anol at 125–135° for 3 days, followed by evaporation to dryness and extraction with ether. Examination of the infrared spectrum of the residue (4.0 g.) showed it to be a mixture of **18** and the hydrochloride of 2-methyl-3-phenylquinoxaline. The latter was removed by further extraction with hot 2 *N* hydrochloric acid to give a solid residue which proved to be identical with compound **18** prepared as described above, as judged by a comparison of both infrared and ultraviolet spectra.

2-Methyl-3-phenylquinoxaline-1 N-Oxide (19).—A solution of 4.4 g. of 2-methyl-3-phenylquinoxaline in a mixture of 40 ml. of glacial acetic acid and 15 ml. of aqueous hydrogen peroxide (30%) was heated at 56° for 14 hr. The mixture was then concentrated to a small volume under reduced pressure; the residue was adjusted to pH 7 with 10% aqueous potassium hydroxide and extracted with chloroform. Evaporation of the chloroform extract gave an oil which upon extraction with 150 ml. of hot petroleum ether (60–70°) followed by cooling gave 2.2 g. (47%) of product, m.p. 96–100°. Two recrystallizations from methanol raised the melting point to 104–106°.

Anal. Calcd. for $C_{15}H_{12}N_2O$: C, 76.24; H, 5.12; N, 11.86. Found: C, 76.01; H, 5.33; N, 11.81.

The undissolved residue from the petroleum ether extraction was crystallized from ethanol and then from benzene to give yellow crystals of 2-methyl-3-phenylquinoxaline-1,4 di-N-oxide, m.p. 193–195°. This material is reported[12] to melt at 193–194°.

2-Chloromethyl-3-phenylquinoxaline (20).—A mixture of 5.0 g. of 2-methyl-3-phenylquinoxaline-1 N-oxide and 10 ml. of methanesulfonyl chloride was set aside at room temperature. Within 1 hr., the solid had dissolved, and after 24 hr. the solution was poured into a mixture of methylene chloride and water. The organic layer was separated, washed with an excess of aqueous sodium acetate solution, and dried over anhydrous sodium sulfate. Evaporation first under water pump pressure and then at 75° (1.5 mm.) (to remove any excess methanesulfonyl chloride) gave an oily residue which was dissolved in benzene and passed through a column of alumina. Elution with benzene gave 2.75 g. (51%) of 2-chloromethyl-3-phenylquinoxaline, m.p. 110–114°; continued elution with benzene and then with benzene–ether furnished an orange oil (not characterized) and then unchanged 1-N-oxide (0.8 g.). Recrystallization of the product from petroleum ether (60–70°) gave 2.3 g., m.p. 118–120°.

Anal. Calcd. for $C_{15}H_{11}N_2Cl$: C, 70.73; H, 4.35; N, 11.00. Found: C, 70.84; H, 4.25; N, 11.08.

Ethyl α-Carbethoxy-α-methyl-β-(3-phenyl-2-quinoxalyl)propanoate (21).—A mixture of 4.5 g. of 2-chloromethyl-3-phenylquinoxaline, 50 ml. of toluene, and diethyl sodiomethylmalonate (prepared from 3.48 g. of diethyl methylmalonate and 0.46 g. of sodium) was stirred and heated under reflux for 9 hr. and then cooled. Water was added and the organic layer was separated, washed with water, and dried over anhydrous sodium sulfate. After evaporation of solvent and excess diethyl methylmalonate *in vacuo*, the residue was dissolved in benzene and passed through a column of alumina. The initial eluent contained unchanged 2-chloromethyl-3-phenylquinoxaline, m.p. 114–117°; the middle fractions (1.5 g., m.p. 82–117°) contained a mixture of starting material and product; continued elution with benzene then gave 2.5 g. (39%) of the desired product, m.p. 98–99°. The analytical sample, m.p. 100–101°, was prepared by recrystallization from petroleum ether (30–60°), followed by recrystallization from aqueous methanol.

Anal. Calcd. for $C_{23}H_{24}N_2O_4$: C, 70.40; H, 6.19; N, 7.14. Found: C, 70.48; H, 6.11; N, 7.17.

α-Carboxy-α-methyl-β-(3-phenyl-2-quinoxalyl)propanoic Acid (22).—A mixture of 1.96 g. of the diethyl ester was heated under reflux with 10 ml. of 2 *N* sodium hydroxide and 10 ml. of ethanol for 3 hr.; the ethanol was then removed by evaporation under reduced pressure. The residual solution was treated with Norite and the filtrate acidified with hydrochloric acid. The precipitate which formed was collected by filtration, dried, and extracted with benzene to give the desired product; yield 1.33 g., m.p. *ca.* 130° dec. The analytical sample, m.p. *ca.* 120–122° dec., was prepared by recrystallization from aqueous ethanol. It retained some solvent of crystallization even after prolonged drying *in vacuo* at 56°.

