

TABLE I  
 2,4-DINITROPHENYLHYDRAZONES

	C	Analyses, % H	N	M.p., °C.	Yield, %	$[\alpha]_D^{20}$	Crystal form
Calcd. for C <sub>12</sub> H <sub>16</sub> O <sub>9</sub> N <sub>4</sub>	40.0	4.4	15.5				
D-Glucose	39.9	4.5	15.4	122-124	85	+6.5°	Long needles
D-Mannose	39.9	4.3	15.4	180-181	75	-192.0	Leaflets
D-Galactose	39.8	4.5	15.3	178-179	89	+47.7	Amorph. plates
Calcd. for C <sub>11</sub> H <sub>14</sub> O <sub>8</sub> N <sub>4</sub>	40.0	4.2	16.9				
D-Arabinose	40.0	4.2	16.9	181-182	85	-46.0	Square plates
D-Ribose	40.0	4.2	16.9	165-166	80	0.0	Needles
D-Xylose	40.1	4.2	16.8	162-163	90	-22.6	Needles
D-Lyxose	40.1	4.2	16.9	169-170	80	+27.6	Needles

<sup>a</sup> c 0.5% pyridine.

new 2,4-dinitrophenylhydrazones of sugars were prepared for comparative purposes. The only previous report of this type of derivative was that of Glaser and Zuckerman<sup>2</sup> who prepared the 2,4-dinitrophenylhydrazones of glucose and glucoheptose. Essentially, their conditions were used for the preparation of the 2,4-dinitrophenylhydrazones of D-galactose, D-mannose, D-arabinose, D-ribose, D-xylose and D-lyxose. These authors also noted that increased amounts of a red oxidation product were obtained, when the reaction was carried out in dilute alcoholic solution. Consequently, a series of runs were made in absolute ethanol, methanol and methyl cellosolve. No improvement in yield or purity was obtained.

The sugar (0.02 mole) was dissolved in 5 ml. of water, 2,4-dinitrophenylhydrazine (0.02 mole) suspended in 100 ml. of absolute ethanol was added and the mixture was refluxed 12 hours. Except for galactose, which formed a gel,

(2) E. Glaser and N. Zuckerman, *Z. physiol. Chem.*, **167**, 37 (1927).

a clear yellow to red solution was obtained. This was evaporated *in vacuo* to dryness and the residue was extracted with 50 ml. of hot ethyl acetate to remove small amounts of red oxidation product. Except for galactose, the dinitrophenylhydrazones were recrystallized from 80-95% ethanol. No solvent combination was found for the highly insoluble galactose derivative, which would not yield a gel. Consequently, the galactose dinitrophenylhydrazone was purified by several extractions with hot ethyl acetate. The product consisted of amorphous plates which had a constant melting point regardless of how they were prepared. The other dinitrophenylhydrazones were soluble in hot water, hot ethanol and hot methanol, but were insoluble in ethyl acetate, acetone, carbon tetrachloride and benzene. The analyses and physical properties are given in Table I.

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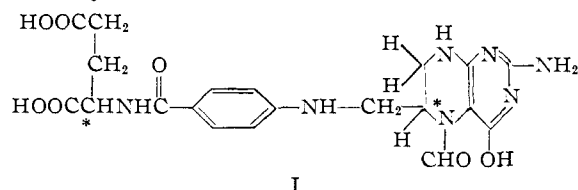
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## COMMUNICATIONS TO THE EDITOR

### DIASTEREOISOMERS OF LEUCOVORIN

Sir:

Leucovorin<sup>1</sup> (5-formyl-*dl*-5,6,7,8-tetrahydropteroyl-L-glutamic acid) (I), prepared from pteroylglutamic acid (PGA) (II) by formylation, reduction and rearrangement in alkali,<sup>2</sup> contains a new asymmetric center at carbon six.<sup>3</sup> Since II has an optically active carbon in the L-glutamic acid moiety, I consisted of a mixture of two diastereoisomers, the *dl* and *ll* forms. We wish to report their separation utilizing the difference in solubility of the calcium salts.



