ylbutyric acid and what appeared to be the ester of an unidentified acid (D). The presence of the latter was suggested by an examination of its infrared spectrum. It is being studied further.

The diester isogermidine crystallized from fraction B (tubes 12–18) (m.p. either 221–222° or 229–230°, depending on which form separated; $[\alpha]^{28}D$ − 63.2° (*c*, 1 in pyridine), −26.0° (*c*, 1 in chloroform)). Calcd. for $C_{34}H_{53}O_{10}N$: C, 64.2; H, 8.40; eq. wt., 635. Found: C, 64.0; H, 8.33; eq. wt., 646, 655. Hydrolysis of isogermidine yielded germine, α-methylbutyric acid and acetic acid. The acids were identified by conversion to their *p*-phenylphenacyl esters.

Fraction C (tubes 19–36) gave crystalline veratetrine (m.p. 269–270° (dec.); $[\alpha]^{26}D$ − 31° (*c*, 1 in pyridine), $[\alpha]^{28}D$ − 2° (*c*, 1 in chloroform)). The analytical data indicate the empirical formula $C_{43}H_{64}O_{16}N$ (calcd. C, 60.7; H, 7.58; N, 1.65; eq. wt., 850.5; found: C, 60.8; H, 7.56; N, 1.4; eq. wt., 840). We have also isolated the same alkaloidal ester from *Veratrum album*. Volatile acid determination, found 3.2 equivalents of acid.

Alkaline hydrolysis of veratetrine yielded the alkamine isoprotoverine (identified by melting point and by comparison of its infrared and ultraviolet absorption spectra with those of authentic isoprotoverine) and an acid fraction. The acids were converted to their *p*-phenylphenacyl esters which were separated chromatographically into the esters of α-methylbutyric acid, acetic acid and the unidentified acid "D," obtained above from germbudine. The weights of the phenylphenacyl esters suggest veratetrine gives on hydrolysis one equivalent each of α-methylbutyric acid and acid D, together with two equivalents of acetic acid.

The hypotensive activity of germbudine, isogermidine and veratetrine have been determined in the anesthetized dog by a modification[4] of the method of Maison and Stutzman.[5] In comparison with a mixed alkaloidal ester preparation from *Veratrum viride* ("Deravine"), which produced a 30% fall in the mean arterial blood pressure of the anesthetized dog at a dose level of 0.2 γ per kg. per min., their relative activities are 0.97, 0.12 and 0.87, respectively.

(4) F. R. Skelton, Marjorie Beck and G. A. Grant, *Fed. Proc.*, Vol. 11, No. 1, Part I, p. 390, March, 1952.

(5) C. L. Maison and J. W. Stutzman, *Arch. Int. Pharmacodyn.*, **85**, 357 (1951).

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RECEIVED MAY 14, 1952

THE ANALYSIS AND SEPARATION OF GLUCURONIC AND GALACTURONIC ACIDS BY ION EXCHANGE[1]

Sir:

Methods for the analysis of uronic acids in biological material have always been rather limited due to the interference caused by sugars. Recently Dische[2] has described a series of color tests, which depend upon the rate and difference in color formation to distinguish between glucuronic and galacturonic acids, polyuronides, and sugars. Roboz[3] has previously shown that galacturonic acid could be separated from other material by adsorption on an anion-exchange resin (IRA-400); then recovered by elution with 25–50% acetic acid. However, no methods reported so far have effected both a qualitative and quantitative determination of the uronic acids, with a complete recovery of the pure individual uronic acids as crystalline materials; such a method will be described here.

In these experiments, a dilute alkaline solution of galacturonic, glucuronic, and mannuronic acids was absorbed quantitatively on the acetate form of the strong base anion exchanger Dowex-1. The uronic acids were eluted with 0.15 *M* acetic acid and the fractions analyzed by a slight modification of the orcinol method as described by Brown[4] for pentose determination (samples were heated at 100° for at least one-half hour). This assay method, as applied here, obeys Beer's law through the concentration range of 2 to 60 μg./ml. of uronic acid.