Anal. Calcd. for $C_{19}H_{16}N_2O_4$: C, 67.84; H, 4.80; N, 8.33. Calcd. for $C_{19}H_{16}N_2O_4 \cdot C_2H_5OH$: C, 65.95; H, 5.81; N, 7.33. Found: C, 66.64, 66.45; H, 6.06, 5.93; N, 6.95, 6.75.

α-Methyl-β-(3-phenyl-2-quinoxalyl)propanoic Acid (23).—The dicarboxylic acid described above (1.33 g.) was heated at 135° for 1 hr. and the residue cooled and partitioned between chloroform and saturated sodium bicarbonate solution. Acidification of the aqueous phase gave 0.90 g. (78%) of the desired propanoic acid, m.p. 164–166°. The melting point was raised to 166–167° by recrystallization from aqueous methanol.

Anal. Calcd. for $C_{18}H_{16}N_2O_2$: C, 73.96; H, 5.52; N, 9.58. Found: C, 73.86; H, 5.51; N, 9.46.

2-Methyl-4-phenylpyrrolo(1,2-*a*)quinoxalin-1(5H)-one (14) by Cyclization of 23.—To a mixture of 30 ml. of acetic anhydride and 6 drops of concentrated sulfuric acid, preheated to 95° on a steam bath, was added 0.60 g. of α-methyl-β-(3-phenyl-2-quinoxalyl)propanoic acid (**23**). The resulting solution was maintained at 95° for 20 min. and then concentrated to a small volume under reduced pressure. Water was added to the residue and the pH adjusted to 5 with sodium carbonate. The precipitate which separated was collected by filtration and crystallized from aqueous ethanol (1:2) to give 0.19 g. of 2-methyl-4-phenylpyrrolo(1,2-*a*)quinoxalin-1(5H)-one, identical in every respect with an authentic sample prepared as described above by decarboxylation of the adduct formed from 2-methyl-3-phenylquinoxaline and maleic anhydride. The cyclodehydration product was further characterized by conversion with phosphorus oxychloride into 1-chloro-2-methyl-4-phenylpyrrolo-(1,2-*a*)quinoxaline, m.p. and mixture m.p. 158–159°.

Dilution of the aqueous ethanolic mother liquor gave 0.2 g. of solid which, by examination of its infrared spectrum, proved to contain unchanged starting material. This was treated with acetic anhydride–sulfuric acid as described above to give an additional 0.11 g. of product; total yield 0.30 g. (53%).

(12) J. K. Landquist and G. J. Stacey, *J. Chem. Soc.*, 2822 (1953).

[CONTRIBUTION FROM THE EVANS CHEMISTRY LABORATORY OF THE OHIO STATE UNIVERSITY, COLUMBUS 10, OHIO]

The Synthesis and Ionization Constants of the Six Aminobenzo[c]phenanthrenes[1]

BY MELVIN S. NEWMAN AND JOCHANAN BLUM[2]

RECEIVED NOVEMBER 15, 1963

2-, 3-, 4-, 5-, and 6-aminobenzo[c]phenanthrene were made by Curtius rearrangement of the corresponding acid azides, followed by alkaline hydrolysis of the resulting isocyanates. 1- and 2-aminobenzo[c]phenanthrene were made by Bucherer reactions from the corresponding hydroxy compounds. The ionization constants of these amines were determined. The ultraviolet absorption spectra of the amines and their hydrochlorides were determined. 3-Methyl- and 4-methylbenzo[c]phenanthrene were made by new routes.

The interest in preparing the six isomeric monosubstituted derivatives of benzo[c]phenanthrene (I) has been discussed previously.[3] The ionization constants of the six carboxybenzo[c]phenanthrenes[4] and of the six hydroxybenzo[c]phenanthrenes[3] have been

(1) This research was supported by a grant from the National Institutes of Health, Bethesda, Md.

(2) Postdoctoral Fellow, 1963.

(3) See discussion: (a) in M. S. Newman and J. Blum, *J. Am. Chem. Soc.*, **86**, 503 (1964); (b) M. S. Newman and D. K. Phillips, *ibid.*, **81**, 3667 (1959).

(4) M. S. Newman and H. Boden, *ibid.*, **83**, 115 (1961).

TABLE I

IONIZATION CONSTANTS OF AMINOBENZO[c]PHENANTHRENES IN 50% ETHANOL AT 26 ± 1°

Isomer	pK_a[a]	Isomer	pK_a[a]
1-Amino	2.78	4-Amino	3.32
2-Amino	3.66	5-Amino	3.10
3-Amino	3.56	6-Amino	3.22

[a] These values are the average of four determinations at wave lengths between 320 and 345 mμ. The maximum deviation was 0.03 unit.

hydrolyze to yield the corresponding hydroxy compounds. However, an attempt to hydrolyze 3-aminobenzo[c]phenanthrene by boiling for 6 hr. in strongly alkaline aqueous dioxane failed to yield any trace of phenol and the amino compound was recovered in almost quantitative yield. We conclude that no imino form is present.

