The other acid-catalyzed cyclizations were carried out in a similar way. The results are summarized in Table I.

Cleavage of 10-cyclohexyl-1,2-benzanthracene (IIa). A mixture of 0.3 g. of the hydrocarbon, IIa, 20 ml. of 48% hydrobromic acid, and 40 ml. of glacial acetic acid was heated under reflux for 24 hr. On cooling, white plates formed which were filtered and recrystallized from 95% ethanol yielding white crystals, m.p. 157–160°, identified as 1,2-benzanthracene (100%).

The other acid-catalyzed cleavage experiments were carried out in a similar way. The results are summarized in Table I.

BLACKSBURG, VA.

[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE[1]]

Ionization Constants of Derivatives of Fluorene and Other Polycyclic Compounds[2]

PRESTON H. GRANTHAM, ELIZABETH K. WEISBURGER, AND JOHN H. WEISBURGER

Received June 27, 1960

Potentiometric and spectrophotometric methods were applied to the determination of the apparent ionization constants, in 70% ethanol, of 120 polycyclic aromatic compounds. These were phenols, carboxylic acids, and chiefly amines derived from naphthalene, biphenyl, phenanthrene, chrysene, pyrene, dibenzofuran, dibenzothiophene, carbazole, diphenyl sulfide, diphenylmethane, and especially from fluorene. The effect of the substituent groups *N,N*-dimethyl-, nitro-, keto-, fluoro-, chloro-, bromo-, iodo-, acetamido-, methoxy-, hydroxy-, and amino- was established. The results are discussed in terms of inductive and steric interactions, resonance and hydrogen bonding in these molecules in relation to their structure.

Useful insight into the chemical and physical properties of molecules in the ground state can be derived from a comparative study of the ionization constants[3] of such molecules.[4,5] Relatively few compounds of the polynuclear aromatic type have been investigated in this regard. It is the purpose of this paper to present and discuss data in this field, especially in respect to derivatives of biphenyl, fluorene, and certain other tri- and tetracyclic compounds. The conclusions derived proved helpful in gaining a better understanding of the intimate molecular structure of the compounds, particularly in terms of inductive and resonance effects in the polynuclear systems. In addition, this study furnished information on the possible relationship of the ionization constants of some of these compounds to their carcinogenicity.[6] In a number of other cases a connection has been found between the pharmacologic activity in a series of related chemicals and their ionization constants.[7] In those instances the specific property assayed depended on whether the ionized or the nonionized species existed and was active at the *p*H of the living host, *i.e.* around *p*H 7.4.

The method used for the determination of the *p*K value involved the potentiometric measurement of the *p*H of a solution containing exactly equivalent amounts of a compound and its salt. This relatively simple procedure, while not of universal applicability,[7a] was found to be sufficiently accurate with the pure substances studied. The known literature values of some of the chemicals examined again in the present study (*cf.* footnotes to Tables) were reproduced without difficulty. In addition, the results obtained were corroborated in a number of cases by the spectrophotometric method. The values observed by this method were within the experimental error of those with the potentiometric procedure if the *p*K fell within the range of 3.50 to 11.80. The *p*K values of compounds outside this range were determined by the spectrophotometric method.

(1) National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

(2) Presented in part before the 125th Meeting of the American Association for the Advancement of Science, Washington, D. C., December 1958.

(3) In this paper all ionization constants refer to the apparent acid constant, pK_a'.

(4) In order to limit the references to a reasonable number, comprehensive reviews or monographs will be cited wherever possible. Similarly, references to the preparation of known compounds will generally not be given. J. H. W. will gladly supply information on particular compounds upon inquiry by interested readers.

(5) (a) H. C. Brown, D. H. McDaniel, and O. Häfliger in E. A. Braude and F. C. Nachod, *Determination of Organic Structures by Physical Methods*, Academic Press, New York, 1955, pp. 567–662. (b) L. N. Ferguson, *Electron Structures of Organic Molecules*, Prentice-Hall, New York, 1952, pp. 189–200. (c) A. Albert, *Heterocyclic Chemistry*, Oxford University Press, Oxford, England, 1959, pp. 336–346. (d) J. Hine, *Physical Organic Chemistry*, McGraw-Hill, New York, 1956, pp. 46–80. (e) G. W. Wheland, *Resonance in Organic Chemistry*, Wiley, New York, 1955, pp. 337–376. (f) B. Pullman and A. Pullman, *Les Théories Électroniques de la Chimie Organique*, Masson et Companie, Paris, 1952, pp. 316–322. (g) L. P. Hammett, *Physical Organic Chemistry*, McGraw-Hill, New York, 1940, Chapters VII and IX.

(6) For reasons of chemical stability the acetyl derivatives, such as *N*-2-fluorenylacetamide, are usually tested. However, animals possess enzyme systems capable of removing the acetyl group. In those cases where both amine and acetyl derivatives were examined the biological effects were usually similar. See E. K. Weisburger, and J. H. Weisburger, *Advances in Cancer Research*, **5**, 331 (1958).

(7) (a) A. Albert, *Pharmacol. Revs.*, **4**, 136 (1952); (b) D. Libermann, *Bull. soc. chim. biol.*, **34**, 1026 (1952); (c) T. C. Butler, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 367 (1955); (d) J. J. Burns, T. F. Yu, P. Dayton, L. Berger, A. B. Gutman, and B. B. Brodie, *Nature*, **182**, 1162 (1958).

Owing to the low solubility in water of most of the compounds, the data by the potentiometric procedure were obtained in 70% ethanol solution, usually at a concentration of 5.26×10^{-3} moles/l. Since the spectrophotometric method gave satisfactory results on more dilute solutions, both water and 70% ethanol were used as solvents in a comparative study involving some of the compounds.

EXPERIMENTAL[8]

Materials. Many of the compounds studied were available to us from previous work (shown as *W* in Tables). Others were kindly donated by Dr. F. E. Ray (shown as *R*), University of Florida, and by Drs. J. A. and E. C. Miller (*M*), University of Wisconsin, who also forwarded several of the compounds prepared by Dr. T. L. Fletcher (*F*), University of Washington, to all of whom we are greatly indebted. A few compounds were commercial (*C*) samples. Some of the amines were supplied as the acetyl derivatives. In these cases hydrolysis by 6–12*N* aqueous or ethanolic hydrochloric acid followed by isolation and recrystallization furnished the desired amines. Purity of the compounds was established by melting point determination, spectroscopy, and other appropriate methods.

Preparation of new compounds. N,N-Dimethylation of isomeric fluorenamines. This reaction was performed as described by Fletcher, *et al.*[9] Briefly, 5 mmoles of the fluorenamine and 6.7 mmoles (about 0.8 ml.) of trimethylphosphate (Aldrich Chemical Company, Milwaukee) were heated progressively in an oil bath at 195° and maintained thereat for 1 hr. Upon cooling to 100°, 6 ml. of 4.2*N* sodium hydroxide solution was added and the mixture refluxed another hour. After addition of 20 ml. of cold water the desired compound was isolated as described below for the various isomers.

N,N-Dimethyl-1-fluorenamine. The oily reaction mixture was extracted with ether. The ether solution was washed to neutrality, dried, and the solvent was distilled off. The residue was distilled *in vacuo* (3–5 mm.) yielding a yellowish liquid which turned to a glass at −80° but refused to crystallize at room temperature. However, conversion to the hydrochloride afforded 1.65 mmoles of white needles, which melted at 192.5–193.5° after four crystallizations from 6*N* hydrochloric acid.

Anal. Calcd. for $C_{15}H_{16}NCl$: C, 73.31; H, 6.56; N, 5.70. Found: C, 73.46; H, 6.51; N, 5.82.

