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]

DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE
WESTERN RESERVE UNIVERSITY
CLEVELAND, OHIO

DAVID A. GOLDTHWAIT[16]
RICHARD A. PEABODY
G. ROBERT GREENBERG

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).

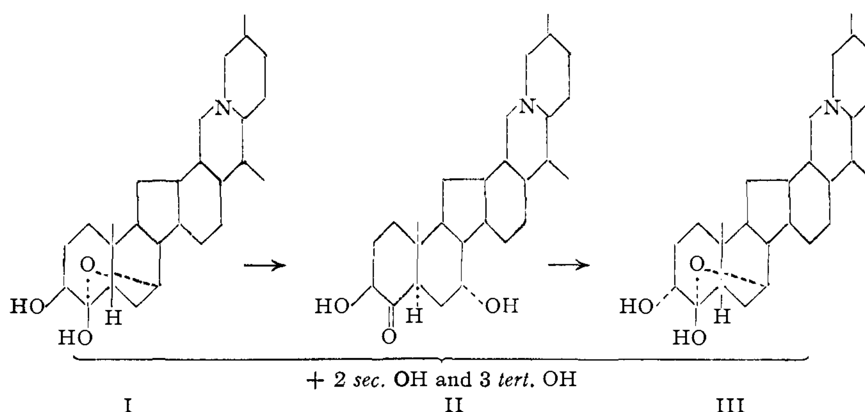
(15) The technical assistance of Mr. Brown Conrow is gratefully acknowledged.

(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).



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).

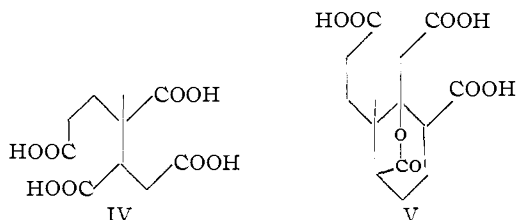
(4) S. M. Kupchan, M. Fieser, C. R. Narayanan, L. F. Fieser and J. Fried, *ibid.*, **76**, 1200 (1954).

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

and pyridine yields germine tetraacetate,[6] stable to chromic acid, m.p. 260–261° dec.; $[\alpha]^{23}D$ −98° (c 1.57, py.). Calcd. for $C_{27}H_{39}O_8N(COCH_3)_4$: C, 62.02; H, 7.59; acetyl, 25.40. Found: C, 61.77; H, 7.50; acetyl, 25.22. Treatment of isogermine under the same conditions affords isogermine tetraacetate, m.p. 222–226°; $[\alpha]^{24}D$ −80° (c 1.63, py.). Found: C, 61.69; H, 7.49; acetyl, 25.85. Acetylation of pseudogermine under the same conditions yields pseudogermine tetraacetate, m.p. 208–220°; $[\alpha]^{24}D$ −59° (c 1.70, py.). Calcd. for $C_{27}H_{39}O_8N(COCH_3)_4 \cdot H_2O$: C, 60.41; H, 7.68; acetyl, 24.75. Found: C, 60.34; H, 7.29; acetyl, 24.44. The fact that all three isomers yield tetraacetates indicates that the ether bridge of the hemiketal system must terminate at a primary or secondary carbon atom.

Moreover acetylation of dihydrogermine[7] leads to dihydrogermine pentaacetate, stable to chromic acid, m.p. 288–290° dec.; $[\alpha]^{28}D$ −64° (c 1.41, py.). Calcd. for $C_{27}H_{40}O_8N(COCH_3)_5$: C, 61.56; H, 7.68; acetyl, 29.82. Found: C, 61.61; H, 7.72; acetyl, 29.82. This result is compatible with the view that sodium-alcohol reduction of germine proceeds by liberation and then reduction of a keto group with concomitant liberation of the hydroxyl group involved in the hemiketal ring. Since this hydroxyl group is acylable, it must be primary or secondary.

The fact that the hemiketal ring in germine is 5-membered[4] indicates that the terminus of the ether bridge of the hemiketal system must be at C_1, C_7, or C_{19}. The formation of the hexanetetracarboxylic acid IV by chromic acid oxidation



of germine[1,5] shows the absence of oxygen from positions C_1 and C_{19} and fixes C_7 as the terminus of the ether bridge. The failure to detect the lactone tricarboxylic acid V among the products of chromic acid oxidation of germine[1,5] is readily explicable on the basis of partial formulation I for germine.

(6) Earlier reports (W. Poethke, *Arch. Pharm.*, **275**, 571 (1938); J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950)) that germine forms a pentaacetate have been found to be in error. Recent acetyl determinations both at Harvard and at the Squibb Institute agree with a tetraacetate formula.