The ionization constants of the six aminobenzo[c]phenanthrenes are listed in Table I. The most notable feature is the weakness of the 1-amino compound. This fact may be attributed to steric hindrance to solvation of the protonated form, just as the weakness of 1-hydroxybenzo[c]phenanthrene as an acid was attributed to steric hindrance to solvation of the anion.[3]

TABLE II

ULTRAVIOLET SPECTRA OF AMINOBENZO[c]PHENANTHRENES AND THEIR HYDROCHLORIDES

Amines[a]	λ_{max}, mμ (log ε)													
1-	213						271.5	287			318	353s	371	391s
	(4.64)						(4.52)	(4.40)			(4.04)	(3.52)	(3.46)	(3.32)
2-	220.5		237	248s	256			280	292		317s		372s	388
	(4.44)		(4.48)	(4.48)	(4.51)			(4.52)	(4.51)		(4.19)		(3.32)	(3.40)
3-	221.5				257s			287			325	337s		384s
	(4.69)				(4.30)			(4.76)			(3.92)	(3.88)		(3.27)
4-	209.5	225.5				264.5			298			348	368s	385s
	(4.64)	(4.51)				(4.56)			(4.53)			(3.77)	(3.70)	(3.48)
5-	218		232s	248s		262.5s			292.5			346		388s
	(4.53)		(4.35)	(4.14)		(4.27)			(4.71)			(3.97)		(3.28)
6-	222		240	247.5	256		274s	283.5	292s		321s		373s	395
	(4.55)		(4.40)	(4.40)	(4.44)		(4.43)	(4.48)	(4.47)		(4.15)		(3.23)	(3.35)
Amine hydrochlorides[b]														
1-	220		231s	247s	256s	267s	276.5	286	297.5s	307.5	321	333s	362	383.5
	(4.64)		(4.25)	(4.02)	(4.15)	(4.39)	(4.70)	(4.83)	(4.20)	(4.02)	(3.99)	(3.67)	(2.75)	(2.86)
2-	218	223s	230.5	244s	254s	264s	273	283	296s	303	316.5	326.5s	354.5	373
	(4.67)	(4.50)	(4.41)	(3.99)	(4.15)	(4.46)	(4.72)	(4.88)	(4.18)	(4.06)	(4.01)	(3.75)	(2.76)	(2.59)
3-	218	223s	229	245s	254s	264s	273	282.5	295s	303	315.5	327s	355	372.5
	(4.61)	(4.47)	(4.32)	(3.91)	(4.12)	(4.39)	(4.71)	(4.86)	(4.11)	(4.05)	(4.09)	(3.59)	(2.55)	(2.31)
4-	219	223s	230	245s	254s	265s	274.5	284.5	296.5s	304.5s	317.5	328s	355	374.5
	(4.65)	(4.53)	(4.39)	(4.05)	(4.16)	(4.41)	(4.75)	(4.88)	(4.25)	(4.11)	(4.04)	(3.77)	(2.68)	(2.46)
5-	218	223.5s	230	245s	254s	264s	273	283	296.5	304	316.5	328s	356.5	374.5
	(4.67)	(4.50)	(4.38)	(3.91)	(4.10)	(4.46)	(4.75)	(4.90)	(4.20)	(4.10)	(4.08)	(3.75)	(2.78)	(2.45)
6-	218.5	223s	229	245s	254s	265s	273	282.5	296.5	303s	316	327s	358	376.5
	(4.64)	(4.50)	(4.32)	(3.97)	(4.21)	(4.53)	(4.47)	(4.84)	(4.20)	(4.11)	(4.03)	(3.72)	(2.67)	(2.39)

[a] In 95% ethanol. [b] In 2 *N* HCl in 80% ethanol.

measured. In this paper, the synthesis and measurement of the basic ionization constants of the six aminobenzo[c]phenanthrenes are described.

The 2-, 3-, 4-, 5-, and 6-aminobenzo[c]phenanthrenes were prepared by Curtius rearrangements of the corresponding acyl azides followed by hydrolysis of the isocyanates. The 1-amino compound was not prepared by this route as treatment of 1-carboxybenzo[c]phenanthrene with thionyl chloride produced 1,8,9-naphthanthr-10-one, identical with a sample prepared by the oxidation of 1-methylbenzo[c]phenanthrene.[4]

1-Aminobenzo[c]phenanthrene could be prepared in 87% yield by the Bucherer reaction[5] from 1-hydroxybenzo[c]phenanthrene.[3] Since 2-hydroxybenzo[c]phenanthrene could also be converted into the 2-amino compound by the Bucherer reaction in 92% yield, this route would probably have worked for the other isomers.