N,N-Dimethyl-3-fluorenamine. Proceeding as with the 1-isomer, the vacuum distillation gave 2.95 mmoles of a yellowish solid, m.p. 59–62°. Four crystallizations from 2 ml. of petroleum ether (b.p. 30–60°) (in a freezer at −15°) left almost white crystals in clusters, m.p. 62–63°.

Anal. Calcd. for $C_{15}H_{15}N$: C, 86.08; H, 7.22; N, 6.69. Found: C, 86.19; H, 7.17; N, 6.66.

N,N-Dimethyl-4-fluorenamine. After the vacuum distillation, 3.1 mmoles of pale yellow liquid which crystallized on standing (m.p. about 30°) was obtained. This material was sublimed *in vacuo*. The sublimate, treated with Norit in dilute hydrochloric acid solution, furnished 2.5 mmoles of white powder, m.p. 34–36°, after neutralization with sodium bicarbonate solution. Four further crystallizations from 1–2 ml. of petroleum ether (at −15°) raised the m.p. to 37°.

Anal. Calcd. for $C_{15}H_{15}N$: C, 86.08; H, 7.22; N, 6.69. Found: C, 86.14; H, 7.16; N, 6.92.

4-Fluorenol. An ice-cold solution of 0.5 g. of 4-fluorenamine in 10 ml. of acetic acid, 10 ml. of water, and 5 ml. of concentrated sulfuric acid was diazotized with a solution of 0.3 g. of sodium nitrite in 3 ml. of water. After 0.5 hr., 0.7 g. of urea was added, and the mixture was stirred 15 min. longer. The solution of the diazonium salt was introduced dropwise into 110 ml. of refluxing 3.5*N* sulfuric acid. Upon cooling 0.15 g. (range 0.09–0.27 g. in various runs) of alkali-soluble material, m.p. 107–109° was obtained. Four crystallizations of 0.57 g. (combined material obtained in several experiments) from water afforded 0.12 g. of pale yellow crystals, m.p. 110–110.5°. The slight coloration was not removed by vacuum sublimation. $\lambda_{max}^{C_2H_5OH}$ 258.5 mμ (ε 18,200), 263.5 (16,400), 268.5 (21,000), 286.5 (7,600), 294 (8,700), and 306 (5,300): λ_{min} 241 (6,600), 262.5 (16,300), 265.5 (16,200), 279 (5,800), 290.5 (6,600), and 302.5 (4,900).

Anal. Calcd. for $C_{13}H_{10}O$: C, 85.69; H, 5.53. Found: C, 86.00; H, 5.80.

4-Bromofluorene. Incidental to the preparation above, the diazonium solution from 0.73 g. of 4-fluorenamine was also decomposed in the presence of cuprous bromide in the usual manner. The resulting oil was taken up in benzene, washed with alkali and water, and percolated through an alumina column. The resulting waxy material was sublimed *in vacuo* (bath temperature 50°) and the sublimate crystallized from methanol-water to give 96 mg. of cream-colored crystals, m.p. 57–58°. Two further crystallizations from methanol left 15 mg. of white prisms, m.p. 60–61°, of 4-bromofluorene (Suzuki, *et al.*[10] reported m.p. 61°).

7-Nitro-2-fluorenecarboxylic acid. A solution of 3 g. of 2-fluorenecarboxylic acid in 40 ml. of glacial acetic acid was cooled to 40°, and 15 ml. of yellow fuming nitric acid (d = 1.49) was added. The mixture was stirred and heated to 100°. At 95° all dissolved and a reaction occurred at 100–105°. The temperature was kept at this level for 5 min., whereupon a precipitate began appearing. The pale yellow material, wt. 1 g., melted at 320–330° (Kofler block). Recrystallization from acetic acid (250 ml./g.) or better dimethylformamide-water (3:1) yielded small pale yellow

(8) Microanalyses were performed by the staff of the NIH Microanalytical Laboratory to whom we are grateful. Competent technical assistance was rendered by Mrs. A. Parker.

(9) T. L. Fletcher, M. E. Taylor, and A. W. Dahl, *J. Org. Chem.*, 20, 1021 (1955).

(10) K. Suzuki, S. Kajigaeshi, and S. Kato, *Yuki Gosei Kagaku Kyôkai Shi*, 16, 304 (1958). We are greatly indebted to Dr. Suzuki for valuable discussion and a sample of his material which showed no depression in melting point and identical infrared spectrum with the compound described here. The reason for repeating this particular experiment at this time was the controversy regarding the correct melting point of 4-bromofluorene. H. F. Miller and G. B. Bachman, *J. Am. Chem. Soc.*, 57, 2447 (1935), reported a melting point of 165°. E. D. Bergmann and E. Loewenthal, *Bull. soc. chim. France*, 1952, 66, found 170°, and J. H. Weisburger, E. K. Weisburger, and H. P. Morris, *J. Am. Chem. Soc.*, 74, 4540 (1952), gave m.p. 112°. Suzuki, *et al.* established that the compound, m.p. 165°, in the hands of Miller and Bachman was really 2,7-dibromofluorene, m.p. 165°. Furthermore, it may be noted that Suzuki, *et al.* observed a melting point of 167–168° for 4-bromo-9-fluorenol (although Miller and Bachman attributed a melting point of 149–150° to this compound). Thus, it is possible that Bergman and Loewenthal dealt with the 9-hydroxy derivative, considering the melting point of their sample and its method of preparation, a relatively short Clemmensen reduction giving a poor yield of product crystallizing from benzene.

Re-examination of our earlier preparation of the supposed 4-bromofluorene, m.p. 112°, revealed that it was really 2-bromofluorene (lit. m.p. 113°) by mixed melting point with an authentic sample[11] and identity of their infrared curves. Our starting material was obviously contaminated with 2-isomer, and in view of the much lower solubility of the 2-bromofluorene, it was the material isolated in the extensive crystallization procedures used earlier. Hence, 4-bromofluorene has a melting point of 61°, as described for the first time by Suzuki *et al.*

needles, m.p. 335° (Kofler) with some sublimation at 310°. $\lambda_{max}^{70\%C_2H_5OH}$ 257 mμ (ε 9,060), 328 (22,700); λ_{min} 228.5 (5,280), 276 (5,340).

Anal. Calcd. for $C_{14}H_9NO_4$: C, 65.88; H, 3.55. Found: C, 65.59; H, 3.81.

A Schmidt reaction in sulfuric acid–chloroform gave 7-nitro-2-fluorenamine, orange material, m.p. 230°, proving the location of the nitro group in the carboxylic acid.

7-Methoxy-3-nitro-2-fluorenamine. One gram of *N*-(7-methoxy-2-fluorenyl)acetamide in 100 ml. of acetic acid was nitrated by the dropwise addition, with efficient stirring, of 2 ml. of a 1:1 mixture of water and concentrated nitric acid at 20°. After continued stirring for 1 hr. the solution was poured on ice yielding 0.99 g. of a yellowish-orange precipitate, m.p. 158–160°. This material was hydrolyzed by refluxing in 20 ml. of a 1:1 mixture of ethanol and 6*N* hydrochloric acid for 0.5 hr. The product was extracted with 150 ml. of 0.3*N* hydrochloric acid, removing the compound nitrated *ortho* to the methoxy group which, however, was not isolated in pure form. The insoluble red residue (0.3 g.) of 7-methoxy-3-nitro-2-fluorenamine melted at 202°. Crystallizations from ethanol-water or benzene–petroleum ether mixtures gave long red needles of the same melting point.

Anal. Calcd. for $C_{14}H_{12}N_2O_3$: C, 65.61; H, 4.72; N, 10.93. Found: C, 65.84; H, 4.94; N, 10.62.