The separation of galacturonic and glucuronic acids in the presence of arabinose and galactose is shown in Fig. 1. The same separation can be achieved with a formate system using 0.01 *M* formic acid as the eluting agent. Mannuronic acid was eluted from the column in the same fraction as the glucuronic acid and a separation of these two could not be effected in the systems reported here. The free sugars, which were not absorbed, were collected in the first fraction and determined colorimetrica ly (galactose was determined by the anthrone method of Dreywood[5] as developed by Morris[6];

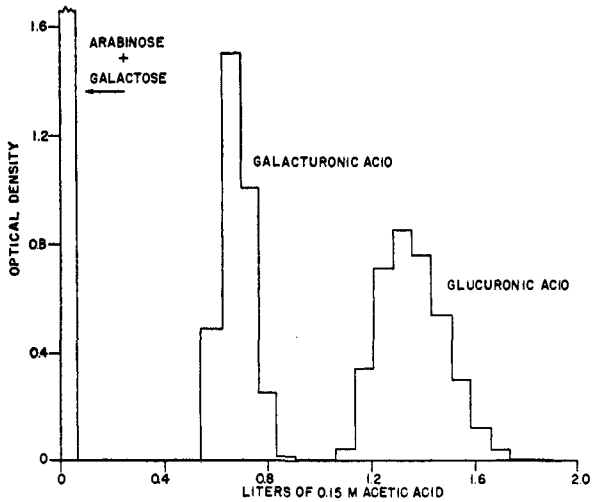

Fig. 1. The separation of galacturonic and glucuronic acids in the presence of sugar: exchanger, 0.85 sq. cm. × 12 cm. Dowex-1, *ca.* 300 mesh, acetate form; eluting agent, 0.15 *M* acetic acid at ~2.5 ml./min.; test material, 5.0 mg. each of arabinose and galactose, 10 mg. each of galacturonic and glucuronic acids in 10 ml. of 0.02 *M* sodium hydroxide [galactose was determined by anthrone method at 620 mμ; the other materials by orcinol method at 660 mμ].

(1) Work performed under Contract W-7405-eng-26 for the Atomic Energy Commission.

(2) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947); **171**, 725 (1947); **183**, 489 (1950); also *Arch. Biochem.*, **16**, 409 (1948).

(3) E. Roboz, Internat. Congr. of Pure and Applied Chem., Abstracts XII, 156 (1951).

(4) A. H. Brown, *Arch. Biochem.*, **11**, 269 (1946).

(5) R. Dreywood, *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946).

(6) D. L. Morris, *Science*, **107**, 254 (1948).

arabinose, by the orcinol method of Brown[4]). If unknown sugars are present in this fraction they can be adsorbed directly on a borate column and analyzed individually by the method of Khym and Zill.[7] Essentially, quantitative recoveries of the sugars and the uronic acids were obtained.

The identity of the peaks was determined by column runs on the individual uronic acids and by isolation and characterization of the uronic acid in the peak. Eluate fractions were evaporated to dryness *in vacuo* and characterized as the benzimidazole derivative according to the procedure of Lohmar, Dimler, Moore, and Link.[8]

(7) J. X. Khym and L. P. Zill, THIS JOURNAL, **73**, 2399 (1951); **74**, 2090 (1952).

(8) R. Lohmar, R. J. Dimler, S. Moore and K. P. Link, *J. Biol. Chem.*, **143**, 551 (1942).

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RECEIVED MARCH 29, 1952

3,5-DIAMINO-*AS*-TRIAZINES AS INHIBITORS OF LACTIC ACID BACTERIA AND PLASMODIA

Sir:

The inhibitory activity of various 2,4-diaminopyrimidines on the growth of *Lactobacillus casei* was interpreted as a competitive antagonism between these substances and folic acid.[1] The formal structural analogy between certain 5-substituted diaminopyrimidines and the known antimalarial chlorguanide (I) led to the suggestions that the diaminopyrimidines might have antimalarial properties and that both the biguanide and pyrimidine might act on plasmodia as antagonists of folic acid and related compounds.[2] Further investigations of the antimalarial properties of the pyrimidines culminated in the synthesis of 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (II)[3] which has a minimum effective dose[4] of about 0.03 mg./kg. corresponding to activity of 60 times chloroguanide against *Plasmodium gallinaceum* and 200 times against *P. berghei*. Furthermore, evidence relating the antimalarial activity of both types of substance to the folic acid metabolism of the parasite was forthcoming.[5,6]

Recent investigations have shown that chlorguanide (I) is converted to 2,4-diamino-1-*p*-chlorophenyl-6,6-dimethyl-1,6-dihydro-1,3,5-triazine (III) *in vivo*,[7] and that this metabolite is an active antimalarial[7,8] and an antagonist of the folic acid group of vitamins in microbiological studies.[8] It is apparent that a real structural analogy between II and III exists and is determinative of the similarity of biological behavior of I and II.

