In attempting to apply this method to the quantitative determination of the azo-dyes, the pigment spots were eluted from the paper with one normal sodium hydroxide and the amount determined with a colorimeter. A complete recovery of the chromatographed spots was not possible, since the azo-dyes of the above compounds are unstable. In chromatograms developed by Lederer's method, recovery was less than 50%, whereas when alkali was used for separation, recovery was about 80%.

The method described in this paper was used in measuring the production of *p*-aminobenzoic acid and anthranilic acid in Neurospora.[5]

Experimental

The azo-dyes were prepared after the method of Schmidt and Kolbl.[6] The arylamines were diazotized, coupled with α-naphthol in alkaline solution, and the resulting dye extracted from acidified solution with ether. The ether solution was washed in distilled water and condensed to a small volume *in vacuo;* then a drop was deposited on a filter paper strip (Whatman #1).

The chromatograph was developed by the ascending technique: a paper strip was suspended from the lid of a glass jar so that it dipped into one-tenth molar sodium hydroxide which covered the bottom of the jar. This solvent was made by diluting a five normal stock solution, which possibly contained some carbonate. The solvent moves rapidly and in a few hours ascends high enough to separate all the spots.

(5) M. Zalokar, *Genetics*, **35**, 700 (1950) (Abstract).

(6) K. H. Schmidt and C. Kolbl, *Z. Physiol. Chem.*, **281**, 7 (1944).

DEPARTMENT OF ZOOLOGY
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NEW COMPOUNDS

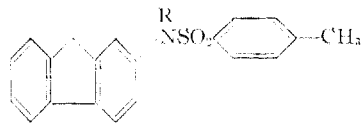
2-Tosylaminofluorene Derivatives[1]

Sodium tosylaminofluorene has been found to react readily with alkyl and acyl halides. These derivatives may be useful in identifying compounds containing active halogen and by hydrolysis monoalkylaminofluorenes may be obtained from some of the derivatives. These alkylamino compounds should prove of value in a study of chemical carcinogenicity. Table I lists these new tosylamino derivatives.

General Procedure.—To 0.01 mole of 2-tosylaminofluorene dissolved in 100 ml. of warm neutral ethyl alcohol was added 10 ml. of 1 *N* aqueous sodium hydroxide followed by 0.011 mole of the halide. The mixture was refluxed for one hour. Then 200 ml. of water was added. After cooling, the mixture was filtered. Sometimes an oil was formed; in this case, the mixture was allowed to stand overnight in the cold room and then filtered. Recrystallizing solvent in all cases was methyl alcohol.

The 2-tosylaminofluorene used was prepared by the following improved procedure: To a solution of 5.4 g. (0.003 mole) 2-aminofluorene in 40 ml. of boiling acetic acid was added 9.5 g. (0.0038 mole) of *p*-toluenesulfonyl chloride. To this boiling milky mixture was added 4.1 g. (0.05 mole) of sodium acetate in small portions over 15 minutes. The mixture was refluxed 15 minutes. Finally water was added slowly to the boiling solution until crystals started to come out. The solution was allowed to cool and filtered. The large crystals were recrystallized from aqueous acetic acid. Yield of colorless crystals, melting at 161–162°, was 8.0–8.5 g. (80–85%). This compound can also be crystallized from aqueous methyl cellosolve or methyl alcohol; lit. m.p. was 157–158°.[3]

TABLE I



RX	Yield, %	M.p., °C.	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
Methyl iodide	95[2]	137–138				
Ethyl iodide	95	185	3.86	3.92	8.81	8.80
n-Propyl bromide	92	125–126	3.71	3.67	8.49	8.52
n-Butyl bromide	65	132–133	3.58	3.65	8.18	8.10
Benzyl chloride	93	200	3.29	3.31	7.53	7.50
Allyl chloride	78	131–132	3.73	3.57	8.53	8.36
Methallyl chloride	74	175–176	3.60	3.81	8.23	8.32
Ethyl chlorocarbonate	50	193	3.44	3.37	7.86	8.05
Acetyl chloride	75	164–165	3.71	3.64	8.49	8.35

(2) F. E. Ray and J. Little, in press.

(3) N. Campbell, W. Anderson and J. Gilmore, *J. Chem. Soc.*, 446 (1940).