Standard solutions. A 0.15*N* solution of potassium hydroxide was prepared with carbon dioxide-free redistilled water and standardized against reagent grade potassium hydrogen phthalate by accepted techniques. Dilution of reagent grade hydrochloric acid with redistilled water and standardization against the potassium hydroxide solution afforded 0.15*N* hydrochloric acid. The ethanol was a benzene-free, fermentation grade alcohol ("Pharmco" brand, 200 proof) obtained from Publicker Industries, Philadelphia.

Instruments. A Cambridge *p*H meter, model R, equipped with glass and saturated calomel electrode assemblies was used for *p*H measurements. The instrument was calibrated against standard buffers of *p*H 4, 7, and 9. The temperature of the solutions was maintained at 25° by partial submersion of the vessel in a thermostated bath.

Ultraviolet and visible spectra were recorded on a Cary spectrophotometer, model 14, employing 1-cm. quartz cells. The temperature of the cell compartment was kept constant at the desired temperature (*cf.* Table II) by circulating water from a bath.

Procedure for the potentiometric determination of the apparent ionization constant. The compound (15 micromoles, usually 2–4 mg.) was weighed accurately into the titration vessel, the top of a Parr style weighing bottle (Kimble Glass Co. weighing bottle stopper no. 15180, with a 24/12 standard joint). After solution in 2.0 ml. of ethanol, by gentle warming if required, 0.8 ml. of carbon dioxide-free redistilled water and 0.5 equivalent (50 microliters, micropipet) of 0.15*N* acid (for amino groups) or base (for carboxy, hydroxy, groups, or amine salts) was added, giving a 70% ethanol solution. The *p*H of this solution was accurately determined at 25°. Instrumental stability was checked by reading the appropriate standard buffers before and after each sample. Duplicate runs for each compound were performed and agreement was within 0.05 *p*H units. The ionic strength of the solutions was not taken into account, but little effect is anticipated with the dilute solutions used.[12]

A few compounds insoluble under these conditions were studied in more dilute solution (2.0–2.5 millimolar).

Ionization constants by the spectroscopic method. The method devised by Flexser, Hammett and Dingwall[13] and used recently in an extensive investigation of a series of aso dyes by Sawicki and Ray[cf. 14,15] was adapted to this study. Three spectra of the solutions of the ionized, nonionized, and a mixture of these forms of a compound were run sequentially on a Cary instrument so that the wave-length scale on the three curves coincided. In this manner the isosbestic points and the general suitability of the curves were readily apparent.[16] Calculations of the ionization constant based on the absorbance of the compound at the three hydrogen ion concentrations with the aid of the equations given by the pre-cited authors, were performed at a number of wave lengths but not near the maxima or minima. The results so obtained showed little scatter for any compound if the proper *p*H values were selected.

RESULTS AND DISCUSSION

The value of the apparent ionization constant obtained at a concentration of approximately 5 mmoles per liter is well within the reliable range of the simplified micromethod described. Indeed, the data for representative compounds with an amino, hydroxy, and carboxy group show little deviation until considerably higher dilutions are employed (Table I). The variation of the constant is more sensitive to the concentration factor with compounds at the extremes of validity of the method, as might be expected owing to the larger influence of hydrolytic phenomena with the weaker acids and bases.

Ionization constants of fluorenamines. Table II presents the *p*K values of the isomeric fluorena-

TABLE I

IONIZATION CONSTANTS OF A COMPOUND WITH AN AMINO, HYDROXY, AND CARBOXY GROUP IN SOLUTIONS OF VARYING CONCENTRATIONS

Compound	Concentration, Mmoles $\times 10^{-3}$/Ml.	pK_a'
2-Fluorenamine	10	4.30
	5	4.31
	2.5	4.32
	1.0	4.44
N-(7-Hydroxy-2-fluorenyl)-acetamide	20	11.59
	10	11.58
	5	11.58
	2.5	11.35
	2.0	11.35
9-Oxo-4-fluorenecarboxylic acid	20	4.98
	10	5.00
	5	5.00
	2.5	5.01
	2.0	5.00
	1.0	5.15

(11) S. D. Ross, M. Finkelstein, and R. C. Petersen, *J. Am. Chem. Soc.*, 80, 4327 (1958).

(12) T. V. Parke and W. W. Davis, *Anal. Chem.*, 26, 642 (1954).

(13) L. A. Flexser, L. P. Hammett, and A. Dingwall, *J. Am. Chem. Soc.*, 57, 2103 (1935).

(14) E. Sawicki and F. E. Ray, *J. Org. Chem.*, 19, 1686 (1954).

(15) J. M. Vandenbelt, C. Henrich, and S. G. Vanden Berg, *Anal. Chem.*, 26, 726 (1954).

(16) A few preliminary experiments were required to select the optimal conditions with a compound of unknown *p*K value. Footnotes to the appropriate Tables show the hydrogen ion concentration so determined for each compound, and used in the calculations.

TABLE II

IONIZATION CONSTANTS OF ISOMERIC FLUORENAMINES AND AMINOFLUORENOLS IN 70% ETHANOL AND IN WATER

	pK_a' of Amino Group				pK_a' of Hydroxy Group
Method	Potentiom.	Spectroscopic			Potentiom.
Temperature	25°	25°	25°	40°	25°
Solvent	70% C_2H_5OH	70% C_2H_5OH	H_2O	H_2O	70% C_2H_5OH
Compound[a]					
1-Fluorenamine	3.60	3.57[b]	3.87[f]	3.67	—
2-Fluorenamine	4.30	4.27[c]	4.64[g]	4.42	—
3-Fluorenamine	4.37	4.39[d]	4.82[h]	4.57	—
4-Fluorenamine	3.4	3.15[e]	3.39[f]	3.52	—
9-Fluorenamine	7.56	—	—	—	—
2-Amino-1-fluorenol	4.54	—	4.82[i]	—	11.25
7-Amino-1-fluorenol	4.38	—	4.63[i]	—	11.66
2-Amino-3-fluorenol	4.50	—	—	—	11.60
7-Amino-3-fluorenol	4.32	—	4.63[i]	—	11.9
2-Amino-5-fluorenol	4.59	—	4.73[i]	—	11.9
2-Amino-7-fluorenol	4.60	—	4.88[i]	—	11.8
2-Amino-9-fluorenol	3.88	—	—	—	—

[a] The last compound was supplied by *M;* all others came from this laboratory (*W*). [b] Conditions for this determination were: Alkaline form (*1*), 0.2 ml. of 2.5 millimolar solution in ethanol, 6.8 ml. of ethanol, and 3 ml. of water; acid form (*2*) substitute 1 ml. of 0.107*N* hydrochloric acid and 2 ml. of water for 3 ml. of water; buffered form (*3*), same but 0.1 ml. hydrochloric acid and 2.9 of water, *p*H measured in final solution 3.32. Conditions for other compounds will be listed in an abbreviated manner, giving the *p*H of *3*, and where necessary, solutions used for *1* or *2*. [c] *3*, pH 4.37. [d] *3*, *p*H 4.56. [e] *3*, pH 3.29. [f] *3*, *p*H 2.99. [g] *3*, *p*H 4.20. [h] *3*, *p*H 4.34. [i] *3*, *p*H 4.26.

mines and aminofluorenols obtained by two different methods in water and 70% ethanol. The potentiometric and the spectrophotometric methods give very similar results where the *p*K is above 3.50 for the amino group. The potentiometric method as used here is not suitable below this value; a *p*H reading of 3.3 to 3.4 is always found irrespective of the true value of the constant, as determined by the spectrophotometric method (*cf.* subsequent tables).

