leads in one purification step from a crude concentrate to a product of high purity. This method is based on the observation[2] that CoA, owing to its character as a sulfhydryl compound,[3] can be precipitated in strong acid solution by Cu_2O in the presence of reduced glutathione (GSH). This step supplemented by available column procedures[4] for initial concentration and for elimination of carrier GSH permits preparation of CoA containing about 20% pantothenic acid from yeast in 10–15% yield.

CoA is adsorbed on a charcoal column from an aqueous extract (100°) of dried brewers' yeast and eluted with 5% pyridine (yield 80%). The eluate is shaken with chloroform, concentrated, and the coenzyme precipitated with 5 volumes of acetone (yield 60–70%). A solution of the acetone powder is again passed through a charcoal column and eluted as before after washing with dilute alkali (yield 80%). The eluate is freed from pyridine and concentrated to contain 5% solids.[5] GSH is added (10–20 mg./ml.) and the *p*H adjusted to 7. After a few minutes 0.05 volume of 10 *N* H_2SO_4 is added and Cu_2O is stirred in slowly as outlined by Hopkins.[6] The precipitate is washed with 0.5 *N* H_2SO_4, then with water until sulfate-free and finally decomposed with H_2S (yield 30–35%). After removal of CuS and H_2S the solution is passed through Dowex 50 (H^+) to remove GSH. The effluent is concentrated and lyophilized (yield 85%, over-all yield 10–15%). In a typical run from 8 kg. of dried yeast, 620 mg. of powder was obtained which analyzed as follows:

	%	Molar ratio
Pantothenic acid		
Microbiological[7]	22	1.0
Spectrophotometric, DPN reduction[8]	21	1.0
Transacetylase[9]	24	1.1
Sulfanilamide acetylation[10]	22	1.0
Adenine, from ultraviolet absorption at 260 mμ	13.7	1.0
Ribose	18.9	1.3
Glutathione, glyoxalase[11]		
GSH	3	0.1
GSSG	4	0.065
L-Glutamic acid, after acid hydrolysis[12]		
Microbiological, *L. arabinosus*	14	0.95
Decarboxylase, *E. Coli*[13]	11	0.75
Phosphorus, Fiske-SubbaRow[14]		
Inorganic	<0.1	
Total	10.3	3.3
Nitrogen, Kjeldahl	13.3	9.5
Sulfur[15]	4.68	1.45
Carbon[15]	32.3	27
Hydrogen[15]	4.83	48
Ash[15]	0	—

Electrophoresis and paper chromatography of such preparations revealed that the bulk of the material and CoA activity moved as a single component, although two to four minor components were detected.

(2) H. Beinert, R. W. Von Korff and D. E. Green, unpublished.

(3) W. H. Devries, W. M. Govier, J. S. Evans, J. D. Gregory, G. D. Novelli, M. Soodak and F. Lipmann, THIS JOURNAL, **72**, 4838 (1950); G. M. Brown, J. A. Craig and E. E. Snell, *Arch. Biochem.*, **27**, 473 (1950); F. Lynen and E. Reichert, *Angew. Chem.*, **63**, 47 (1951).

(4) D. A. Buyske, R. E. Handschumacher, Harvey Higgins, Tsoo E. King, F. M. Strong, V. H. Cheldelin, L. J. Teply and G. C. Mueller, *J. Biol. Chem.*, **193**, 307 (1951); D. A. Buyske, R. E. Handschumacher, Harvey Higgins, and F. M. Strong, unpublished.

(5) The second charcoal treatment may be omitted if a final pantothenic acid content of about 12–15% is satisfactory.

(6) F. G. Hopkins, *J. Biol. Chem.*, **84**, 269 (1929).

(7) J. B. Neilands and F. M. Strong, *Arch. Biochem.*, **19**, 287 (1948).

(8) Unpublished method of R. W. Von Korff.

(9) E. R. Stadman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951).

(10) N. O. Kaplan and F. Lipmann, *ibid.*, **174**, 37 (1948).

(11) G. E. Woodward, *ibid.*, **109**, 1 (1935).