CANCER RESEARCH LABORATORY
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RECEIVED MARCH 21, 1952

Esters of Terephthalic Acid

Four terephthalates were prepared by the following general procedure. The melting points, analyses and yields of the esters are tabulated below.

Experimental.—A mixture of 0.5 g. of terephthalyl chloride and 2 ml. of the alcohol was heated gently over a low flame for 10 minutes. A 10-ml. portion of distilled water was then added and the mixture cooled in an ice-bath until the product had solidified. The material was collected by filtration, washed with 2% sodium carbonate solution, dried, and recrystallized from 95% ethanol.

The authors acknowledge with appreciation the encouragement of Dr. E. Emmet Reid.

TABLE I

Ester	Mol. form of ester	Yield, % based on chloride	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Found C	Found H
Di-*n*-heptyl	$C_{22}H_{34}O_4$	31	36	72.89	9.45	72.84	9.54
Di-*n*-octyl[a]	$C_{24}H_{38}O_4$	34	43	73.81	9.82	74.01	9.92
Di-*n*-nonyl	$C_{26}H_{42}O_4$	34	46	74.60	10.11	74.85	10.24
Di-*n*-decyl	$C_{28}H_{46}O_4$	39	57	75.30	10.37	75.28	10.63

[a] J. B. Cohen and H. S. de Pennington, *J. Chem. Soc.*, 113, 63 (1918), report this compound but give no constants.

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RECEIVED APRIL 18, 1952

2,4-Dinitrophenylhydrazones of Some Hexoses and Pentoses[1]

In the course of identification of the carbohydrate residue in the degradation products of nucleic acids, a number of

(1) This investigation was supported by research grant C-1308 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

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

TABLE I

2,4-DINITROPHENYLHYDRAZONES

	Analyses, % C	H	N	M.p., °C.	Yield, %	$[\alpha]^{23}D^{a}$	Crystal form
Calcd. for $C_{12}H_{16}O_9N_4$	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 $C_{11}H_{14}O_8N_4$	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

[a] *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[2] 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, 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.

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

BIOLOGY DIVISION
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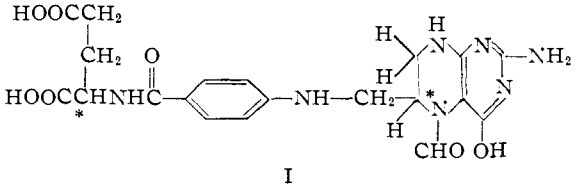
RECEIVED MARCH 20, 1952

COMMUNICATIONS TO THE EDITOR

DIASTEREOISOMERS OF LEUCOVORIN

Sir:

Leucovorin[1] (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,[2] contains a new asymmetric center at carbon six.[3] Since II has an optically active carbon in the L-glutamic acid moiety, I consisted of a mixture of two diastereoisomers, the *d*L and *l*L forms. We wish to report their separation utilizing the difference in solubility of the calcium salts.



I

A 10% aqueous solution of calcium leucovorin (I, Table I) on cooling deposited a calcium salt with $[\alpha]^{26}D$ −2.82° and microbiological activity for *Leuconostoc citrovorum* of 1.4 mg./mg., compared with anhydrous I as the standard; in this manner 22 g. of calcium *l*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 $C_{20}H_{21}N_7O_7Ca \cdot 4H_2O$: C, 41.2; H, 4.98; N, 16.9; —CHO, 4.97; Ca, 6.86; H_2O, 12.4. Found: C, 41.6; H, 5.30; N, 16.6; —CHO, 5.28; Ca, 6.86; H_2O, 12.2 (by weight loss at 100°/2 mm.).

Polarographically,[4] 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 *p*H 2 for 24 hours at 25°. III was similar to a barium salt of citrovorum factor (CF) (IV) from liver[5] in these respects. At a concentration of 10 mg./l. in 30% ethanol containing 0.03% NH_3 III exhibited a maximum at 285–286 mμ (% T = 29.5) and a minimum at 242–244 mμ (% T = 77.0).

(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**, 3252 (1952).
(4) W. Allen, *et al.*, *ibid.*, **74**, 3264 (1952).
(5) M. Silverman and J. C. Keresztesy, *ibid.*, **73**, 1897 (1951); J. C. Keresztesy and M. Silverman, *ibid.*, **73**, 5510 (1951).