The ionization constants are 0.14–0.43 (average 0.28) units higher in water as compared to 70% ethanol. This slight effect of solvent on the *p*K of the amino group has also been reported by others.[17, cf. 5] On the other hand, acidic groups are weakened considerably when going from water to 70% ethanol solution, as evidenced by the relatively high *p*K values for the phenolic hydroxy group and the carboxylic acids (*see* Tables). This fact, likewise, has been observed in other series of compounds.[5]

A rise in temperature from 25 to 40° decreases the *p*K of 1-, 2-, and 3-fluorenamine by 0.20, 0.22, and 0.25 units, which corresponds to heats of ionization 5.7, 6.3, and 7.1 kcal./mole, respectively, values of the same order of magnitude as that reported by Elliot and Mason[18] for 2-fluorenamine (5.7) based on the temperature range of 0.2 to 20°. In contrast 4-fluorenamine exhibits an anomalous pattern in that the *p*K increased by 0.13 units in the 15° temperature interval.

Of the four isomeric fluorenamines, the order of decreasing basicity is the 3-, 2-, 1-, and 4- derivative. In turn, these four compounds can be assembled into two classes. On the one hand are the 3- and 2- derivatives, which have similar *p*K values of 4.39 and 4.27, and on the other the 1- and 4- isomers, which have lower *p*K values of 3.57 and 3.15, respectively. In the latter two compounds, the amine function is attached to carbon atoms *ortho* to the ring annelation, a *peri* position, suggesting that this location is responsible for their lower proton affinity. Such *ortho* effects have been reported in a number of other cases which are fully discussed in recent reviews[5] (*cf.* also the known examples of the naphthylamines, the *p*K values of which in 70% ethanol are listed in Table III). The slightly higher *p*K of 1-fluorenamine, as compared to the 4-isomer, may be attributed to the electron-releasing hyperconjugative action of the 9-methylene group, *ortho* to the 1-position, but *meta* to the 4-position. Likewise, the higher *p*K of 3-fluorenamine, can be explained by its location *para* to the 9-carbon, whereas the 2-derivative is *meta*. However, the 9-carbon does enhance the basicity even of the amino group located *meta* to the 9-position, since the 2- and 4-fluorenamines exhibit higher *p*K values than the comparable 4- and 2-biphenylamines (Table III).

The *N,N*-dimethyl derivatives of the hindered amines (2-biphenylamine, 1-, and 4-fluorenamine) are more basic than the corresponding unalkylated compounds (Table III), owing presumably to steric inhibition of resonance. The dimethylamino grouping is apparently deformed from a coplanar

(17) J. M. Vandenbelt, C. H. Spurlock, M. Giffels, and M. W. Eash, *Science*, 121, 646 (1955).

TABLE III

IONIZATION CONSTANTS OF VARIOUS AROMATIC AMINES IN 70% ETHANOL SOLUTION

Compound	Source	pK_a'	Method
1-Naphthylamine[i]	C	3.60	P
2-Naphthylamine[j]	C	3.85	P
1,2,3,4-Tetrahydro-2-naphthylamine	W	9.13	P
2-Biphenylamine[k]	M	2.94	S[a]
N,N-Dimethyl-2-biphenylamine	W	3.71	P
3-Biphenylamine[l]	M	3.89	P
3-Amino-4-phenylacetanilide	M	2.80	S[a]
4-Biphenylamine[m]	M	3.94	P
N,N-Dimethyl-4-biphenylamine	M	3.66	P
2-Methyl-4-biphenylamine	M	4.23	P
2′-Methyl-4-biphenylamine	M	4.03	P
4′-Methyl-4-biphenylamine	M	3.95	P
p-Terphenyl-4-amine	M	3.98	P
2′-Fluoro-4-biphenylamine	M	3.77	P
2-Phenanthrylamine[n]	M	3.74	P
3-Phenanthrylamine[n]	M	3.79	P
2-Chrysenamine	M	3.58	P
1-Pyrenamine[o]	M	2.77	S[a]
3-Dibenzofuranamine[p]	M	3.35	S[a]
2-Dibenzothiophenamine	M	3.94	P
3-Dibenzothiophenamine	M	3.52	P
4′-Amino-2-biphenylcarboxylic acid	R	3.92	P
4-Aminodiphenylsulfide	M	2.86	S[a]
3-Dibenzothiophenamine 5-oxide	M	2.57	S[a]
3-Dibenzothiophenamine 5-dioxide	M	1.25	S[b]
3-Aminocarbazole	M	5.75	P
N,N-Dimethyl-1-fluorenamine	W	4.41	P
N-Methyl-2-fluorenamine	R	4.02	P
N,N-Dimethyl-2-fluorenamine	W	3.99	P
N,N-Dimethyl-3-fluorenamine	W	4.43	P
N,N-Dimethyl-4-fluorenamine	W	3.59	P
7-Methoxy-2-fluorenamine	W	4.45	P
1-Aminofluorenone	W	−0.32	S[c]
2-Aminofluorenone	M	2.40	S[d]
3-Aminofluorenone	W	0.86	S[e]
4-Aminofluorenone	W	1.42	S[f]
7-Nitro-1-fluorenamine	W	3.50	P
3-Nitro-2-fluorenamine	W	−1.2	S[g]
7-Methoxy-3-nitro-2-fluorenamine	W	−0.9	S[g]
5-Nitro-2-fluorenamine	W	3.59	P
7-Nitro-2-fluorenamine	W	3.57	P
7-Nitro-3-fluorenamine	W	3.86	P
5-Nitro-4-fluorenamine	W	1.91	S[f]
7-Nitro-4-fluorenamine	W	2.19	S[d]
1-Fluoro-2-fluorenamine	W	2.59	S[h]
3-Fluoro-2-fluorenamine	W	2.69	S[a]
3-Iodo-2-fluorenamine	W	1.97	S[f]
4-Fluoro-2-fluorenamine	F	3.05	S[d]
5-Fluoro-2-fluorenamine	F	3.93	P
6-Fluoro-2-fluorenamine	W	3.71	S[a]
8-Fluoro-2-fluorenamine	W	3.54	S[h]
1-Fluoro-4-fluorenamine	W	2.95	S[h]
3-Fluoro-4-fluorenamine	W	2.71	S[a]
7-Fluoro-2-fluorenamine	M	3.53	S[h]
7-Chloro-2-fluorenamine	W	4.00	P
7-Bromo-2-fluorenamine	W	4.00	P
7-Iodo-2-fluorenamine	W	3.90	P
N-(7-Amino-2-fluorenyl)-acetamide	W	4.31	P
N-(7-Amino-3-fluorenyl)-acetamide	W	4.10	P

[a] Form *S*, pH 2.72. [b] *S*, pH 0.96. We are grateful to Dr. R. Bates, National Bureau of Standards, for discussions concerning the measurement of low pH values, which was performed by zero displacement of the pH meter with the appropriate buffers. [c] *S*, pH −0.25, obtained by the addition of 3 ml. of 6*N* H_2SO_4 to 7 ml. of ethanol solution; *Z* was similarly made with 3 ml. of 25*N* H_2SO_4. [d] *S*, pH 2.50. [e] *S*, pH 0.75. [f] *S*, pH 1.45. [g] *S*, pH −0.73, obtained by using 3 ml. of 12.5*N* H_2SO_4, and *Z* as under *c*. [h] *S*, pH 3.00. [i] In water pK is 3.92.[5a] [j] In water pK is 4.11[5a] (found in this study: 4.15). [k] pK is 3.78 in water,[5a] 3.03 in 50% ethanol[18a] at 20°. [l] pK is 4.18 in water,[5a] 3.82 in 50% ethanol.[18a] [m] pK is 4.27 in water,[5a] 3.81 in 50% ethanol.[18a] [n] pK values are 3.60 and 3.59, respectively, in 50% ethanol.[18a] [o] pK is 2.91 in 50% ethanol.[18a] [p] Sawicki and Ray[18c] reported a pK of 3.3.

position with the ring system by the adjacent hydrogen atoms, resulting in a decrease in the base-weakening resonance (*cf.* the excellent discussion of this phenomenon by Ferguson,[5b] and Brown, *et al.*[5a]). The *N,N*-dimethyl derivatives of the unhindered amines, such as 2- and 3-fluorenamine, and 4-biphenylamine show little change in the pK value, or a slight lowering.