In the case of the hydroxybenzo[c]phenanthrenes, the possibility of their existence as ketonic tautomers was considered because of the steric effects involved. However, no evidence of such tautomers was found.[3a] The ready conversion of the two hydroxybenzo[c]phenanthrenes to the amino compounds above described may involve the ketonic tautomers as these are postulated intermediates in the Bucherer reaction.[5] With this in mind, we reasoned that if the aminobenzo[c]phenanthrenes could exist in tautomeric equilibrium with the imino form, the latter should readily

(5) "Organic Reactions," Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 105.

The ionization constants of the 2- and 3-amino compounds are comparable to those for 2- and 3-aminophenanthrene[6] (pK_a = 3.60 and 3.59, respectively, at 20°) and the ionization constants of 4-, 5-, and 6-aminobenzo[c]phenanthrenes lie in the region of 1- and 9-aminophenanthrene[6] (3.23 and 3.19, respectively) as might be expected since these amines are on carbons adjacent to a ring fusion and are hence subject to about the same steric effect.

The ultraviolet spectra of the six aminobenzo[c]phenanthrenes and their hydrochlorides are recorded in Table II. The hydrochlorides of the six amino compounds have spectra almost identical with those of the corresponding methyl compounds[7] in alcohol.

Additional quantities of 3-methyl- and 4-methylbenzo[c]phenanthrene were needed for oxidation to the corresponding carboxy derivatives.[4] In the Experimental part, new syntheses of these methyl compounds are described.

(6) J. J. Elliott and S. F. Mason, *J. Chem. Soc.*, 2352 (1959). In an article which appeared while in press, P. H. Gore and A. N. Lubiensky, *ibid.*, 6056 (1963), report pK_a for the five aminophenanthrenes and the two aminotriphenylenes. Their value for 4-aminophenanthrene, 3.30, is higher than we would expect in view of a value of 2.75 for 1-aminotriphenylene.

(7) G. M. Badger and I. S. Walker, *ibid.*, 3238 (1954); see also spectra recorded in A.P.I. Research Project 44, 1955, pp. 597–602, by Shell Development Co. workers.

Experimental[8]

The synthesis of 3-methylbenzo[c]phenanthrene followed the routes previously developed.[9]

Diethyl *p*-Methylbenzhydrylmalonate.—To a well-stirred solution of 85 g. of diethyl benzalmalonate[10] in 150 ml. of dry benzene containing 1.5 g. of cuprous bromide was added the Grignard reagent prepared from 57 g. of *p*-bromotoluene in 150 ml. of ether. The temperature was held at 0–10° for 30 min. during the addition and the mixture was then left overnight at room temperature. After the usual work-up there was obtained 102 g. (90%) of an almost colorless viscous oil, b.p. 178–180° at 1.5 mm., which solidified on standing in the cold to a solid,[11] m.p. 48.5–49.7°. When the reaction was carried out at 20° in the absence of cuprous bromide, the yield was 46%.

2-(*p*-Methylbenzhydryl)-1,3-propanediol.—Reduction of the above malonate with lithium aluminum hydride in ether–benzene for 2 hr. essentially as described[9a] for another case afforded the pure diol, m.p. 87–88°, as colorless small prisms on crystallization from cyclohexane in 88% yield.

Anal. Calcd. for $C_{17}H_{20}O_2$: C, 79.7; H, 7.8. Found[m]: C, 79.5; H, 7.8.

2-(*p*-Methylbenzhydryl)-1,3-propanediol Bismethanesulfonate.—The above diol was converted into the bismethanesulfonate, m.p. 101–102°, when recrystallized from alcohol, in 97% yield essentially as described for another case.[9b]

Anal. Calcd. for $C_{19}H_{24}O_6S_2$: C, 55.3; H, 5.8; S, 15.5. Found[m]: C, 55.4; H, 6.0; S, 15.7.

3-(*p*-Methylbenzhydryl)-glutaronitrile.—The above bismethanesulfonate was converted into the glutaronitrile, m.p. 137–138°, when recrystallized from benzene–Skellysolve B (petroleum ether, b.p. 65–70°) in 89% yield essentially as described.[9b]

Anal. Calcd. for $C_{19}H_{18}N_2$: C, 83.2; H, 6.6; N, 10.2. Found[m]: C, 83.1; H, 6.5; N, 10.0.

3-(*p*-Methylbenzhydryl)-glutaric Acid.—Hydrolysis of the above dinitrile essentially as described[9b] afforded the glutaric acid in 81% yield as colorless finely divided crystals, m.p. 161–162°, on crystallization from benzene.

Anal. Calcd. for $C_{19}H_{20}O_4$: C, 73.1; H, 6.4. Found[m]: C, 73.3; H, 6.5.