(12) This amount of glutamic acid obviously cannot be contained in the glutathione present, but may still have been derived in some manner from the glutathione used in the preparation. The data available do not permit the conclusion that glutamic acid is a component of the CoA molecule. In fact a similar subsequent preparation contained 24% P.A. but less than 2% glutamic acid.

(13) W. W. Umbriet and I. C. Gunsalus, *J. Biol. Chem.*, **159**, 333 (1945).

(14) B. L. Griswold, F. L. Humoller and A. R. McIntyre, *Anal. Chem.*, **23**, 192 (1951).

(15) Microanalyses by C. W. Beazley, Micro-Tech Laboratories, Skokie, Illinois.

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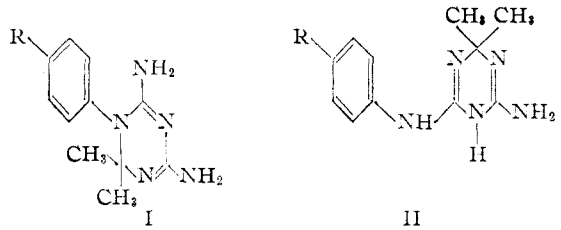
A SERIES OF NEW, BIOLOGICALLY SIGNIFICANT DIHYDROTRIAZINES

Sir:

We wish to report the synthesis (by E. J. M.) of a hitherto unreported class of compounds (I) with anti-vitamin and anti-malarial activity. In the course of a program of synthesis of arylbiguanides,[1] a new compound (m.p. 189–191°; $\lambda_{max.}^{H_2O}$ 236 mμ, log ε 4.24; *Anal.* Calcd. for $C_{14}H_{19}N_5O_2 \cdot HCl$: C, 51.61; H, 6.19; N, 21.50. Found: C, 51.97; H, 6.12; N, 21.22) was produced by the condensation of ethyl *p*-aminobenzoate, dicyandiamide and concentrated hydrochloric acid in acetone. The structure Ia·HCl, 4,6-diamino-1-(*p*-carbethoxyphenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine hydrochloride, is proposed for this substance. This reaction is general for a ring-substituted aniline hydrochloride, dicyandiamide or N^1-monosubstituted dicyandiamide and a number of ketones or aldehydes. Another synthesis of compounds with structure I has been developed through condensation of arylbiguanides with ketones or aldehydes under acid conditions.

(1) Synthesis of a group of arylbiguanides was undertaken originally in these laboratories at the suggestion of M. M. Pechet as one part of a broad program in the chemotherapy of cancer initiated by Sidney Farber.



I II

a, R = $COOC_2H_5$
b, R = Cl
c, R = H

The action of alkali or ammonia on Ib·HCl (m.p. 210–215°; $\lambda_{max.}^{H_2O}$ 241 mμ, log ε 4.16; *Anal.* Calcd. for $C_{11}H_{14}N_5Cl\cdot HCl$: C, 45.84; H, 5.25; N, 24.31. Found: C, 45.98; H, 5.17; N, 24.48) affords IIb[2,3] (m.p. 130–133°; $\lambda_{max.}^{H_2O}$ 255 mμ, log ε 4.25), 6-amino-2-(*p*-chloroanilino)-1,4-dihydro-4,4-dimethyl-*s*-triazine,[4] by an irreversible intramolecular rearrangement. IIb·HCl (m.p. 128–131°; $\lambda_{max.}^{H_2O}$ 255 mμ, log ε 4.24; *Anal.* Calcd. for $C_{11}H_{14}N_5Cl\cdot HCl$: C, 45.84; H, 5.25; N, 24.31; Cl, 24.61. Found: C, 45.67; H, 5.21; N, 24.53; Cl, 24.70), isomeric with Ib·HCl, results on treatment of IIb with hydrochloric acid.

Many compounds of structure I inhibit *Streptococcus faecalis 8043* and *Lactobacillus arabinosus 17-5* in standard pteroylglutamic acid and niacin assay systems,[5] whereas those of structure II are relatively inert. For example, Ib·HCl (0.05–0.20 gamma/ml.) inhibits 0.001–0.01 gamma/ml. of pteroylglutamic acid or niacin in corresponding microbiological systems, whereas IIb produces only partial inhibition versus 0.001 gamma/ml. of pteroylglutamic acid or niacin in concentrations of 100–1000 gamma/ml. Ib·HCl also inhibits *Lactobacillus casei 7469* and *Leuconostoc citrovorum 8081* in systems containing 0.001–0.01 gamma/ml. of riboflavin[5] and 0.6 unit/ml. of citrovorum factor[6] respectively, but only in concentrations of 100 gamma/ml. or more. Other derivatives of structure I exhibit similar activities, although the minimal inhibiting dose varies with substitution in the molecule while, in general, derivatives of structure II are inert.

