not been determined independently, but the alkaline end-point of the titration has been established. This enables one to determine the total number of both acid and basic groups by titration. Again there is complete agreement with analysis. No anomalous groups are observed.

TABLE I

NUMBER OF CATIONIC GROUPS

	Assumed mol. wt.	Analyses[a] Arginine, histidine and lysine	Terminal N groups	Tota	Titration
Ribonuclease	13,500	17.3	1[b]	18.3	19[e]
Lysozyme	14,200	16.8	1[c]	17.8	19[f]
β-Lactoglobulin	39,000	40.6	3	43.6	45[g]
Ovalbumin	45,000	41.0	0	41.0	41[h]
Human serum albumin	65,000	92.5	1[d]	93.5	94[i]

[a] Except where otherwise indicated analytical data were taken from the compilation by Tristram (ref. 4). See ref. 3. [b] C. B. Anfinsen, R. R. Redfield, W. L. Choate, J. Page and W. R. Carroll, *J. Biol. Chem.*, **207**, 201 (1954). [c] Several references are given in ref. *f*. [d] H. van Vunakis and E. Brand, Abstracts, 119th Meeting, Am. Chem. Soc., 1951, p. 28c. [e] C. Tanford and J. Hauenstein, in preparation. [f] C. Tanford and M. L. Wagner, THIS JOURNAL, **76**, 3331 (1954). [g] R. K. Cannan, A. H. Palmer and A. C. Kibrick, *J. Biol. Chem.*, **142**, 803 (1942). [h] R. K. Cannan, A. C. Kibrick and A. H. Palmer, *Ann. N. Y. Acad. Sci.*, **41**, 243 (1941). [i] C. Tanford, THIS JOURNAL, **72**, 441 (1950).

As regards the six proteins here discussed, it can therefore be said with certainty that no significant fraction of the theoretically possible sites have undergone an acyl shift during titration to *p*H 2.

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Identification of Amines. I. N-(Arylaminomethyl)-phthalimides

BY MELDRUM B. WINSTEAD AND HAROLD W. HEINE

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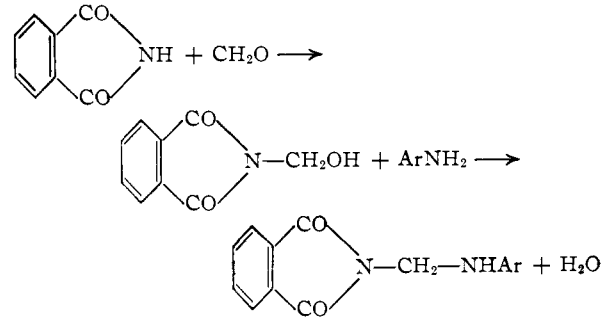
Work in this Laboratory has shown that the reaction of primary aromatic amines with phthalimide and formalin to produce N-(arylaminomethyl)-phthalimides is a general reaction and one that is particularly useful for the identification of aromatic amines. A survey of the literature has revealed that only N-(piperidinomethyl)-phthalimide[1a,1b] and N-(morpholinomethyl)-phthalimide[1a] have been prepared by this method. N-(Morpholinomethyl)-phthalimide also has been synthesized by treating N-hydroxymethylmorpholine with phthalimide.[2] In addition two N-(arylaminomethyl)-phthalimides, N-(anilinomethyl)-phthalimide and N-(phenylhydrazinomethyl)-phthalimide, have been synthesized by the treatment of aniline and phenylhydrazine, respectively, with N-bromomethylphthalimide.[1b]

A possible mechanism of this reaction involves the initial formation of N-hydroxymethylphthalimide which subsequently reacts with the amine, *i.e.*



Thus in one experiment the present investigators treated N-hydroxymethylphthalimide with aniline in an aqueous alcohol solution and produced a 70% yield of N-(anilinomethyl)-phthalimide.

Alternately the formaldehyde may react initially with the aromatic amine to form the N-hydroxymethyl intermediate which would subsequently react with phthalimide to form the desired derivative.[2] Work is in progress to determine which of the two postulated mechanisms is correct.

The N-(arylaminomethyl)-phthalimides are easily made and readily purified. In many cases they precipitate from the reaction medium in a high state of purity and in good yields. All of the derivatives melt above 110° and below 235°. In addition the melting point of each derivative is distinctly different from that of the starting reagents. The melting points of isomeric derivatives vary from thirty to fifty degrees with only three exceptions.