5,6,6a,7,8,12b-Hexahydro-3-methylbenzo[c]phenanthrene-5,8-dione.—To 400 g. of stirred polyphosphoric acid[12] at 140° was added 20.0 g. of finely powdered glutaric acid (see previous paragraph). After 75 min. at 140° the mixture was poured into 2 l. of water and the product isolated in the usual way. On recrystallization from benzene–cyclohexane using decolorizing charcoal there was obtained 11.5 g. (66%) of pale yellow diketone, m.p. 201–202° (infrared absorption at 5.95 μ, 1680 cm.$^{-1}$).

Anal. Calcd. for $C_{19}H_{16}O_2$: C, 82.6; H, 5.8. Found[m]: C, 82.5; H, 5.9.

The yield of diketone was essentially the same in other runs in which the temperature was varied between 120 and 160°.

5,6,6a,7,8,12b-Hexahydro-3-methylbenzo[c]phenanthrene-5,8-diol.—A solution of 0.5 g. of sodium borohydride and 0.2 g. of sodium hydroxide in 50 ml. of methanol was added to a suspension of 8.0 g. of the above dione in 100 ml. of methanol. After stirring for 5 min. a clear yellow solution was obtained. After 2 hr. the mixture was just acidified with hydrochloric acid and most of the methanol removed under reduced pressure. Dilution with water yielded the crystalline diol, which, after crystallization from benzene–Skellysolve B, was obtained in 96% yield as a light tan powder, m.p. 96–99°. In different runs, products which melted as high as 118° were obtained. However, for further work no attempt to isolate a pure stereoisomer was made. The mixture, m.p. 96–99°, was analyzed.

Anal. Calcd. for $C_{19}H_{20}O_2$: C, 81.4; H, 7.1. Found[m]: C, 81.4; H, 7.6.

3-Methylbenzo[c]phenanthrene.—In the best of several experiments, 1.00 g. of the above diol was refluxed in 8 ml. of xylene in a flask equipped with a phase-separating head. On addition of 5 mg. of iodine, dehydration started. After the color of the solution faded one more small crystal of iodine was added. This procedure[9b] was repeated during 7 days, a total of 50 mg. of iodine being used in all. The organic material was then isolated in the usual manner and was heated at 240–250° for 30 min. with 0.15 g. of sulfur. A small amount of zinc dust was added and heating at 180° continued for 10 min. The product was then isolated after chromatography over alumina using benzene–Skellysolve B mixtures for elution. The crude product was recrystallized from ethanol to yield 0.65 g. (75%) of 3-methylbenzo[c]phenanthrene, m.p. 68.9–69.5°. This is a polymorphic form of this hydrocarbon, the previously listed[13] m.p. being near 54°. The picrate, m.p. 134–135°, had the same m.p. as previously listed.[13] On seeding a sample of the lower melting polymorph with the higher, the m.p. rose to that of the higher form.

When working on a larger scale, the crude hydrocarbon was distilled at 224–225° at 1 mm. The yield was the same as that obtained in the small scale run.

4-Methylbenzo[c]phenanthrene.—A hot solution of 7.0 g. of 4-keto-1,2,3,4-tetrahydrobenzo[c]phenanthrene[3a] in 100 ml. of benzene was added to excess methylmagnesium iodide in ether. After refluxing for 3 hr. the mixture was worked up as usual to yield an oil which was heated at 310–320° under nitrogen with 2.0 g. of 5% palladium-on-charcoal (Engelhard Industries product) for 5 hr. After chromatography over alumina and crystallization from alcohol there was isolated 5.85 g. (85%) of 4-methylbenzo[c]phenanthrene,[13] m.p. 64.6–65.6°.

2-, 3-, and 4-Carboxybenzo[c]phenanthrenes.—These acids were prepared by oxidation of the corresponding methyl derivatives as described.[14] Increasing the reaction time from 65 to 84 hr. increased the yield of the 3-carboxy compound from 59 to 89%, but did not change the yields of the 2- (64 to 70%) and 4-isomers appreciably.

5- and 6-carboxybenzo[c]phenanthrenes were made essentially as described from β-(1-bromo-2-naphthyl)-α-phenylacrylic acid and α-(1-bromo-2-naphthyl)-β-phenylacrylic acid. Improved yields of these two acids were obtained by the use of potassium, in place of sodium, salts. For example, a mixture of 53 g. of benzaldehyde, 89 g. of 1-bromo-2-naphthylacetic acid, 75 g. of dry potassium carbonate, and 150 g. of acetic anhydride was stirred at reflux for 3 hr. in a 2-l. flask (because of the copious foaming). The resulting clear brown solution was worked up to yield 106 g. (89%) of α-(1-bromo-2-naphthyl)-β-phenylacrylic acid,[15] m.p. 205–206°.

