[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CENTRAL STATE COLLEGE]

p-Phenylazobenzoyl Chloride for Identification and Chromatographic Separation of Colorless Compounds. II. Amines[1]

E. O. WOOLFOLK AND E. H. ROBERTS[2]

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Thirty-eight N-substituted amides of *p*-phenylazobenzoic acid have been prepared by the interaction of *p*-phenylazobenzoyl chloride with aliphatic and aromatic primary amines and certain types of secondary amines. The amides are formed in good yields and in pure condition, and appear to be convenient derivatives for the identification of these amines. These amides are colored and certain mixtures of them can be separated by chromatographic adsorption.

This investigation of *p*-phenylazobenzoyl chloride as a reagent for the identification and chromatographic separation of amines is a continuation of a similar study of that substance as a reagent for alcohols.[3] The reagent reacts with amines to form colored derivatives which are suitable for purposes of identification and for separation of certain mixtures of them by chromatographic adsorption.

The derivatives form readily in fair yields on gently refluxing *p*-phenylazobenzoyl chloride with the amine in the presence of pyridine. They are easily crystallized and give convenient sharp melting points after two recrystallizations. However, traces of *p*-phenylazobenzoic acid were occasionally occluded by the derivatives and were removed chromatographically on activated alumina in order to obtain good analytical values. Whereas most reagents for aliphatic primary and secondary amines and some aromatic amines yield oily or inconveniently low-melting derivatives, all of the amines tested in this study yielded satisfactory solids. Their melting points are much higher, where comparison is possible, than those of the corresponding derivatives obtained from the common reagents. These derivatives are also of exceptional merit in that their melting points differ sufficiently, that they could be used very satisfactorily as checks on the identification of the individual amine.

The reaction appeared to be a general one; thirty-two primary amines and the secondary amines such as morpholine and piperidine, and four of the mixed aliphatic aromatic type reacted satisfactorily. Aminophenols reacted with the reagent to produce acylation on the amino group only. The product was soluble in alkali; reprecipitation of the derivative on acidification, indicated that only the amino group had been attached. Previous attempts in our laboratory to bring about reaction between *p*-phenylazobenzoyl chloride and phenols under similar conditions have proved unsuccessful.

The *p*-phenylazobenzoyl chloride was found to react more rapidly with amines than with water, and aqueous solutions of amines may be treated quite satisfactorily for the preparation of derivatives.

The new N-substituted-*p*-phenylazobenzamides that have been characterized are recorded in Table I.

The derivatives, which are brilliantly colored in varying shades of red, have a distinctive advantage in that they afford a method of separating mixtures of colorless, nonfluorescing amines by chromatographic adsorption. On activated alumina 24 binary mixtures involving 32 different derivatives and 3 ternary mixtures involving 9 derivatives have been chromatographed. The chromatograms were developed with benzene and mixtures of 1 to 5% ethyl acetate in benzene. The resulting separations are given in Table II. Eleven pairs were separated sufficiently to make two zones visible with a colorless zone between. Four pairs formed a continuous band. Sectioning of this with subsequent elution yielded an almost homogeneous top and bottom section with intervening sections of varying composition. Nine pairs did not give satisfactory separation under the conditions employed. The first member of each pair listed in Table II, wherein separation was obtained, was the most strongly adsorbed derivative. As was expected, in the separation of the binary mixtures in which the components were not isomers, the higher molecular weight derivative formed the lower or least readily adsorbed band.

The ternary mixtures which were studied, as indicated in Table II, were mixtures of the derivatives of *ortho*, *meta*, and *para* isomers. In two instances the derivative of the *ortho* isomer was the least strongly adsorbed and separated from a mixture of the derivatives of the *meta* and *para* isomers. One ternary mixture gave no separation. The results of these experiments might have been anticipated from an analysis of the separations obtained in chromatographing the three possible combinations of binary mixtures within an *ortho*, *meta*, and *para* series.

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(2) Present address: School of Graduate Studies, Department of Chemistry, Wayne University.

(3) Woolfolk, Beach, and McPherson, *J. Org. Chem.*, **20**, 391 (1955).