Inhibition induced in pteroylglutamic acid systems by appropriate concentrations of compounds of structure I differs from that obtained with 4-aminopteroylglutamic acid (0.1 gamma/ml. or more) in that it is irreversible by 0.1–200 gamma/ml. of pteroylglutamic acid, adenine, or guanine. Reversal is obtained with citrovorum factor (0.1 unit/ml.), leucovorin (1.0 unit/ml.), dihydropteroylglutamic acid (0.01 gamma/ml.), N^{10}-formylpteroylglutamic acid (0.01 gamma/ml.), thymine (10 gamma/ml.), certain components of nucleic acid or high concentrations of ascorbic acid (200 gamma/ml.). When substituted for pteroylglutamic acid in *Streptococcus faecalis 8043* systems, citrovorum factor (0.000001 unit/ml.), leucovorin (0.001 unit/ml.), dihydropteroylglutamic acid (0.001 gamma/ml.) and N^{10}-formylpteroylglutamic acid (0.00001 gamma/ml.) are inhibited only by those concentrations required to inhibit *Leuconostoc citrovorum 8081* (*circa* 100 gamma/ml.). No significant inhibition was observed versus thymine.

Preliminary tests indicate that several compounds of structure I exhibit activity against experimental avian malaria, although compounds of structure II are comparatively inactive. For example, Ib·HCl is active against *Plasmodium lophurae* in the duck at oral dosages of approximately 3.13 mg./kg. and Ic·HCl at approximately 12.5 mg./kg. Ib·HCl is about six times as active as quinine, twice as active as atabrine or paludrine, and half as active as plasmochin in the duck.[7]

Further extensive studies on the scope and mechanism of the reaction and on the biological activity and mode of action of these compounds are in progress.

(2) S. Birtwell, F. H. S. Curd, J. A. Hendry and F. L. Rose, *J. Chem. Soc.*, 1645 (1948).

(3) N. N. Crounse, *J. Org. Chem.*, **16**, 492 (1951).

(4) Or a tautomer of this structure.

(5) Assoc. Vitamin Chemists, Inc., "Methods of Vitamin Assay," 2nd ed., Interscience Publishers, New York, N. Y., 1951.

(6) H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(7) We are indebted to J. H. Williams and his colleagues of the Lederle Laboratories, Pearl River, New York, for the anti-malarial assays.

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QUANTITATIVE CALCULATION OF THE BEHAVIOR OF ELUTION BANDS IN THE SEPARATION OF RARE EARTHS ON ION-EXCHANGE COLUMNS

Sir:

This Laboratory, in a series of papers, has described procedures for separating rare earths on ion-exchange columns. In the accompanying letter a quantitative theory for describing this phenomenon in the *p*H range between 5.5 and 7.3 for 0.1% citric acid has been outlined. In the course of this work, some very interesting quantitative relationships concerning the movement of bands on the column have been observed. Since the high-capacity resins used have a constant equivalent capacity for the ions adsorbed, if the number of equivalents of rare earth on the column is known, then the length of the original rare earth band can be calculated. If this band is eluted down the column with a citrate solution of known *p*H, the band widens out until it reaches an equilibrium value and from then on the front edge of the band travels at the same rate as the rear edge. Within this band the rare earths separate into individual bands proportionate to the amount of rare earth present and in an order starting with the heavier rare earths and proceeding to the lighter rare earths. Under equilibrium conditions these bands are exceedingly sharp. However, in practice they tend to be blurred due to channeling and non-equilibrium conditions. Under very carefully controlled procedures they can be made very sharp. We expect to publish shortly detailed experimental results and calculations showing that it is possible to calculate to better than 5%, and in most cases to better than 1 to 2%, the length of the equilibrium band on the column, the rate at which the front and rear edges