The condensation of 1-bromo-2-naphthaldehyde with potassium phenylacetate afforded β-(1-bromo-2-naphthyl)-α-phenylacrylic acid in 82% yield.[16]

1- and 2-Aminobenzo[c]phenanthrenes.—In a steel pressure vessel were placed 1.5 g. of 1-hydroxybenzo[c]phenanthrene,[3a] 15 ml. of pure dioxane, and a solution prepared by passing 30 g. of sulfur dioxide into 150 ml. of 28% ammonium hydroxide. The mixture was heated at 180° for 16 hr. The organic product was then isolated as usual; the crude product was dissolved in 15 ml. of boiling alcohol and treated with 10 ml. of concentrated hydrochloric acid. On cooling, the amine hydrochloride was collected by filtration and washed with benzene. A solution of the amine hydrochloride in boiling alcohol was decolorized with charcoal, and the colorless filtrate treated with aqueous sodium carbonate to yield 1-aminobenzo[c]phenanthrene in 87% yield as slightly yellow-green crystals, m.p. 169–170°, after crystallization from benzene.

In a similar way 2-hydroxybenzo[c]phenanthrene was converted into 2-aminobenzo[c]phenanthrene, m.p. 67–68°, in 92% yield.

2-, 3-, 4-, 5-, and 6-Aminobenzo[c]phenanthrenes.—In a typical reaction 5.0 g. of a carboxybenzo[c]phenanthrene was heated

(8) All melting points are uncorrected. The term "worked up in the usual manner" means that a solution of the products in an organic solvent, usually ether–benzene, was washed with acid and/or alkali as required and saturated sodium chloride solution, and was then dried by shaking with, or filtering through, anhydrous magnesium sulfate. The solvents were then removed and the residue purified by crystallization, distillation, or chromatography.

Analyses marked [b] by A. Bernhardt Laboratories, Mülheim, Germany; [g] by Galbraith Laboratories, Knoxville, Tenn.; and [m] by Microanalysis Laboratories, Wilmington, Del.

(9) (a) M. S. Newman and M. Wolf, *J. Am. Chem. Soc.*, **74**, 3225 (1952); (b) M. S. Newman and R. M. Wise, *ibid.*, **78**, 450 (1956).

(10) C. F. H. Allen and F. W. Spangler, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 377.

(11) The same compound has been prepared in 23% yield by G. A. Holmberg, *Acta Acad. Aboensis Math. Phys.*, **16**, No. 6 (1948); *Chem. Abstr.*, **45**, 558c (1951), who reports m.p. 48–49°.

(12) We thank the Victor Chemical Co., Chicago, Ill., for a generous gift of polyphosphoric acid.

(13) C. L. Hewett, *J. Chem. Soc.*, 1286 (1938), gives 54.0–54.5°; M. S. Newman, H. V. Anderson, and K. H. Takemura, *J. Am. Chem. Soc.*, **75**, 347 (1953), give 54.4–55.4°.

(14) M. S. Newman and H. Boden, *J. Org. Chem.*, **26**, 1759 (1961).

(15) Compare ref. 17 (54% yield) when sodium carbonate was used.

(16) C. L. Hewett, *J. Chem. Soc.*, 293 (1940), obtained a 72% yield using sodium phenylacetate.

with 50 ml. of pure thionyl chloride at reflux for 3 hr. The excess reagent was removed under reduced pressure and the residue was rinsed with low boiling petroleum ether (Skellysolve F). The crude acid chlorides thus obtained (see Table III) were then dissolved in from 200 to 600 ml. of pure acetone, as determined by solubility, and cooled rapidly to about 5°. Before crystallization occurred a solution of 5.0 g. of sodium azide in 25 ml. of cold water was added at once and the mixture was shaken violently by hand, with occasional cooling, for about 20 min. Then 1 l. of water was added and the mixture left to stand until the organic precipitate had completely solidified (usually 15–20 min.). The crude azide was collected, dried, and recrystallized from cyclohexane or benzene–cyclohexane, keeping the temperature below 65°. The properties of the azides are listed in Table III.

TABLE III

BENZO[c]PHENANTHRENECARBOXYLIC ACID CHLORIDES AND AZIDES

Isomer	M.p.,[a] °C.	Dec. pt.,[b] °C.	Yield, %	Anal.,[c] % C	H	N[f]
2	123	89	92	76.4	3.8	14.0[g]
3	120	116	95	76.8	3.7	14.0[m]
4	141	130	87	76.8	3.7	13.9[m]
5	108[d]	81[e]	96	76.6	3.9	14.0[g]
6	124	93	97	76.5	3.5	14.2[g]

[a] Approximate m.p. of crude acid chloride. [b] Approximate m.p. of acid azide—all decomposed rapidly at the m.p. [c] Calcd. for $C_{19}H_{11}N_3O$: C, 76.7; H, 3.7; N, 14.1. [d] J. L. Everett and C. L. Hewett, *J. Chem. Soc.*, 1159 (1940), report m.p. 110–111° for this acid chloride. [e] M. S. Newman and A. I. Kosak[17] reported m.p. 69–72° dec. for this azide. [f] The superscripts g and m have the same meaning as in footnote 8.