TABLE I

AMIDES OF *para*-PHENYLAZOBENZOIC ACID

Amine Used	M.P., °C.[a,b] Corrected	Yield,[c] %	Formula	Nitrogen[d] Calc'd	Found
Primary Aliphatic					
Allylamine	130.0–131.0	32	$C_{16}H_{15}N_3O$	15.83	15.61
n-Amylamine	134.5–135.5	60	$C_{18}H_{21}N_3O$	14.23	14.63
Isoamylamine	120.0–121.0	54	$C_{18}H_{21}N_3O$	14.23	14.17
Benzylamine	194.0–194.5	50	$C_{20}H_{17}N_3O$	13.32	13.16
n-Butylamine	143.0–145.5	80	$C_{17}H_{19}N_3O$	14.94	15.76
Isobutylamine	148.5–149.5	73	$C_{17}H_{19}N_3O$	14.94	14.58
tert-Butylamine	195.0–196.5	69	$C_{17}H_{19}N_3O$	14.93	14.71
Cyclohexylamine	228.0–229.0	70	$C_{19}H_{21}N_3O$	13.67	13.60
Ethylamine	161.5–162.0	59	$C_{15}H_{15}N_3O$	16.58	16.43
n-Heptylamine	139.5–140.0	71	$C_{20}H_{25}N_3O$	12.99	12.84
Methylamine	149.0–150.0	77	$C_{14}H_{13}N_3O$	17.56	17.20
n-Propylamine	152.5–154.0	49	$C_{16}H_{17}N_3O$	15.71	15.60
Isopropylamine	193.5–194.0	49	$C_{16}H_{17}N_3O$	15.71	15.78
Primary Aromatic					
o-Aminophenol	188.0–189.0	45	$C_{19}H_{15}N_3O_2$	13.24	13.13
m-Aminophenol	234.0–235.0	50*	$C_{19}H_{15}N_3O_2$	13.24	13.10
p-Aminophenol	274.5–275.5	42	$C_{19}H_{15}N_3O_2$	13.24	13.16
Aniline	214.5–216.0	49	$C_{19}H_{15}N_3O$	13.94	14.09
m-Bromoaniline	181.5–183.0	45*	$C_{19}H_{15}BrN_3O$	11.02	10.84
p-Bromoaniline	253.0–254.0	69	$C_{19}H_{15}BrN_3O$	11.02	11.10
o-Chloroaniline	165.5–166.5	78*	$C_{19}H_{14}ClN_3O$	12.51	12.67
p-Chloroaniline	245.0–246.0	48	$C_{19}H_{14}ClN_3O$	12.51	12.43
2,6-Diethylaniline	233.0–234.0	36	$C_{23}H_{23}N_3O$	11.75	11.71
o-Ethoxyaniline	116.0–116.5	37	$C_{21}H_{19}N_3O_2$	12.16	12.28
m-Ethoxyaniline	160.5–162.0	39*	$C_{21}H_{19}N_3O_2$	12.16	12.15
p-Ethoxyaniline	220.0–221.0	49	$C_{21}H_{19}N_3O_2$	12.16	12.13
o-Methoxyaniline	116.5–117.5	85	$C_{20}H_{17}N_3O_2$	12.68	12.88
p-Methoxyaniline	225.0–226.5	64	$C_{20}H_{17}N_3O_2$	12.68	12.68
1-Naphthylamine	198.0–199.0	61	$C_{23}H_{17}N_3O$	11.96	11.98
2-Naphthylamine	222.0–224.5	57	$C_{23}H_{17}N_3O$	11.96	11.62
o-Toluidine	175.5–177.0	52*	$C_{20}H_{17}N_3O$	13.32	13.16
m-Toluidine	168.5–170.0	42*	$C_{20}H_{17}N_3O$	13.32	13.31
p-Toluidine	212.0–213.5	62	$C_{20}H_{17}N_3O$	13.32	13.17
Secondary					
n-Butylaniline	105.0–106.0	45*	$C_{23}H_{23}N_3O$	11.85	11.98
Ethylaniline	71.0– 71.5	39*	$C_{21}H_{19}N_3O$	12.75	12.81
Isoamylaniline	116.0–116.5	37*	$C_{24}H_{25}N_3O$	11.31	11.67
Methylaniline	113.0–114.0	77*	$C_{20}H_{17}N_3O$	13.32	13.36
Morpholine	124.0–124.5	72	$C_{17}H_{17}N_3O_2$	14.26	14.29
Piperidine	104.5–105.5	78	$C_{18}H_{19}N_3O$	14.32	14.18

[a] Melting points taken on Kofler Micro Hot Stage. [b] Melting points of the *p*-phenylazobenzoyl derivatives were compared with the melting points of the derivatives of corresponding amines which are listed by The Staff of Hopkins and Williams Research Laboratory [*Organic Reagents for Organic Analysis*, 2nd ed., Chemical Publishing Co., Inc., Brooklyn, N. Y., *1950, pp. 171–192] in their tables of melting points. The melting points of the p-phenylazobenzoyl derivatives were higher* than those of corresponding amines formed with the common reagents, such as acetic anhydride, benzene sulphonyl chloride, and benzoyl chloride; but lower and more convenient in several instances than the derivatives formed from the less common reagents such as 1-chloro-2,4-dinitrobenzene, α-naphthyl isocyanate, *p*-nitrophenylisocyanate, picric acid, picryl chloride, phenyl isothiocyanate, *phthalic anhydride, p-toluenesulfonyl chloride, and 1,3,5-trinitrobenzene.* [c] Yields marked with asterick are on purified product, otherwise reported on crude product. [d] Microanalyses by the Dumas Method were performed by the Du Good Chemical Laboratories, St. Louis, Missouri.