A 10% aqueous solution of calcium leucovorin (I, Table I) on cooling deposited a calcium salt with  $[\alpha]_D^{20}$  -2.82° and microbiological activity for

(1) J. A. Brockman, *et al.*, *THIS JOURNAL*, **72**, 4325 (1950).

(2) B. Roth, *et al.*, *ibid.*, **74**, 3247 (1952).

(3) D. B. Cosulich, *et al.*, *ibid.*, **74**, 3352 (1952).

*Leuconostoc citrovorum* of 1.4 mg./mg., compared with anhydrous I as the standard; in this manner 22 g. of calcium *l*-leucovorin (III) in an impure state was obtained from a total of 88.5 g. of I. After three recrystallizations from water, with charcoal and Magnesol decolorizations, III was obtained with a rotation (Table I) which did not change after a fourth recrystallization.

*Anal.* Calcd. for C<sub>20</sub>H<sub>21</sub>N<sub>7</sub>O<sub>7</sub>·Ca·4H<sub>2</sub>O: C, 41.2; H, 4.98; N, 16.9; —CHO, 4.97; Ca, 6.86; H<sub>2</sub>O, 12.4. Found: C, 41.6; H, 5.30; N, 16.6; —CHO, 5.28; Ca, 6.86; H<sub>2</sub>O, 12.2 (by weight loss at 100°/2 mm.).

Polarographically,<sup>4</sup> I and III were identical. The microbiological assays (Table I) show that III was about twice as active as I and exhibited a decrease in PGA activity after treatment with acid at pH 2 for 24 hours at 25°. III was similar to a barium salt of citrovorum factor (CF) (IV) from liver<sup>5</sup> in these respects. At a concentration of 10 mg./l. in 30% ethanol containing 0.03% NH<sub>3</sub> III exhibited a maximum at 285-286 mμ (% T = 29.5) and a minimum at 242-244 mμ (% T = 77.0).

(4) W. Allen, *et al.*, *ibid.*, **74**, 3264 (1952).

(5) M. Silverman and J. C. Keresztosy, *ibid.*, **73**, 1897 (1951); J. C. Keresztosy and M. Silverman, *ibid.*, **73**, 5510 (1951).

At present calcium *dL*-leucovorin (V) has not been obtained in a pure state; however, a fraction has been obtained with a high rotation, lowered microbiological activity, and polarographic activity<sup>4</sup> identical with I (see Table I). The purification of V is now in progress.

TABLE I

	I <sup>a</sup>	III	IV <sup>c</sup>	V
$[\alpha]_D$	+14.26	-15.1		+28.3
$c, \%$ <sup>b</sup>	3.42	1.82		3.53
CF assay, <sup>c</sup>	800	1640	1340	576
after acid	40	40	40	31
PGA assay, <sup>d</sup>	550	1070	890	247
after acid	1050	970	700	721

<sup>a</sup> As the calcium salt. <sup>b</sup> Concentration in water calculated for the anhydrous calcium salt. <sup>c</sup> In  $\gamma$ /mg., using anhydrous I as the standard for *Le. citrovorum* 8081. <sup>d</sup> In  $\gamma$ /mg., with II as the standard for *S. faecalis* R. <sup>e</sup> The solution of citrovorum factor (1 mg./ml.) was furnished by Dr. John C. Keresztesy.

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#### HIGH ENERGY HELIUM-ION IRRADIATION OF FORMIC ACID IN AQUEOUS SOLUTION<sup>1</sup>

Sir:

Several years ago Fricke, Hart and Smith<sup>2</sup> measured the amounts of hydrogen and carbon dioxide formed by X-ray induced reactions in aqueous solutions of formic acid over a wide range of pH values and solute concentrations. To account for the observed gas yields, they suggested that under certain conditions both oxalic acid and formaldehyde may be formed in addition to hydrogen and carbon dioxide. Recently, as part of a general study<sup>3,4</sup> of the radiation induced synthesis of organic substances in aqueous solutions, we have found that oxalic acid, formaldehyde and at least eight other organic compounds are produced by high energy helium-ion bombardment of hydrogen-saturated aqueous formic acid solutions. The present preliminary communication describes briefly the experimental techniques and observations. A more detailed report of this work will be forthcoming.