9-Fluorenamine, in which the amine function is attached at the saturated 9-methylene carbon atom, is considerably more basic than its purely aromatic congeners, with a pK of 7.56. However, it is appreciably less proton-attracting than the corresponding open ring analog, benzylamine, with an estimated pK of about 9 in 70% ethanol (9.34 in water). Since the inductive effects in benzylamine and in 9-fluorenamine would tend to operate similarly, it would seem that the considerably lower proton affinity of the latter compound is due to hyperconjugative resonance phenomena between substituents at the 9-position and the remainder of the molecule. This concept is borne out by data with other 9-substituted derivatives, to be discussed later.

Ionization constants of other aromatic amines. The pK values of the naphthylamines in 70% ethanol are also about 0.30 units lower than the values in water (Table III). As discussed by Brown, *et al.*[5a] resonance as well as steric effects play a role in giving 1-substituted naphthalenes higher acidities than 2-substituted ones. Reduction of the ring bearing the amine function yields the strong base, 1,2,3,4-tetrahydro-2-naphthylamine, pK 9.13.

There is little difference between the constants of 3- and 4-biphenylamines. Substitution of an acetamino residue *ortho* to the 3-amino group is strongly base-weakening. 2-Methyl-4-biphenylamine has a pK quite similar to that of its analog, 2-fluoren-

(18) (a) J. J. Elliott and S. F. Mason, *J. Chem. Soc.*, 2352 (1959). (b) The values for the fluorenamines would appear to be somewhat higher than that observed in the precise and careful work of E. E. Sager and I. J. Siewers, *J. Research Natl. Bur. Standards*, **45**, 489 (1950) on the heat of dissociation of 4-aminobenzophenone in water, which was 19,000 joules deg.$^{-1}$ mole^{-1} or 4.54 kcal. mole^{-1} on the basis of determinations from 10 to 40° in 5-degree steps. (c) E. Sawicki and F. E. Ray, *J. Am. Chem. Soc.*, **75**, 2519 (1953).

amine. The base strengthening effect of the methyl group may, however, be somewhat more complex in this instance than that due to the 9-methylene in the fluorene derivative. In addition to its hyperconjugative inductive action, the methyl group also may force the unsubstituted phenyl ring out of the plane of the substituted ring, resulting in a reduced resonance interaction. This effect may well be measured in large part by the increased basicity of the 2′-methyl derivative over that of 4-biphenylamine itself, *i.e.*, by 0.09 units, since methyl, or carboxy, or phenyl groups in the 4′-position have little effect by themselves. Thus, inductive and resonance interactions between substituents in the two phenyl rings in biphenyl are rather weak, a conclusion also reached by Kreiter, *et al.*[19] On the other hand, such exchanges are somewhat more pronounced in the planar and more rigid fluorene molecule.

Just as 2- and 3-fluorenamine have similar *p*K values, the corresponding 2- and 3-phenanthrene derivatives showed the closely related constants of 3.74 and 3.79. Likewise, the 3-derivative has a higher *p*K owing to interaction with the electrons from 9,10 bond, *para* to the 3-carbon. 2-Aminochrysene has a lower *p*K than the phenanthrene derivative, because of the greater electron withdrawing power of the larger ring system. Elliott and Mason[18a] felt that "the conjugate acid of the larger amine had the smaller entropy of dissociation and less endothermic heat of dissociation." 1-Pyrenamine has the low constant of 2.77 not only because of the multiple ring system, but also because the amino group is in the *peri* position.

The oxygen and sulfur heterocyclic analogs of fluorenamine manifest lower *p*K values. The oxygen in 3-dibenzofuranamine, with the amino group in a *meta* relationship to the hetero atom, possesses a larger depressing effect than sulfur in 3-dibenzothiophenamine in accord with findings in other heterocyclic systems. 2-Dibenzothiophenamine, in which the sulfur is in a *para* position to the amine function, exhibits a higher *p*K than the 3-isomer, but the open analog 4-aminodiphenyl sulfide has an even lower *p*K. The 5-thio oxide and dioxide derivatives are much stronger acids than the thiophene derivatives. However, other things being equal, the sulfur to oxygen bond is less acid-strengthening than the carbon to oxygen bond in the corresponding fluorenone (see below), indicating that resonance phenomena are transmitted better through the carbon than through the sulfur atom. In contrast to the lowering of the proton affinity by oxygen and sulfur, a nitrogen atom *para* to the amino group as in 3-aminocarbazole is considerably base-strengthening. Carbazole itself is a very weak base,[20] so it would be logical to assume that the proton acceptor is the amino nitrogen rather than the ring nitrogen. However, it is also possible that the amino nitrogen increases the hydrogen ion affinity of the ring nitrogen. Thus, the position of the proton in 3-aminocarbazole is uncertain. Similar cases in other heterocyclic systems are on record.[5a,f]

Substituted fluorenamines. Derivatives of 2-fluorenamine monohydroxylated in any position except the 9-carbon are stronger bases than the parent compound (Table II). The base-strengthening effect on the amine function at carbon-2 is least with hydroxy groups at the 6- and 8-positions between which resonance interactions occur to the smallest extent. On the other hand, the strongest proton acceptor results when the hydroxy group is at the 7-position, an extended *para* position favoring exchanges of charges. A methoxy group at that carbon is not nearly as active, increasing the *p*K by only 0.15 units (Table III) as compared to 0.30 for the hydroxy group. If the electronegative hydroxy group is located at the 9-position, however, where it is not phenolic, it has a fairly large base-weakening effect on the amine function located at the 2-position. A keto group at the 9-position exerts an even larger effect, lowering the *p*K of the *meta* amino groups at the 2- and 4-positions by 1.90 and 1.73 units, respectively. Furthermore, the *p*K of the amino group at the 3-carbon, *para* to the keto function at the 9-position is reduced by over 3.5 units, presumably because of extensive resonance and inductive interactions. The amino group in 1-aminofluorenone is almost 10^4 times weaker than in the corresponding fluorene derivative, for the same reasons and with the additional attenuation due to hydrogen bonding across the *peri* locations of the functional groups. Except for the nitro group, the ketonic oxygen exhibits the highest base-weakening power.

The powerful electron-withdrawing ability of the unhindered nitro group serves as a sensitive indicator of resonance phenomena in the fluorene ring system. It exerts its most pronounced action in an *ortho* position. 3-Nitro-2-fluorenamine is the least basic of the amines studied in this series, with a *p*K of -1.2. The low value may be ascribed to a combination of factors, consisting of inductive and electronic withdrawal of electrons, hydrogen bonding, and possibly a steric inhibition of the approach of the proton. In this case a methoxy residue located at the extended *para* carbon, the 7-position, enhances the basicity by 0.3 units, a some-

(19) V. P. Kreiter, W. A. Bonner, and R. H. Eastman, *J. Am. Chem. Soc.*, 76, 5770 (1954).

