior of II, it is considered that the excess phosphate represents an organic impurity.

(12) J. X. Khym and W. E. Cohn, THIS JOURNAL, **76**, 1818 (1954).

(13) J. T. Park and M. J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).

(14) B. Alexander, G. Landwehr and A. M. Seligman, *ibid.*, **160**, 51 (1945).

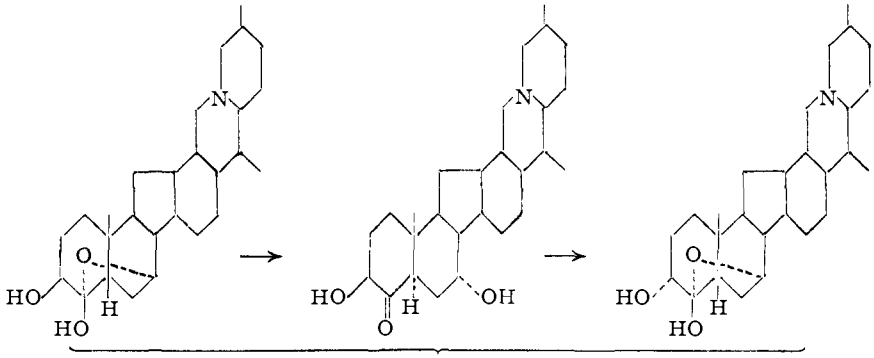
(15) The technical assistance of Mr. Brown Conrow is gratefully acknowledged.

(16) Oglebay Fellow in Medicine, 1952–1954.

THE STRUCTURE OF RINGS A AND B IN GERMINE

Sir:

We should like to propose the following partial structures for germine (I), isogermine (II), and pseudogermine (III).



I II III

Recent studies by Jacobs and his collaborators[1,2,3] have established that germine possesses the same skeletal structure as cevine. We recently reported that germine and pseudogermine contain the same α-ketol-5-membered hemiketal system and differ only in the orientation of the hydroxyl group of the α-ketol system.[4] The close parallel of the isomerization reactions of germine to those of veracevine indicates that the location of the α-ketol system is the same in germine and its isomerization products as in veracevine and its isomerization products.[5] We should like now to present evidence which favors C_7 as the terminus of the ether bridge of the hemiketal system in germine, rather than C_9, the terminus in veracevine.[5]

Acetylation of germine with acetic anhydride

(1) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **148**, 57 (1943).

(2) W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.*, **18**, 765 (1953).

(3) S. W. Pelletier and W. A. Jacobs, THIS JOURNAL, **76**, 2028 (1954).

(4) S. M. Kupchan, M. Fieser, C. R. Narayanan, L. F. Fieser and J. Fried, *ibid.*, **76**, 1200 (1954).

(5) D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).