In a typical run a solution of 4.0 g. of a dried azide in 50 ml. of *p*-cymene was rapidly heated (with stirring) to a temperature about 20 to 30° above its initial decomposition point (see Table III). The temperature was maintained until all gas evolution ceased (5 to 30 min.). A prepared mixture of 2.5 g. of barium hydroxide, 2.5 g. of powdered potassium hydroxide, and 1 ml. of water was added and the mixture vigorously stirred at 190–200° for 30 min. After cooling somewhat (to about 120°), 100 ml. of water was added carefully. The cooled mixture was then filtered and the organic matter was taken into benzene and worked up as usual (without ether). The crude material thus obtained was distilled under vacuum to yield orange colored distillates. These were triturated with and crystallized from benzene to yield crystalline amines (see Table IV) which are sensitive to air and should be protected by working under nitrogen as much as possible. The amines were pale yellow colored solids. The crude 2-amino compound was difficult to purify and hence was converted into the hydrochloride (see above) for purification. The hydrochlorides of the other amines were prepared by treating alcoholic solutions of the amines with excess concentrated hydrochloric acid, followed by recrystallization from alcohol or alcohol–ether. The yields of hydrochlorides were almost quantitative (see Table IV).

The six amino compounds were converted into the corresponding acetylamino derivatives in almost quantitative yields by heating with acetic anhydride in benzene for short times. These derivatives (see Table V) were recrystallized from benzene or xylene. The amines were also characterized as the 2,4,7-trinitrofluorenone (TNF) derivatives[18] (see Table V) formed in benzene and recrystallized from benzene or xylene.

Interestingly, we were unable to prepare a TNF derivative of 1-acetylaminobenzo[c]phenanthrene although deep red TNF derivatives of all of the other acetylamino compounds were formed readily. Concentrated solutions of TNF and the 1-acetylamino compound gave red coloration in several solvents but no red complex was obtained as either TNF, the 1-acetylamino compound, or both separated on standing.

Ionization Constant Determination.—The ionization constants of the six aminobenzo[c]phenanthrenes were determined in much

(17) See M. S. Newman and A. I. Kosak, *J. Org. Chem.*, **14**, 375 (1949), for a previous preparation of the 5-amino compound (not obtained as a solid) and other derivatives of it.

(18) M. Orchin and E. O. Woolfolk, *J. Am. Chem. Soc.*, **68**, 1727 (1946).

TABLE IV

AMINOBENZO[c]PHENANTHRENES AND HYDROCHLORIDES

Isomer	M.p.,[a] °C.	Yield,[b] %	Anal.[c] C	H	N	Cl
1	169–170	87[d]	88.8	5.4	5.9[b]	
	200		77.0	5.2	5.1	12.9[b]
2	67–68	73 (92[d])	88.6	5.4	5.7[g]	
	220		77.2	5.0	5.0	12.6[g]
3	104–105	57	88.7	5.4	5.8[m]	
	230		77.1	4.8	4.9	12.5[g]
4	101–102	61	89.0	5.4	5.5[g]	
	212		77.1	5.1	5.2	12.7[b]
5[17]	90–91	83	88.8	5.4	5.9[b]	
	220		77.4	4.8	5.1	12.7[b]
6	121–122	76	89.0	5.4	5.6[g]	
	241		77.0	5.2	4.8	12.6[g]

[a] The first m.p. is that of the amine, the second the approximate m.p. (dec.) of the hydrochloride. [b] The yields refer to the over-all yield of reasonably pure amine from acid chloride, except for those marked [d]. [c] The first analysis is for the amines. Calcd. for $C_{18}H_{13}N$: C, 88.8; H, 5.4; N, 5.8. The second analysis is for the amine hydrochloride. Calcd. for $C_{18}H_{14}ClN$: C, 77.3; H, 5.0; N, 5.0; Cl, 12.7. The superscripts b, g, m on the analysis are explained in footnote 8. [d] These percentages refer to the yield of amine from the hydroxy compound by the Bucherer reaction.