(7) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **149**, 451 (1943).

DEPARTMENT OF CHEMISTRY
HARVARD UNIVERSITY
CAMBRIDGE 38, MASS. AND
THE SQUIBB INSTITUTE
FOR MEDICAL RESEARCH
NEW BRUNSWICK, N. J.

S. MORRIS KUPCHAN
MARY FIESER
C. R. NARAYANAN
LOUIS F. FIESER
JOSEF FRIED

RECEIVED AUGUST 16, 1954

CHOLESTEROL—A PRECURSOR OF TETRAHYDROCORTISONE IN MAN

Sir:

Cholesterol has been shown to serve as a precursor of two adrenal steroid hormones (hydrocortisone and corticosterone) *in vitro*.[1,2] This communication demonstrates that cholesterol is converted in man to an adrenal steroid metabolite, pregnane-3-α-17-α-21-triol-11,20-dione (THE), probably via Δ^4-pregnene-17-α-21-diol-3,11,20-trione (cortisone).[3] This appears to be the first report of the conversion of cholesterol to an 11-oxygenated steroid in man.

A solution was made of 4.24 μc. of 4-C^{14}-cholesterol[4] (5 μc/mg.) and 33.8 μc. of cholesterol-*t*[5,6] (7.3 μc./mg.) in 2.3 g. of propylene glycol and added to 30 ml. of sterile human plasma. The mixture was given intravenously to a male arthritic patient. Cholesterol with two labels was chosen in this experiment in order to determine whether or not the side chain of cholesterol-*t* is utilized in steroid hormone production. A 24-hour urine sample was collected on the second day after the administration of the radiocholesterol and a crude neutral steroid extract prepared.[7] The extract was subjected to paper partition chromatography[8] for seven days and the steroid, with the same mobility as that of THE, was then eluted and rechromatographed for five days in the same system.[9] The lower of two well-defined areas[10] (corresponding to the movement of pure THE[11]) was cut out and eluted with methanol. An aliquot was mixed with pure THE and found to travel as a single spot in the above solvent system.[12] The remainder, 2.8 mg., was converted to the diacetate and mixed with 10.09 mg. of pure THE diacetate, m.p. 233–234°. Following vacuum sublimation and five recrystallizations from methanol, the THE diacetate melted at 232–233.5°. The ultraviolet spectrum of this material in sulfuric acid after two hours was identical with that given by pure THE diacetate treated in the same manner.[13] The mixture of labeled THE diacetate and carrier, 5.01 mg. (Sample A) was dissolved in 50 ml. of toluene containing 100 mg. of 2,5-diphenyloxazole.[14]

(1) A. Zaffaroni, O. Hechter and G. Pincus, THIS JOURNAL, **73**, 1930 (1951).

(2) O. Hechter, M. M. Solomon, A. Zaffaroni and G. Pincus, *Arch. Biochem. Biophys.*, **46**, 201 (1953).

(3) S. Burstein, K. Savard and R. I. Dorfman, *Endocrinology*, **52**, 448 (1953).

(4) Procured from Radioactive Products, Inc., Detroit, Michigan.

(5) This was generously supplied by R. G. Gould, Los Alamos Scientific Laboratory.

(6) Oppenhauer oxidation and equilibration with alkali indicated that 37.5% of the tritium was located at positions 2, 3, 4 and 6. The remainder of the tritium probably was located at positions 24, 25, 26 and 27 (see D. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **198**, 861 (1952)).

(7). B. Baggett, R. A. Kinsella, Jr., and E. A. Doisy, *ibid.*, **203**, 1013 (1953).

(8) R. B. Burton, A. Zaffaroni and E. H. Keutman, *ibid.*, **188**, 763 (1951).

(9) For the rechromatography the sheets were impregnated with propylene glycol diluted with methanol 1:2 and 1 mg. of steroid per sheet was applied.

(10) In this run the lower area was 6 to 8 cm. from the starting line, while in later 7-day runs it appeared from 13 to 15 cm. from the origin.

(11) We are grateful to Dr. Karl Pfister of Merck, Inc., for supplies of this steroid.

(12) In other runs aliquots were removed at this point, mixed with authentic THE and run for six days in the benzene–formamide system.[8] On development only one spot was observed. Other aliquots were converted to THE diacetate and were found to have the same mobility as pure THE diacetate in the benzene-formamide system.

(13) A. Zaffaroni, THIS JOURNAL, **72**, 3828 (1950).

(14) F. N. Hayes and R. G. Gould, *Science*, **117**, 480 (1953).