(20) Although the *p*K of carbazole has apparently not been measured (*cf.* Pullman and Pullman, *loc. cit.*[5f] p. 325) it seems to be very weakly basic indeed. Thus, the compound dissolves in 84% sulfuric acid, but the salt hydrolyzes to yield free carbazole upon dilution to about 25% sulfuric acid [*cf.* R. E. Glegg, *Anal. Chem.*, 28, 532 (1956)]; T. Nakajima and B. Pullman, *Bull. soc. chim. France*, 1958, 1502, and B. Pullman, *J. Chem. Soc.*, 1621 (1959), have discussed the basicity of the nitrogen atoms in purines and pyrimidines.

what larger effect than that seen in the absence of the nitro group. Advantage has been taken of the low basicity of 3-nitro-2-fluorenamine to separate this compound by virtue of its insolubility in dilute acid, from the more basic, soluble 7-nitro isomer.[21] As may be expected, a nitro group at the 7- and 5-positions lowers the proton affinity of an amino group at 2 and 4 quite appreciably. Moreover, in 5-nitro-4-fluorenamine, it is probably that hydrogen bonding can occur (extended *ortho* position) even though the 4- and 5-carbons of fluorene are rigidly held at a greater distance than the corresponding positions in biphenyl.[22] The comparatively smaller reduction in the ionization constant by a nitro group at the 7-position on amine functions at the 3- and 1-positions is a reflection of negligible resonance interactions between these positions. The diminution in *p*K observed can be attributed mainly to inductive effects.

The recent synthesis in three laboratories[23] of all the possible monofluoro-2-fluorenamines, as well as 1- and 3-fluoro-4-fluorenamine has permitted a study of the effect of fluorine on the basicity of the amine function. The largest base-weakening action is noted when the fluorine atom is in an *ortho* position, but the effect is not nearly as pronounced as in the nitro derivatives. The halogen in the same ring is a more effective depressant of the *p*K, even in a *meta* position, than in any position in the second ring.[23c,24] In this latter case, the pattern is not clear-cut in that it does not follow the expected behavior if resonance phenomena were of paramount importance. Thus, fluorine at the 8- and 7-positions has a larger base-weakening action than the halogen at the 6-carbon, but, curiously, substitution at the 5-carbon is least effective. It would seem that inductive effects are more significant in these cases, since halogen at the 5- and 7-position should lower the basicity of a 2-amino group more than halogen at the 6- and 8-carbons if resonance were to play any appreciable role. Moreover, 3-fluoro-4-fluorenamine has a lower *p*K value than the 1-fluoro derivative which is also consistent with this view.

The effect of halogens in the 7-position on the strength of the amino group at the 2-position was determined. Chlorine and bromine both depress the *p*K by 0.30 units, while iodine decreases it by 0.40 units. In contrast, fluorine has an appreciably larger action (0.77 units), although in other examples fluorine usually was the least effective of the halogens in lowering the proton affinity of an aromatic or heterocyclic amine.[5a] In an *ortho* position such as in 3-, however, iodine yields an amine with lower *p*K than fluorine does, presumably as a result of a larger steric inhibition by iodine to the approach of a hydrogen ion.

Diamino derivatives. An amino group in a suitable location of a multiple ring system can strengthen another amino group by inductive and resonance effects in addition to the operation of statistical factors.[5g] Thus, 1,2-naphthalenediamine exhibits a first ionization constant of 4.29 (Table IV), appreciably higher than that of 2-naphthylamine, 3.85. On the other hand, the 2,3-diamine has *p*K value of 3.99, only slightly larger than that of the monoamine. That localization of double bond character occurs in naphthalene derivatives between carbons 1 and 2, and of single bond properties between 2 and 3, is well known. The *p*K data reflect this condition whereby pronounced exchanges of charges occur between amino groups at 1 and 2, but not to the same extent between 2 and 3.

TABLE IV

FIRST AND SECOND[a] IONIZATION CONSTANTS OF AROMATIC DIAMINES IN 70% ETHANOL SOLUTION

Compound	Source	$pK_1'_a$	$pK_2'_a$
1,2-Naphthalenediamine	C	4.29	<3.5
2,3-Naphthalenediamine	C	3.99	<3.5
1,8-Naphthalenediamine	C	4.08	<3.5
2,2'-Biphenyldiamine	W	3.81	<3.5
Benzidine[b]	C	4.63	3.48
4,4'-Methylenedianiline	C	4.81	3.71
4,4'-Methylenebis(*N*,*N*-dimethyl)aniline	C	4.63	3.57
1,7-Fluorenediamine	W	4.47	<3.5
2,3-Fluorenediamine	W	4.49	<3.5
2,5-Fluorenediamine	W	4.52	<3.5
2,7-Fluorenediamine	W	4.97	3.5
4,5-Fluorenediamine	W	5.16	<3.5

[a] The value of pK_2' was not determined when it was less than 3.5, the limit of accuracy of the potentiometric method as used here. [b] pK_1 and pK_2 in water is 4.97 and 3.75 respectively.

Another type of influence is exemplified by 1,8-naphthalenediamine, with a *p*K of 4.08, as compared to a *p*K value of 3.60 for the corresponding monoamine. The two amino groups in the former compound are located at positions of low resonance or direct inductive interactions. Hence, the enhancement of the basicity caused by the introduction of the second amino group may be ascribable to a

(21) O. Diels, E. Schill, and S. Tolson, *Ber.*, 35, 3284 (1902); A. Eckert and E. Langecker, *J. prakt. Chem.*, 118, 263 (1928).

(22) (a) G. M. Brown and M. H. Bortner, *Acta Cryst.*, 7, 139 (1954); D. M. Burns and J. Iball, *Nature*, 173, 635 (1954); *Proc. Roy. Soc.* (*London*), A 227, 200 (1955). (b) Fischer-Hirschfelder models suggest that nitro and amino, and nitro and carboxy groups at the 4- and 5-carbons overlap. Two amino groups are very close together but do not prevent free rotation, and obviously the same situation prevails for an amino group and a hydrogen atom.

(23) (a) J. A. Miller, R. B. Sandin, E. C. Miller, and H. P. Rusch, *Cancer Research*, 15, 188 (1955). (b) T. L. Fletcher, W. H. Wetzel, M. J. Namkung, and H.-L. Pan, *J. Am. Chem. Soc.*, 81, 109 (1959). (c) K. Suzuki, E. K. Weisburger, and J. H. Weisburger, *J. Org. Chem.*, 24, 1511 (1959).

(24) Advantage was taken recently of the larger base weakening effect of fluorine *ortho* to an amino group as compared to other positions to confirm the structure of a series of isomeric fluorofluorenamines.[23c]

favored steric location, whereby a single proton may be captured and shared by the two amino groups. A similar explanation may hold for the relatively high first ionization constant of 2,2′-biphenyldiamine, 3.81, in relation to that of 2-biphenylamine, 2.94.

Another compound of this type is 4,5-fluorenediamine, which as a result of this neighboring group participation is considerably stronger than the corresponding monoamine, 4-fluorenamine.[22b] Indeed, the 4,5-diamine has the highest pK of the diamines examined in this study, if exception is made of the heterocyclic 3-aminocarbazole.

The symmetrical 2,7-fluorenediamine possesses a higher first ionization constant (4.97) than the related monoamine, 2-fluorenamine (4.30). The second amino group exhibits a larger base-strengthening action in this system than in the open benzidine, wherein resonance effects would be the predominant factor, and in 4,4′-methylenedianiline (4,4′-diaminodiphenylmethane) in which inductive phenomena would be the more important. Hence, both of these mechanisms may be expected to contribute in the 2,7-fluorene derivative. Incidentally, *N*-methylation of the methylenedianiline gives a compound with a lower pK, since the amino groups are in an unhindered position. Resonance interactions are very weak between the 1- and 7-carbons of fluorene, so that the slightly higher pK of the 1,7-diamine as compared to 2-fluorenamine may be almost entirely related to inductive factors. An amino group at the 3- and at the 5-carbon has an unexpectedly low base-strengthening action on the amine function at 2, in view of the ease of transmission of both inductive and resonance effects between these positions. Incidentally, it is not too unreasonable to assume that it is the amino group at the 2- (or 7-) carbon which captures the first proton in the unsymmetrical 1,7 and 2,5-diamines, in view of the obvious differences in basic strength of amino groups in the unhindered 2- as compared to the hindered 1- and 4- (or 5-) positions. On the other hand, the situation is not as clear-cut with the 2,3-diamine, so that it is impossible to decide, with the data at hand, which of the two amino groups in this compound ionizes first. Actually, an equilibrium between the two forms may exist.

