TABLE I

C-5 SUBSTITUTED BENZALHYDANTOINS AND C-5 SUBSTITUTED BENZYLHYDANTOINS

Hydantoin	Formula	Yield, %	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
2,4-Dichlorobenzal	$C_{10}H_6O_2N_2Cl_2$	60	310–312	46.71	46.91	2.35	2.51
2,4-Dichlorobenzyl	$C_{10}H_8O_2N_2Cl_2$	56	226–227	46.35	46.51	3.11	3.15
2-Ethoxybenzal	$C_{12}H_{12}O_3N_2$[a]	68	222–223	62.08	62.18	5.17	5.21
2-Ethoxybenzyl	$C_{12}H_{14}O_3N_2$	80	171–172	61.50	61.91	5.98	5.98
3,4-Diethoxybenzal	$C_{14}H_{16}O_4N_2$[a]	65	245–247	60.83	61.06	5.83	5.84
3,4-Diethoxybenzyl	$C_{14}H_{18}O_2N_2$[b]	75	186–187	60.40	60.09	6.50	6.48
4-Hydroxy-3-ethoxybenzal	$C_{12}H_{12}O_4N_2$	76	263–265	58.07	58.03	4.88	4.87
4-Hydroxy-3-ethoxybenzyl	$C_{12}H_{14}O_4N_2$[b]	60	194–195	57.57	57.43	5.63	5.60
4-Hydroxy-3,5-dimethoxybenzal	$C_{12}H_{12}O_5N_2$[c]	45	304–306	54.50	54.39	4.57	4.49
4-Hydroxy-3,5-dimethoxybenzyl	$C_{12}H_{14}O_5N_2$[b]	60	225–226	54.11	53.67	5.29	5.30

[a] Yellow needles made by procedure used for dichlorobenzalhydantoin. [b] White crystals made by procedure used for ethoxybenzylhydantoin. [c] Yellow crystals made by procedure used for 4-hydroxy-3-ethoxybenzalhydantoin.

acetic anhydride was gently refluxed on an oil-bath for 3 hours. After 2 hours a yellow solid separated from the liquid and soon accumulated. The heating was continued another hour. The mixture was cooled, filtered, and the solid product washed with 50 ml. of ethanol and 50 ml. of water. The dried product weighed 11.1 g. An additional 2.1 g. separated from the filtrate; total yield 60%. Crystallization from acetic acid gave pale yellow crystals.

5-(2,4-Dichlorobenzyl)-hydantoin.—A mixture of 10 g. of 5-(2,4-dichlorobenzal)-hydantoin, 15 ml. of glacial acetic acid, 5 ml. of acetic anhydride and 70 ml. of hydriodic acid (sp. gr. 1.5) was refluxed for 2 hours on an oil-bath at 118°. The mixture was distilled with steam to remove acid, cooled, and filtered; yield 5.7 g. (56%). The product crystallized from acetic acid as white needles.

5-(2-Ethoxybenzyl)-hydantoin.—Five grams of 5-(2-ethoxybenzal)-hydantoin in 50 ml. of 1 *N* NaOH was hydrogenated at room temperature in the presence of 5 g. of Raney nickel in a Parr hydrogenator at 40 p.s.i. for 5 hours. The catalyst was filtered off and filtrate was made acid with dilute hydrochloric acid; 4.1 g. (80%) of white precipitate was obtained. Recrystallization from hot water yielded white platelets.

5-(4-Hydroxy-3-ethoxybenzal)-hydantoin.—Twenty-five grams of 4-hydroxy-3-ethoxybenzaldehyde, 15 g. of hydantoin, 25 ml. of pyridine and 12 ml. of diethylamine were refluxed gently for 8 hours; the mixture was allowed to stand overnight and the tan colored precipitate was filtered and washed with cold ethanol; yield 28.2 g. (76%). The product was recrystallized from dilute acetic acid.

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Paper Chromatography of Azo-dyes from Arylamines and Sulfanilamide[1]

BY M. ZALOKAR

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A diazotized arylamine, coupled with different phenolic compounds, gives a typical and very sensitive color reaction. This reaction is currently used in the determination of *p*-aminobenzoic acid and sulfanilamide in biological material.[2] The fact that it cannot discriminate among different isomers of arylamines leads to difficulties when the major problem is that of determining *p*-aminobenzoic acid in the presence of either anthranilic acid or sulfanilamide.

Azo-dyes resulting from the coupling of α-naphthol with the diazotized arylamine were separated on paper chromatograms by two different methods. In the first method, the chromatograms were developed with tenth molar sodium hydroxide. The spots could be identified by their position and difference in color: the faster *o*-aminobenzoic acid derivative was orange in color, fluorescent in ultraviolet light, and turned yellow in strong alkali; the slower *m*-aminobenzoic acid spot was orange; the *p*-aminobenzoic acid derivative was pink and stayed so in strong alkali. The sulfanilamide spot could not be separated from the *p*-aminobenzoic acid spot.

Spraying these chromatograms with indicator solutions showed that the ascending solvent separated into different *p*H zones. A neutral zone ran first, an alkaline zone, having a *p*H of about 11, followed, and last was a zone having the alkalinity of the hydroxide used (*p*H 13). The formation of a neutral water zone running ahead of the alkaline solution was observed first by Schoenbein.[3] The second zone was due to sodium carbonate, which separated from the solvent, and which was produced by a reaction of the ascending solvent with atmospheric carbon dioxide. These *p*H zones suggested that the separation of the chromatographed spots might depend upon different adsorption rates of the dyes at a given *p*H and upon their displacement at various *p*H values. This is supported by the fact that in weaker alkali (0.05 *N*) the movement of the spots was slower, while in stronger alkali (0.2 *N*) they were shifted near the upper margin of the liquid. In a carbon dioxide atmosphere the ascending hydroxide was neutralized, giving carbonate in which the spots remained stationary.

The other method of separating azo-dyes of arylamines and sulfanilamide was by partition chromatography, as described by Lederer for the separation of indicators.[4] The following table gives *RF* values for different compounds which were prepared as above. Amyl alcohol, shaken with a 10% solution of concentrated ammonia, was used as a solvent.

TABLE I

Azo-dye of	*RF*
o-Aminobenzoic acid	0.63
m-Aminobenzoic acid	.09
p-Aminobenzoic acid	.05
Sulfanilamide	.50

(1) Supported by Initiative 171 Funds of the State of Washington. Present address: National Institutes of Health, Bethesda, Md.

(2) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).

(3) C. F. Schoenbein, *Chem. Centr., N. F.*, **6**, 881 (1861).

(4) M. Lederer, *Science*, **112**, 504 (1950).

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).

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NEW COMPOUNDS

2-Tosylaminofluorene Derivatives[1]

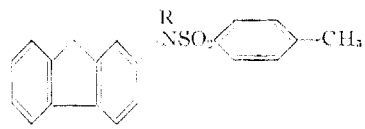
Sodium tosylaminofluorene has been found to react readily with alkyl and acyl halides. These derivatives may be useful in identifying compounds containing active halogen and by hydrolysis monoalkylaminofluorenes may be obtained from some of the derivatives. These alkylamino compounds should prove of 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]

(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.

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 chloro-carbonate	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).

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