(1) G. H. Hitchings, G. B. Elion, H. VanderWerff and E. A. Falco, *J. Biol. Chem.*, **174**, 765 (1948).

(2) E. A. Falco, G. H. Hitchings, P. B. Russell, and H. VanderWerff, *Nature*, **164**, 107 (1949).

(3) E. A. Falco, L. G. Goodwin, G. H. Hitchings, I. M. Rollo and P. B. Russell, *Brit. J. Pharm.*, **6**, 185 (1951).

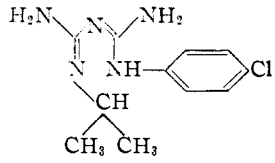
(4) I. M. Rollo, *Nature*, **168**, 332 (1951).

(5) J. Greenberg and E. M. Richeson, *J. Pharm. Exp. Therap.*, **99**, 444 (1950).

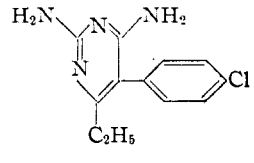
(6) J. Greenberg, *Proc. Soc. Exper. Biol. Med.*, **71**, 306 (1949).

(7) H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi and F. L. Rose, *Nature*, **168**, 1080 (1951).

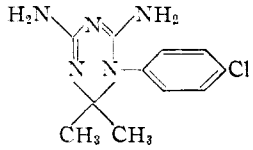
(8) E. J. Modest, G. E. Foley, M. M. Pechet and S. Farber, THIS JOURNAL, **74**, 855 (1952).



I

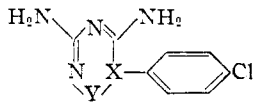


II



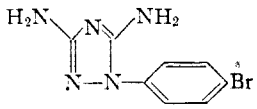
III

The structural feature common to II and III is the unit IV. The significance of this structural unit, in various heterocyclic systems, has been under investigation in these laboratories for some time. For example, 3,5-diamino-1-*p*-bromophenyl-1,2,4-triazole (*p*-bromophenylguanazole) (V) and several 3,5-diamino-6-phenyl-1,2,4-triazines (VI) have been synthesized, and their biological activities have been investigated. (**3,5-Diamino-1-*p*-bromophenyltriazole**, m.p. 210°. *Anal.* Calcd. for $C_8H_8N_5Br$: C, 36.8; H, 3.2. Found: C, 36.6; H, 3.3. **3,5-Diamino-6-(3′4′-dichlorophenyl)-1,2,-4-triazine**, m.p. 219–220°. *Anal.* Calcd. for $C_9H_7N_5Cl_2$: C, 42.2; H, 2.7. Found: C, 42.4; H, 3.0. **3,5-Diamino-6-(4′-chlorophenyl)-1,2,4-triazine**, m.p. 218–220°. Calcd. for $C_9H_8N_5Cl$: C, 48.9; H, 3.6. Found: C, 48.6; H, 3.6.)

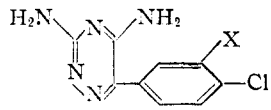


IV

X = N, C, C—CH_2, C—O
Y = C, N



V



VI

X = Cl or H

Biologically the asymmetrical triazine (VI, X = H) closely resembles the pyrimidine (II) and the symmetrical dihydrotriazine (III). Thus it is a competitive antagonist of folic and folinic acids in the growth of *L. casei* and of folinic acid (synthetic "Leucovorin") in the growth of *Leuconostoc citrovorum*, with an inhibition index in the latter system of approximately 20,000 (at half-maximal growth). With VI, as with II and III, the minimum concentration for inhibition of *Streptococcus faecalis* is several hundred-fold greater when folinic acid is supplied than when the organisms are grown with folic acid. This is in contrast to the finding with *L. casei* where folic and folinic acids are essentially equivalent over a wide range of concentration of the inhibitor. The antimalarial activities of VI and its congeners are quite high. The activity of the 3,4-dichlorophenyl analog of VI against *P. berghei* is 230 times that of chlorguanide; it is, however, less active than chlorguanide in *P. gallinaceum* infections.

The biological activities of the guanazole (V) are