The preparation of N-(arylaminomethyl)-phthalimides of additional primary and secondary aromatic amines as well as N-(alkylaminomethyl)-phthalimides of primary and secondary aliphatic amines now is being investigated.

Experimental[3]

Preparation of N-(Arylaminomethyl)-phthalimide Derivatives.—Three grams (0.0204 mole) of phthalimide is suspended in 35 ml. of boiling 80% ethanol. Two milliliters of 37% formaldehyde is added and the solution is refluxed until all of the phthalimide has dissolved. Next a solution of 0.023 mole of the aromatic amine dissolved in 5–10 ml. of 80% ethanol is added. The alcoholic solution turns yellow or orange almost immediately after the amine has been added. The alcoholic solution is refluxed for one-half hour. If the aromatic amine contains a *meta*-directing group the refluxing period is increased to one hour. In a few cases the derivative is not too soluble in boiling 80% ethanol and begins to precipitate during the refluxing period.

The reaction medium is thoroughly chilled, and the product is filtered and dried. Many of the derivatives are brightly colored compounds which generally precipitate in a high state of purity. The derivative is recrystallized from 80% ethanol. Those derivatives which are only slightly soluble in this solvent are best recrystallized from a mixture of dioxane and petroleum ether (65–110°).

Table I lists the N-(arylaminomethyl)-phthalimide derivatives prepared.

N-Hydroxymethylphthalimide.[4]—One hundred and two grams (0.70 mole) of phthalimide, 52 ml. of formalin and

(1) (a) M. B. Moore and R. T. Rapala, THIS JOURNAL, **68**, 1657 (1946); (b) F. Sachs, *Ber.*, **31**, 3233 (1898).

(2) W. I. Weaver, J. K. Simons and W. E. Baldwin, THIS JOURNAL, **66**, 222 (1944).

(3) All melting points are corrected.

(4) S. R. Buc, THIS JOURNAL, **69**, 254 (1947).

TABLE I

N-(ARYLAMINOMETHYL)-PHTHALIMIDE DERIVATIVES

Amine used	M.p., °C.	Yield, %	Formula	Nitrogen, % Calcd.	Found
Aniline[a]	144.5–145	86	$C_{15}H_{12}N_2O_2$	...	...
o-Bromoaniline	117–118.5	47	$C_{15}H_{11}BrN_2O_2$	8.45	8.53
m-Bromoaniline[b]	167.5–168.5	83	$C_{15}H_{11}BrN_2O_2$	8.45	8.53
p-Bromoaniline[b]	215–216	91	$C_{15}H_{11}BrN_2O_2$	8.45	8.45
2-Bromo-4-methylaniline	117–119	43	$C_{16}H_{13}BrN_2O_2$	8.11	8.14
o-Chloroaniline	141–141.5	32	$C_{15}H_{11}ClN_2O_2$	9.77	9.69
m-Chloroaniline	169	50	$C_{15}H_{11}ClN_2O_2$	9.77	9.56
p-Chloroaniline[b]	207–208	73	$C_{15}H_{11}ClN_2O_2$	9.77	9.59
2,4-Dichloroaniline	139–140		$C_{15}H_{10}Cl_2N_2O_2$	8.72	8.70
p-Iodoaniline[b]	168–169 d.	58	$C_{15}H_{11}IN_2O_2$	7.41	7.30
o-Toluidine	137–138	72	$C_{16}H_{14}N_2O_2$	10.52	10.45
m-Toluidine	138–139	40	$C_{16}H_{14}N_2O_2$	10.52	10.47
p-Toluidine[b]	174.5–175.5	85	$C_{16}H_{14}N_2O_2$	10.52	10.56
2,4-Dimethylaniline	130–132.5	67	$C_{17}H_{16}N_2O_2$	10.00	10.08
2,5-Dimethylaniline	131–133	84	$C_{17}H_{16}N_2O_2$	10.00	10.01
o-Anisidine	112–112.5	90	$C_{16}H_{14}N_2O_3$	9.93	10.15
p-Anisidine	145	87	$C_{16}H_{14}N_2O_3$	9.93	9.72
o-Phenetidine	119–120	85	$C_{17}H_{16}N_2O_3$	9.46	9.66
p-Phenetidine	152–153	63	$C_{17}H_{16}N_2O_3$	9.46	9.55
p-Aminoacetanilide	211	73	$C_{17}H_{15}N_3O_3$	13.59	13.70
p-Aminoacetophenone[c]	165–165.5	52	$C_{17}H_{14}N_2O_3$	9.52	9.66
2-Aminobiphenyl	193–193.5	66	$C_{21}H_{16}N_2O_2$	8.53	8.32
4-Aminobiphenyl[b]	189–189.5	99	$C_{21}H_{16}N_2O_2$	8.53	8.72
Diphenylamine[d]	169.5–170	12	$C_{21}H_{16}N_2O_2$	8.53	8.59
2-Aminopyridine[e]	184	23	$C_{14}H_{11}N_3O_2$	16.60	16.52
Anthranilic acid[b,c]	188–189 d.	56	$C_{16}H_{12}N_2O_4$	9.46	9.71
m-Aminobenzoic acid[b,c]	200–200.5 d.	73	$C_{16}H_{12}N_2O_4$	9.46	9.44
p-Aminobenzoic acid[b,c]	232 d.	50	$C_{16}H_{12}N_2O_4$	9.46	9.54
Ethyl *p*-aminobenzoate[c]	176.5	47	$C_{18}H_{16}N_2O_4$	8.63	8.43
Carbazole[e]	241–242	27	$C_{21}H_{14}N_2O_2$	8.59	8.62