EXPERIMENTAL

Amines. Amines of commercially available grades were used without further purification.

Preparation of N-substituted amides. A mixture of acid chloride (approximately 0.1 g.), amine (excess), and a few milliliters of pyridine were refluxed gently for 1 hour. The red solution was poured with stirring into ice and 10% sodium carbonate solution. The amides which crystallized were filtered, washed with water, 1% hydrochloric acid and water, and air-dried. Those amides which separated as oils were extracted with ether. The ether extracts were washed with water and a saturated sodium chloride solution, dried over sodium sulfate, and the ether was removed. The crude amide was dissolved in the minimum volume of benzene or a mixture of Skellysolve-B and benzene and chromatographed on activated alumina on which the free acid was strongly adsorbed. In the case of the aminophenols, chromatography also removed an appreciable quantity of dark

TABLE II

CHROMATOGRAPHIC SEPARATION OF MODEL MIXTURES OF AMIDES OF *para*-PHENYLAZOBENZOIC ACID

BINARY MIXTURES	
Separated into Zones	
1-Naphthylamine	2-Naphthylamine
Aniline	*o*-Methoxyaniline
Morpholine	Piperidine
p-Aminophenol	*o*-Aminophenol
Allylamine	Isopropylamine
p-Chloroaniline	*o*-Chloroaniline
Benzylamine	Cyclohexylamine
p-Ethoxyaniline	*o*-Ethoxyaniline
m-Ethoxyaniline	*o*-Ethoxyaniline
Aniline	2,6-Diethylaniline
p-Toluidine	*o*-Chloroaniline
Forming Continuous Band	
Piperidine	Methylaniline
p-Toluidine	*o*-Toluidine
n-Butylaniline	Isoamylaniline
m-Aminophenol	*o*-Aminophenol
No Separation	
Aniline	Methylaniline
n-Butylamine	Isobutylamine
n-Amylamine	Isoamylamine
Ethylaniline	*n*-Butylaniline
m-Ethoxyaniline	*p*-Ethoxyaniline
o-Toluidine	*m*-Toluidine
m-Bromoaniline	*p*-Bromoaniline
m-Toluidine	*p*-Toluidine
m-Aminophenol	*p*-Aminophenol

TERNARY MIXTURES

o-Ethoxyaniline was separated from a mixture of the *meta* and *para* isomer.
o-Aminophenol was separated from a mixture of the *meta* and *para* isomer.
No separation obtained for an *ortho-*, *meta-* and *para-*toluidine mixture.

polymeric material from the derivatives. The amide was eluted with absolute ethanol and the solvent was removed. The yield of varying shades of red-colored amides ranged from 32 to 85%. The amides crystallized from dilute alcohol (50–95%) as soft, fine needles or fluffy solids, exhibiting varying degrees of static charge.

N-Methyl p-phenylazobenzamide. A mixture of 0.1044 g. of acid chloride and 10 ml. of a 25% aqueous solution of methylamine was refluxed for one hour. A red solid was present during the entire period of refluxing. The red solid was filtered and air-dried. Yield 77%, m.p. 145.6–147.8°. On chromatographing and recrystallizing from 95% ethanol, this material gave very soft red needles melting at 149.0–150.0°.

Chromatographic separations. A typical chromatographic separation of a mixture of two or three components was conducted as described below. A column 20 mm. by 40 cm. was used. Activated alumina (grade F-20) was packed in the column by filling it several times with the adsorbent while tapping the sides of the column with cork rings and tamping the surface of the adsorbent with a plunger. The adsorbent then was wetted with Skellysolve-B.

The mixture of amides (10 to 20 mg. of each component) was dissolved in the minimum volume of warm benzene and was adsorbed on the column. The chromatogram was developed by passing benzene and solutions of 1% up to 5% of ethyl acetate in benzene through the adsorbent. The adsorbent was pushed out and cut into bands or the bands were dug out of the column by a long narrow spatula, and eluted with absolute ethanol. When a continuous band was obtained, the band was arbitrarily cut into several sections. The pure components were obtained from the top and bottom sections and the intervening sections were mixtures. The eluents were concentrated, filtered into a tared flask, and the last traces of solvent were removed *in vacuo* under a stream of nitrogen. Melting points of the residues were determined.

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