COMMUNICATIONS TO THE EDITOR

TRANSFORMATION OF INOSINIC ACID TO ADENYLIC AND GUANYLIC ACIDS IN A SOLUBLE ENZYME SYSTEM[1]

Sir:

We have shown recently that a soluble enzyme extract from rabbit bone marrow was capable of synthesizing compounds of adenine and guanine from inosinic acid (IMP) in the presence of DPN.[2] Activity was lost upon dialysis and restored by addition of boiled extract. We have now found that the boiled extract may be replaced by appropriate amino donors. Three reaction sequences have been observed in the biosynthesis of adenine and guanine nucleotides from IMP in the dialyzed extract: (1) An enzyme, inosinic acid dehydrogenase, catalyzes the oxidation of IMP to xanthosine 5′-phosphate (XMP) by DPN. (2) XMP is aminated to guanosine 5′-phosphate (GMP) in the presence of *l*-glutamic acid or *l*-glutamine, ATP and Mg^{++}. (3) IMP is aminated to adenosine 5′-phosphate (AMP) in the presence of *l*-aspartic acid and a high energy phosphate source.

When IMP was incubated with the dialyzed extract and DPN, a xanthine derivative accumulated. This was isolated by chromatography on Dowex-1-formate using the elution gradient technique of Hurlbert, *et al.*,[3] and purified by rechromatography on Dowex-1-acetate. Its specific activity (168,000 counts C^{14}/min./μmole) was equal to the mean IMP value. It was identified as XMP by (1) absorption spectrum (maxima at 263 mμ and 235 mμ at *p*H 2); (2) analytical values of 1.02 moles P and 0.94 mole ribose per mole of xanthine; (3) ready hydrolysis with 5′-nucleotidase of *Crotalus adamanteus* venom; and (4) a positive Schiff periodate test[4] indicating the 2′- and 3′-positions were unsubstituted.

Amino donors were identified by incubating dialyzed extract (0.8 ml.) with 2 μmoles of DPN, 10 μmoles of glucose, 0.5 μmole of IMP-8-C^{14}, and, in place of boiled extract, 5 μmoles of one of the following: *l*-aspartic acid, *l*-asparagine, *l*-glutamic acid, *l*-glutamine, *l*-alanine, *l*-arginine, ammonium chloride, and glycine. Of these, it was found that aspartic acid replaced the boiled extract for adenine synthesis, and glutamic acid or glutamine for guanine formation.

Optimal activity in the amination of IMP required the addition of aspartic acid, 3(−)phosphoglycerate (PGA), K^+, Mg^{++}, and inorganic PO_4. Phosphopyruvate can be substituted for PGA but, as indicated in Table I, ATP is not an adequate substitute for PGA, and addition of ADP as a phosphate acceptor has an apparent inhibiting rather than a stimulating effect. The other nucleoside triphosphates tested (UTP and GTP) were also inferior to PGA. In previous experiments,[2] we have used glucose plus DPN to satisfy the energy requirement for amination.

TABLE I

REQUIREMENT FOR HIGH ENERGY PHOSPHATE IN THE AMINATION OF IMP

Each vessel had a total volume of 1.0 ml. containing 0.75 ml. dialyzed extract, 0.5 μmole IMP-8-C^{14}, 10 μmoles *l*-aspartate, 5 μmoles $MgCl_2$, 5 μmoles potassium phosphate, 50 μmoles tris buffer, pH 7.4, and the additions shown below. Incubated at 37° for 30 min.

Additions	μmoles adenine formed
None	0.000
PGA, 10 μmoles	.16
ATP, 3.5 μmoles	.022
UTP, 2 μmoles	.029
GTP, 2 μmoles	.018
PGA + ADP, 3 μmoles	.078
PGA + UDP, 2 μmoles	.19

The requirements for the amination of XMP are glutamic acid or glutamine, ATP and Mg^{++}. As shown in Table II, in contrast to IMP amination, PGA alone is ineffective unless ADP is also added, and ATP completely replaces PGA + ADP.

TABLE II

CONDITIONS FOR THE AMINATION OF XMP

Extract and tris buffer as in Table I with 0.2 μmole XMP-8-C^{14}, 5 μmoles *l*-glutamate, 5 μmoles *l*-glutamine, 5 μmoles $MgCl_2$, and additions shown below.