[a] Sachs[1b] reported m.p. 144–145° (Beilstein, "Handbuch der Organischen Chemie," Vol. XXI, fourth edition, p. 477). [b] Recrystallized from dioxane–petroleum ether (65–110°). [c] Reaction medium refluxed one hour. [d] Reaction medium refluxed 2.75 hours. [e] Reaction medium refluxed two hours.

350 ml. of water are refluxed until a clear solution results. The hot solution is filtered and cooled overnight, and the white, crystalline product obtained is filtered and dried. The yield is 112.7 g. (90%), m.p. 138–141° (reported 137–141°).

N-(Anilinomethyl)-phthalimide.—Two and one-half grams (0.0141 mole) of N-hydroxymethylphthalimide is dissolved in 25 ml. of boiling 80% ethanol. One and one-half grams (0.0161 mole) of aniline is added, and the solution immediately turns yellow. The reaction medium is refluxed for 30 minutes, cooled, and the yellow crystalline product is collected; yield 2.5 g. (70%), m.p. 145–145.5°.

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2-Hydroxy-3-acetylaminofluorene, A Metabolite of 3-Acetylaminofluorene in the Rat[1]

BY ELIZABETH K. WEISBURGER

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As part of a study of the comparative carcinogenic effects of positional isomers of 2-acetylaminofluorene, it was desired to test the activity of 3-acetylaminofluorene. The intermediate, 3-aminofluorene, had been synthesized previously by Hayashi and Nakayama[2] and also by Campbell and Stafford[3] but by procedures which could not readily be adapted for the preparation of relatively large quantities of 3-aminofluorene.

In the present investigation the methods of Bradsher and Jackson[4] for the preparation of 5-nitro-2-cyanobiphenyl and of Ray and Barrick[5] for the synthesis of 3-aminofluorenone from the cyanobiphenyl were used. The 3-aminofluorenone was readily reduced to 3-aminofluorene by use of the Huang-Minlon[6] modification of the Wolff–Kishner reaction. The melting points of the 3-aminofluorene and the acetyl derivative agreed with those reported by Hayashi and Nakayama.[2] However, the ultraviolet absorption spectra showed maxima and minima at lower wave lengths which were not reported by the Japanese authors.

If hydroxylation occurred during metabolism of 3-acetylaminofluorene, three possible compounds might result. *o*-Hydroxylation could yield either

(1) Presented before the Biological Chemistry Division at the 126th National Meeting, New York, September, 1954.

(2) M. Hayashi and A. Nakayama, *J. Soc. Chem. Ind. Japan*, Suppl. binding, **36**, 127B (1933).

(3) N. Campbell and W. H. Stafford, *J. Chem. Soc.*, 299 (1952).

(4) C. K. Bradsher and W. J. Jackson, Jr., THIS JOURNAL, **74**, 4880 (1952).

(5) F. E. Ray and J. G. Barrick, *ibid.*, **70**, 1492 (1948).

(6) Huang-Minlon, *ibid.*, **68**, 2487 (1946).