Air-free aqueous solutions of C<sup>14</sup>-labelled formic acid<sup>5</sup> were irradiated in all-glass target cells with the 40 Mev. helium-ion beam of the 60-inch cyclotron at the Crocker Laboratory. Hydrogen gas was bubbled through the solution during exposure and then passed through dilute sodium hydroxide solution to recover carbon dioxide which was subsequently assayed as BaC<sup>14</sup>O<sub>3</sub>. After irradiation the target solution was distilled to dryness *in vacuo* at room

(1) The work reported in this paper was performed under Contract W-7405-eng-48A with the United States Atomic Energy Commission.

(2) H. Fricke, E. J. Hart and H. P. Smith, *J. Chem. Phys.*, **6**, 229 (1938).

(3) W. M. Garrison, D. C. Morrison, J. G. Hamilton, A. A. Benson, and M. Calvin, *Science*, **114**, 416 (1951).

(4) W. M. Garrison, and G. K. Rollefson, *Discussions Faraday Soc.*, **11**, (in press) (1952).

(5) We wish to thank Dr. Bert M. Tolbert for supplying the HC<sup>14</sup>-OOH used in these experiments.

temperature. The HC<sup>14</sup>HO in the distillate was isolated and assayed as the methone-formaldehyde derivative.<sup>3</sup> A two-dimensional paper chromatogram of the non-volatile fraction was prepared<sup>6</sup> and radioautographed. One major spot typical of oxalic acid and eight other spots of lesser intensity could be seen in the original radioautogram. The region containing the major part of the activity was eluted with 0.1 *N* hydrochloric acid. The activity in an aliquot of this solution co-precipitated quantitatively on lanthanum oxalate after repeated washing and recrystallization from dilute nitric acid. To further identify this major non-volatile product as oxalic acid a second aliquot was cochromatographed with added oxalic acid on a silica column by a partition chromatography method similar to one recently reported.<sup>7</sup> An exact correspondence of oxalic acid titer and C<sup>14</sup> activity in the eluant was observed. A 300-microcurie sample of the HC<sup>14</sup>-OOH used in these experiments was analyzed in exactly the same way as the bombarded solutions; the control showed no activity other than HC<sup>14</sup>-OOH. Decay of the activity in the products could not be detected over a period of several months.

Each of the 10 ml. target solutions contained 300 microcuries of HC<sup>14</sup>OOH and were 0.009 *N* in total formic acid. The helium ions incident on the solution had an energy of 35 Mev. Bombardments were made at a beam current of 0.10 microampere for a period of 3.0 minutes to give a total exposure of 0.005 microampere hour. Yield data are summarized in Table I.

TABLE I

Product	G (molecules/100 e.v.)	
	1	2
Carbon dioxide		0.75
Oxalic acid	0.023	0.027
Formaldehyde	$0.61 \times 10^{-3}$	$0.56 \times 10^{-3}$

We wish to thank Mrs. Jeanne Gile-Melchert and Mrs. Harriet Powers for their assistance in the column separations, Mr. Boyd Weeks for his help in the target assembly, and the staff of the 60-inch cyclotron at the Crocker Laboratory for the bombardments.

(6) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Hass and W. Stepka, *THIS JOURNAL*, **72**, 1710 (1950).

(7) W. A. Bulen, J. E. Varner and R. C. Burrell, *Anal. Chem.*, **24**, 187 (1952).

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#### DIFFERENCES IN THE QUANTITATIVE AMINO ACID COMPOSITION OF INSULINS ISOLATED FROM BEEF, PORK AND SHEEP GLANDS

Sir:

Recently insulin preparations from different animal species have been successfully fractionated by countercurrent distribution.<sup>1</sup> Each preparation appeared to contain a major, or A, component

(1) E. J. Harfenist and L. C. Craig, *THIS JOURNAL*, **74**, 3083 (1952).