Additions	μmoles guanine formed
None	0.001
PGA, 10 μmoles	.007
ADP, 1.5 μmoles	.035
PGA + ADP	.14
ATP, 3.5 μmoles	.14
UTP, 1.6 μmoles	.011

In the foregoing experiments free purines were isolated after acid hydrolysis. In order to demonstrate that the hydroxypurines were indeed aminated in the nucleotide form, chromatography was carried out on Dowex-1-acetate without hydrolysis. Table III demonstrates that the IMP to AMP and

TABLE III

ADENINE AND GUANINE NUCLEOTIDES FORMED FROM IMP AND XMP

10 ml. reaction volumes with reactants as indicated in Tables I and II; incubated at 37° for 60 min.

	μmoles	counts/min./μmole
IMP added	8.1	34,100
IMP recovered	0.8	30,300
AMP	1.4	25,500
ADP	2.4	25,000
ATP	2.7	24,400
XMP added	3.9	40,400
XMP recovered	1.3	39,100
GMP	1.2	25,700

(1) Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and by the U. S. Atomic Energy Commission under Contract AT (30-1)-1818.

(2) R. Abrams and M. Bentley, *Arch. Biochem. and Biophys.*, in press.

(3) R. B. Hurlbert, H. Schmitz, A. F. Brumm and V. R. Potter, *J. Biol. Chem.*, **209**, 23 (1954).

(4) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (**1950**).

XMP to GMP reactions occurred with no extensive dilution of specific activity.

The mechanisms of the amination reactions are being investigated. Carter and Cohen[5] have reported the formation of adenylosuccinic acid from AMP and fumaric acid. It seems likely that this compound is an intermediate in AMP formation from IMP, and that the corresponding guanylo-glutaric acid is an intermediate in GMP formation from XMP.

(5) C. E. Carter and L. H. Cohen, THIS JOURNAL, **77**, 499 (1955).

THE INSTITUTE OF RESEARCH
MONTEFIORE HOSPITAL — RICHARD ABRAMS
PITTSBURGH 13, PA. — MARIAN BENTLEY

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RAUWOLFIA ALKALOIDS. XXII. FURTHER OBSERVATIONS OF THE STEREOCHEMISTRY OF RESERPINE

Sir:

Four lines of evidence have been offered recently in support of Ia as representing the stereochemistry of reserpine[1,2]: (1) the C-3 epimerization of reserpine, (2) molecular rotational differences indicating β-orientation of the C-3 hydrogen, 16-carbomethoxyl and 18-acyloxy groups, (3) the inability of 3-isoreserpic acid to lactonize and (4) data strengthening the presently accepted structures of allo- and 3-epialloyohimbane. We now wish to describe new findings demonstrating conclusively the validity of Ia.

When 3-iso-reserpinol (II)[3] was treated with *p*-toluenesulfonyl chloride in pyridine at room temperature overnight, a substance crystallized directly from the reaction mixture in high yield. An inspection of its properties made it evident that the substance must be a quaternary salt. It has a high melting point (320–330° (dec.)) and is virtually insoluble in chloroform. Analysis indicated it to be a mixed tosylate–chloride salt. *Anal.* Calcd. for $C_{22}H_{29}N_2O_2^+ \cdot 0.6\ SO_3C_7H_7^- \cdot 0.4Cl^-$: C, 66.86; H, 7.09; N, 5.96; S, 4.09; Cl, 3.02. Found: C, 66.64; H, 7.10; N, 6.02; S, 3.89; Cl, 2.87. Addition of an excess of sodium iodide to a hot aqueous solution gave an immediate precipitate of the crystalline iodide salt (m.p. 360–365° (dec.)). *Anal.* Calcd. for $C_{22}H_{29}N_2O_2^+ \cdot I^-$: C, 54.98; H, 6.10. Found: C, 55.33; H, 6.17. A free base extractable in chloroform could not be liberated from the salt with dilute ammonia. The infrared spectrum of the mixed tosylate–chloride salt showed the bands characteristic of the *p*-tosylate ion[4] and the presence of chloride ion was revealed by an instantaneous precipitate with silver nitrate. Formulation of this quaternary salt as III requires a *cis* relationship of the hydrogens at C-15 and C-16 restricting the stereochemistry of reserpine to Ia. Similarly, reserpinol (Ib)[5] gave a quaternary tosylate best characterized as its iodide salt (m.p. 315–316° (dec.)).