Isomeric fluorenols and derivatives. The ionization constant of the phenolic hydroxy group of the four isomeric fluorenols is relatively independent of structure. Thus, 1-, 2-, 3-, and 4-fluorenols have pK values of 11.39, 11.56, 11.76, and 11.71, respectively, in 70% ethanol (Table V).[25] It would appear that the electron cloud around the oxygen atom, which influences the ease of proton release is affected little by comparatively minor variations in the electron distribution of the polynuclear ring system in the ground state.[26] Moreover, the small size of the hydroxy group renders it rather insensitive to steric effects, which play such a prominent role in the case of the amines and of the carboxylic acids (see below). The slightly higher acidity of 1-fluorenol as compared to the 4-isomer suggests that hydrogen bonding might occur to a small extent from the 9- to the 1-position but not from the 5- to the 4-position. The lower acidity of the 3-derivative, relative to the 2-isomer, presumably results from the hyperconjugative action of the 9-methylene group in a *para* position.

TABLE V

IONIZATION CONSTANTS OF FLUORENOLS IN 70% ETHANOL SOLUTION

Compound[a]	pK_a'
1-Fluorenol	11.39
2-Fluorenol[b]	11.56
3-Fluorenol[c]	11.76
4-Fluorenol	11.71
N-(*N*-Hydroxy-2-fluorenyl)acetamide	10.96
N-(1-Hydroxy-2-fluorenyl)acetamide	10.40
N-(1-Hydroxy-4-fluorenyl)acetamide	11.12
N-(2-Hydroxy-3-fluorenyl)acetamide	10.43
N-(3-Hydroxy-2-fluorenyl)acetamide	10.58
N-(5-Hydroxy-2-fluorenyl)acetamide[d]	11.82
N-(6-Hydroxy-2-fluorenyl)acetamide	11.79
N-(7-Hydroxy-2-fluorenyl)acetamide	11.58
N-(8-Hydroxy-2-fluorenyl)acetamide	11.53
2-Nitro-1-fluorenol	8.69
4-Nitro-1-fluorenol	8.46
7-Nitro-1-fluorenol	10.74
3-Nitro-2-fluorenol	8.62
7-Nitro-2-fluorenol[e]	10.76
2-Nitro-3-fluorenol	9.12
7-Nitro-3-fluorenol	11.16
2,4-Dinitro-3-fluorenol	5.88
2-Nitro-3-hydroxyfluorenone	5.42
7-Nitro-4-fluorenol	10.98

[a] All but one of the compounds in this Table were prepared in this laboratory (*W*). We are grateful to Drs. Miller (*M*) for supplying a sample of *N*-(*N*-hydroxy-2-fluorenyl)acetamide.[27] [b] pK in water is 9.51.[19] [c] A pK value of 11.76 was also found by the spectroscopic method. [d] As under *c*, pK 11.93. [e] pK in water is 8.94.[19]

An amino group in an *ortho* position to the phenolic hydroxy group facilitates the proton release as a result of hydrogen bonding, while in all other positions the amino substituent acts in the opposite fashion (Table II). Likewise, an acetamino residue serves to increase the acidity of a phenol by about 1 pK unit when in an *ortho* position, also because of hydrogen bonding, but shows little effect in other positions (Table V). This difference could conceivably be employed in separating the *ortho* isomers of *N*-(hydroxyfluorenyl)acetamide from

(25) V. P. Kreiter *et al.*[19] also observed values in a narrow range, 9.40–9.51, for 3- and 4-hydroxybiphenyl, and 2-hydroxyfluorene. Their measurements were performed in water. The acid-weakening effect encountered when shifting from water to 70% ethanol, as in our determinations, is more than 2 units.

(26) In contrast, the spectra of the four fluorenols are markedly dissimilar, indicating appreciable differences in the structures of those molecules in the excited state.

the others by means of partition in suitably buffered solvent systems. *N*-Hydroxy-2-fluorenylacetamide is weaker than the *ortho*-derivatives, but stronger than the other isomers. All of these compounds are produced during the metabolism of *N*-2-fluorenylacetamide in animals.[6,27]

The nitro group has a powerful acid-strengthening effect and appears to accentuate the minor differences among the phenolic derivatives of fluorene. Thus, even though 2- and 3-fluorenol have quite similar *p*K values, 3-nitro-2-fluorenol has an appreciably higher acidity than the isomeric 2-nitro-3-fluorenol; in the latter compound the 9-methylene in the *para* position again functions to lessen the acid strength. On the other hand, a keto group in the 9-position results in a very considerable increase in the acidity. For this reason 3-hydroxy-2-nitro-9-fluorenone with a *p*K of 5.42 is soluble in aqueous bicarbonate solution. Indeed, the keto group in the *para* position is more potent in causing a proton release from the hydroxy group than a second nitro group in the *ortho* position. 2,4-Dinitro-3-fluorenol has a constant of 5.88. In general, a nitro group in an *ortho* or *para* position (in the same ring) decreases the *p*K of the phenols by about 3 units. A much smaller effect is observed when the nitro group is situated in a different ring from the hydroxy function. The least interaction occurs from the 7- to the 3-position, in which case resonance makes only an unimportant contribution.

Carboxylic acids. Biphenylcarboxylic acids are more acidic than benzoic acid (Table VI). In the 4-isomer, the increase in *p*K is rather small (0.09 units) resting mainly on differences in inductive and resonance effects between the biphenyl and phenyl residues. In the 2-derivative the additional factors of steric hindrance and nonplanarity enter, resulting in a somewhat more acidic compound. These differences in *p*K values are minimized in 70% ethanol. Thus, in water the drop in *p*K from benzoic to 2-biphenylcarboxylic acid is 0.74 units whereas in 70% ethanol it amounted to only 0.41 units.

An acetamino residue at the 4-carbon, *meta* to the carboxy in 2-biphenylcarboxylic acid is acid-strengthening. On the other hand, at the 4'-position in the other ring it is weakening, but an amino group at the same position results in a slightly more potent acid, perhaps because of some zwitterion participation.

The ionization constant of 2-fluorenecarboxylic acid is close to that of benzoic acid. It is slightly higher than that of 4-biphenylcarboxylic acid in which the carboxy function is in the same position relative to the second phenyl ring. In the fluorene derivative the acid-strengthening effect of the phenyl group is counterbalanced by the inductive weakening action due to the 9-methylene radical in a *meta* position. This latter effect is even more pronounced in the *para* and the *ortho* position making the 3- and 1- acids appreciably weaker than the 2-compound. In the 1-isomer, hydrogen bonding from the hydrogens at the 9-position may also play a role, since without these influences the carboxy group at a *peri* position would be expected to be stronger. Thus, the 4-derivative, less exposed to such moderating factors, is the strongest of the four fluorenecarboxylic acids.