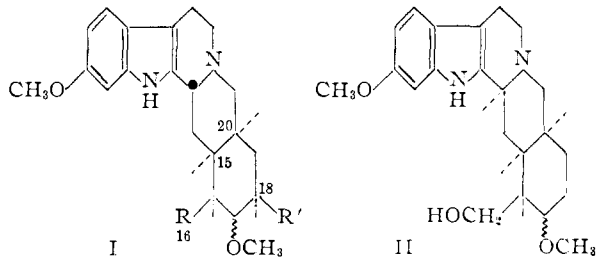
(1) C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F. St. André, *Experientia*, in press.

(2) E. Wenkert and L. H. Liu, *ibid.*, in press.

(3) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, THIS JOURNAL, in press.

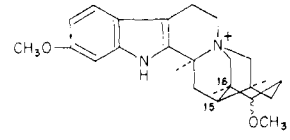
(4) P. A. Diassi, F. H. Weisenborn, C. M. Dylion and O. Wintersteiner, *ibid.*, **77**, 2028 (1955).

(5) C. F. Huebner, H. B. MacPhillamy, A. F. St. André and E. Schlittler, *ibid.*, **77**, 472 (1955).



I II

a: R = —COOCH₃, R′ = —OCOC₆H₂(OCH₃)₃
b: R = —CH₂OH, R′ = H.



III

RESEARCH DEPARTMENT
CIBA PHARMACEUTICAL PRODUCTS, INC.
SUMMIT, NEW JERSEY — C. F. HUEBNER

DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE — E. WENKERT
AMES, IOWA

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STUDIES IN THE SYNTHESIS OF THE ANTIRACHITIC VITAMINS. III. THE SYNTHESIS OF 1-CYCLOHEXYLIDENE-2-[5′-METHOXY-2′-METHYLENECYCLOHEXYLIDENE-1′]-ETHANE

Sir:

Several years ago we reported[1] the synthesis of a simple model (I) of vitamin D. However, subsequent work in this Laboratory[2] showed that the method used was impractical for the synthesis of vitamin D_2 or D_3. In view of work undertaken in other laboratories[3] we wish to report at this time the synthesis of 1-cyclohexylidene-2-[5′-methoxy-2′-methylenecyclohexylidene-1′]-ethane (III) using a method which can easily be adapted for the synthesis of all vitamin Ds.

Cyclohexylidene acetaldehyde (6.8 g.)[4,5] prepared by the chromic acid oxidation[6] of 1-ethenylcyclohexanol-1 was allowed to condense with 14 g. of 4-methoxycyclohexanone with stirring under nitrogen in 900 cc. of methanol containing 4 g. of sodium hydroxide and 10 cc. of water. After twelve hours the mixture was acidified and from it was obtained 6.1 g. (58.6%) of the ketone (II), recrystallized from ligroin, m.p. 79.5–80.5°. *Anal.* Calcd. for (II): C, 76.92; H, 9.46; mol. wt., 234. Found: C, 76.77; H, 9.45; mol. wt. (in exaltone), 224; ε (309 mμ), 29,100. The infrared spectrum shows strong bands for the dienone. 2,4-Dinitrophenylhydrazone, m.p. 178–179°. *Anal.* Calcd. for $C_{21}H_{26}N_4O_5$: C, 60.86; H, 6.33; N, 13.52. Found: C, 61.05; H, 6.68; N, 13.66.

The ketone (II) was allowed to react with freshly

(1) N. A. Milas and W. L. Alderson, Jr., THIS JOURNAL, **61**, 2534 (1939).

(2) Unpublished results.

(3) I. T. Harrison, B. Lythgoe and S. Trippett, *Chem. and Ind.*, April, 507 (1955).

(4) J. B. Aldersley, G. N. Burkhardt, A. E. Gillam and N. C. Hindley, *J. Chem. Soc.*, 10 (**1940**).

(5) V. K. Paranjpe, N. L. Phalnikar and B. V. Bhide, *J. Univ. Bombay*, **28**, 38 (1949–50).

(6) R. Kuhn and C. Grundmann, *Ber.*, **70**, 1897 (1937).