TABLE VI

IONIZATION CONSTANTS OF POLYNUCLEAR CARBOXYLIC ACIDS IN 70% ETHANOL SOLUTION

Compound	Source	pK_a'
Benzoic acid[a]	C	6.41
2-Biphenylcarboxylic acid[b]	W	6.00
4-Acetamino-2-biphenylcarboxylic acid	R	5.82
4'-Acetamino-2-biphenylcarboxylic acid	R	6.25
4'-Amino-2-biphenylcarboxylic acid	R	6.13
4-Biphenylcarboxylic acid	C	6.32
1-Fluorenecarboxylic acid	W	6.65
2-Fluorenecarboxylic acid	W	6.39
3-Fluorenecarboxylic acid	W	6.52
4-Fluorenecarboxylic acid	W	5.87
9-Oxo-1-fluorenecarboxylic acid	W	5.30
9-Oxo-2-fluorenecarboxylic acid	W	5.83
9-Oxo-3-fluorenecarboxylic acid	W	5.46
9-Oxo-4-fluorenecarboxylic acid	W	5.00
7-Nitro-1-fluorenecarboxylic acid	W	6.29
7-Nitro-2-fluorenecarboxylic acid	W	5.82
7-Nitro-3-fluorenecarboxylic acid	W	6.08
5-Nitro-4-fluorenecarboxylic acid	W	6.24
7-Nitro-4-fluorenecarboxylic acid	W	5.41

[a] *p*K in water and 50% ethanol is 4.20 (found in this study, 4.19) and 5.73,[5a] respectively; *cf.* also reference 17. [b] *p*K in water is 3.46.[5a]

Just as amines and phenols derived from fluorenone were stronger acids, the 9-keto function also facilitates proton release from the carboxylic acids. As might be predicted, the acid strength of a carboxy group increases most in the *ortho* position (1.35 units), somewhat less in the *para* position (1.06) and 0.87 units in the 4- and 0.56 units in the 2-position, both *meta* to the keto group.

Nitro groups in the ring not bearing the carboxy function likewise enhance the acidity, the effect being a fraction larger from the 7- to the 2- and 4-positions (some resonance contribution) than from the 7- to the 1- or 3-positions. Oddly, a nitro group at the 5-carbon weakens a carboxy at 4-, possibly because of steric considerations involving hydrogen bonding and attributable to interatomic distances of just the right order of magnitude.[22b,28] For like reasons, substituents at the 4-position of fluorene exhibit somewhat unusual properties,[29] and further physical-chemical investigations of such compounds are of considerable interest.

(27) J. H. Weisburger, E. K. Weisburger, P. H. Grantham, and H. P. Morris, *J. Biol. Chem.*, **234**, 2138 (1959); J. W. Cramer, J. A. Miller, and E. C. Miller, *J. Biol. Chem.*, **235**, 250 (1960); J. A. Miller, J. W. Cramer, and E. C. Miller, *Cancer Research*, **20**, 950 (1960).

CONCLUSIONS

In addition to the relevant points already discussed this section will briefly comment on some of the salient factors derived from this study, especially in respect to fluorene. The ionization constants, reflecting conditions in the molecule in the ground state, suggest that interactions between rings are considerably less than might be expected on the basis of the profound alterations, as a function of structural changes, in the ultraviolet (and visible) spectra, which serve as indicators of molecules in the excited state.[30]

The substituent groups vary greatly in their ability to alter the ionization constants. The following order, arranged in decreasing proton-releasing power was observed, over-all, in the series of fluorene compounds studied: $-NO_2$, $-9-SO_2$, $9-CO$, $9-SO$, $-NH_3^+$, $9=O$, $-F$, $-I$, $-Cl$, $-Br$, $-NHCOCH_3$, $-H$, $-OCH_3$, $-OH$, $-NH_2$. A substituent in an *ortho* position exhibits, generally, a considerably larger effect than the same group in another position. Substituents in the same ring, even in a *meta* relationship for the most part are more effective than when they are located in different rings.

The ionization constants of the isomeric 1-, 2-, 3-, and 4-fluorenamines, 3.57, 4.27, 4.39, and 3.15, respectively, bear no relation to the carcinogenicity of these compounds in rats. Thus, the 2-isomer is carcinogenic, the 1- and 3-derivatives are considerably weaker, and 4-fluorenamine is inactive, according to the presently available data.[31,cf. 6] This lack of a correlation may mean that (1) the compounds examined themselves are not the substances directly involved in eliciting the carcinogenic action, (2) the differences in ionization constants play no role in allowing the penetration of the compounds at the physiological *p*H into the cells where metabolism and tumorigenesis take place. The latter point is quite likely since at *p*H of 7.4 all of the above compounds would be almost completely nonionized. Hence it would appear that point (1) is also a true statement. Other studies[cf. 6,27] suggest the same conclusion, namely that metabolism of the amines is required to elicit the carcinogenic intermediate.

BETHESDA 14, MD.

(28) However, an excellent study by E. Berliner and E. H. Winicov, *J. Am. Chem. Soc.*, **81**, 1630 (1959), suggests that factors other than hydrogen bonding may play a role in the relatively weak acid character of the 5-nitrofluorene-4-carboxylic acid. They noted that 7-nitro-1-naphthoic acid and the 8-nitro-2-isomer were the weakest of the respective nitronaphthoic acids, even though the substituents were in conjugated positions (as they are in our fluorene derivative). In the two exceptional acids of Berliner and Winicov, intramolecular hydrogen bonding cannot be implicated.

(29) For example, whereas 4-fluorenamine diazotizes in the normal manner, the yields of compounds resulting from the replacement of the diazonium derivative by hydroxy or bromine are as a rule quite poor. Likewise, the 4-substituted fluorenes are usually the lowest melting of any of the isomeric derivatives.

(30) R. A. Friedel and M. Orchin, *Ultraviolet Spectra of Aromatic Compounds*, Wiley, New York, 1957; R. A. Friedel, *Appl. Spectroscopy*, **2**, 13 (1956).

(31) H. R. Schinz, H. Fritz-Niggli, T. W. Campbell, and H. Schmid, *Oncologia*, **8**, 233 (1955); H. P. Morris, C. A. Velat, B. P. Wagner, M. Dahlgard, and F. E. Ray, *J. Natl. Cancer Inst.*, **24**, 149 (1960).

[CONTRIBUTION FROM THE FOOD MACHINERY AND CHEMICAL CORPORATION
CHEMICAL RESEARCH AND DEVELOPMENT CENTER]

Reaction of (4,5), (8,9)-Diepoxytricyclo[5.2.1.0^{2,6}]decane with Hydrogen Bromide in Glacial Acetic Acid[1]

A. J. DURBETAKI

Received June 9, 1960

Dicyclopentadiene dioxide was shown to react with hydrogen bromide in glacial acetic acid to give 5-bromotricyclo-[5.2.1.0^{2,6}]decane-4,9-dihydroxy-8-acetate[2] instead of the corresponding dibromohydrin. The structure of the product was inferred from infrared spectra and the formation of corresponding derivatives.

A series of epoxy derivatives with the tricyclo[5.2.1.0.^{2,6}]decane skeletal structure were synthesized and the characteristic position of bands attributable to the oxirane oxygen functional groups were studied. No absorption peaks in the 11.8 μ region were observed for compounds that had no epoxy group on the bicyclo [2.2.1.] heptane ring. Likewise, bands in the 12 μ region were absent for compounds with no oxirane oxygen on the cyclopentane ring. According to these results, assignment of the 852 cm.$^{-1}$ and 834 cm.$^{-1}$ bands of dicyclopentadiene dioxide to the oxirane oxygen of the bicyclo-[2.2.1.]heptane and the cyclopentane ring respectively was made.

The synthesis of dicyclopentadiene dioxide was first reported by Wieland and Bergel.[3] Recent commercial availability of this compound has focused attention on the reactivity of the oxirane oxygen groups and its corresponding assay by means of hydrohalogenation methods.[4,5]

When most epoxides are treated with hydro-

(1) This was presented before the 136th meeting of the American Chemical Society in Atlantic City, N. J., September 13, 1959.