TABLE V

TNF AND ACETYL AMINOBENZO[c]PHENANTHRENES

Isomer	M.p.,[a] °C.	Anal.[b] C	H	N
1	234–235	84.1	5.1	5.0[b]
	180–181	66.7	3.4	10.2[b]
2	168–169	83.9	5.2	4.7[g]
	208–209	66.7	3.4	10.2[b]
	198–199	66.1	3.6	9.2[b]
3	206–207	84.0	5.3	5.1[m]
	191–192	66.8	3.2	9.7[m]
	194–195	65.8	3.6	9.0[m]
4	196–197	84.0	5.4	4.8[g]
	193–194	66.9	3.3	10.2[b]
	193–194	65.9	3.4	9.5[b]
5[17]	216–217	84.1	5.4	5.0[b]
	195–196	66.5	3.4	10.1[b]
	212–213	66.0	3.3	9.4[b]
6	214–215	84.2	5.4	4.8[g]
	208–209	66.6	3.3	9.8[g]
	191–193	65.7	3.5	9.1[g]

[a] The first melting point is that of the acetyl derivative, the second, that of the TNF derivative, and the third, that of the acetylamino compound. The calcd. analyses are as follows. For the acetylaminobenzo[c]phenanthrenes: Calcd. for $C_{20}H_{15}NO$: C, 84.2; H, 5.3; N, 4.9. For the TNF derivatives of the aminobenzo[c]phenanthrenes: Calcd. for $C_{31}H_{18}N_4O_7$: C, 66.7; H, 3.2; N, 10.0. For the TNF derivatives of the acetylaminobenzo[c]phenanthrenes: Calcd. for $C_{33}H_{20}N_4O_8$: C, 66.0; H, 3.3; N, 9.3. [b] The superscripts b, g, m are referred to in footnote 8.

the same way as used for the hydroxybenzo[c]phenanthrenes.[3a] The following solutions were prepared: (a) 6.0 to 8.0 mg. of the free amines was dissolved in 50 ml. of 50% by weight ethanol–water, (b) 5 ml. of solution (a) was diluted to 50 ml. with 0.1 *N* perchloric acid, (c) 5 ml. of solution (a) was diluted to 50 ml. with 0.1 *N* sodium hydroxide, (d, e) 5 ml. of solution (a) was diluted to 50 ml. with a suitable buffer solution. The absorbancies of solutions (b), (c), (d), and (e) were measured on a Beckman Model DU spectrophotometer. The pH values of the buffered solutions (d) and (e) were measured on a Beckman pH meter, Model N, before and after the absorbancy readings and were found to be constant to ±0.01 pH unit (pH 3.55 for (d) and pH 2.86 for solution (e)). Application of the equation

$$pK_a = pH + [(A_B - A_{NaOH})/(A_{HClO_4} - A_B)]$$

where pH is the value obtained from the buffered solution and

A_B, A_{NaOH}, A_{HClO_4} are the absorbancies of the buffer solution in question and of solutions (b) and (c), respectively, at a chosen wave length.

The measure of the pK_a for each amine was carried out at four wave lengths in the 320–345 mμ region. The values agreed to ±0.03 unit. The average values are listed in Table I.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

The Use of Esters of N-Hydroxysuccinimide in Peptide Synthesis[1]

BY GEORGE W. ANDERSON, JOAN E. ZIMMERMAN, AND FRANCIS M. CALLAHAN

RECEIVED DECEMBER 7, 1963

A number of N-hydroxysuccinimide esters of acylamino acids have been synthesized. These compounds are crystalline solids which react readily with amino acids or peptides or their esters. Peptide formation under aqueous conditions goes well. Because of the water solubility of N-hydroxysuccinimide, these esters appear to be more generally useful than the analogous esters of N-hydroxyphthalimide.

In recent years, *p*-nitrophenyl esters of acylamino acids have been of great value in the synthesis of peptide derivatives.[2] Other activated esters such as cyanomethyl esters[3] have been promising, and most recently esters of N-hydroxyphthalimide[4a,b] have been shown to be valuable intermediates. The latter are particularly interesting because of their high reactivity, and in addition they are readily crystallized. It seemed worthwhile to us to explore the field of hydroxylamine compounds for derivatives with still better properties. We are particularly interested in derivatives which give readily removable by-products; in the field of simple hydroxylamine derivatives, water solubility appeared to be an attractive possibility. In contrast to N-hydroxyphthalimide, N-hydroxysuccinimide[5] is water soluble, and we have found that acylamino acid esters of this compound meet the important requirements of crystallinity and high reactivity. Consequently we have undertaken an extensive investigation of these derivatives.

N-Hydroxysuccinimide has previously been best prepared *via* the O-benzyl derivative[6] or by fusion of succinic anhydride with hydroxylamine.[7] We have employed a simplification of the latter procedure using hydroxylamine hydrochloride to give a 44% yield of pure N-hydroxysuccinimide, with a melting point of 99–100° (see Experimental, example 1).

The dicyclohexylcarbodiimide method of ester synthesis, which has been used for the synthesis of esters of N-hydroxyphthalimide[4] and *p*-nitrophenyl esters,[8,9] has been used for the synthesis of the N-hydroxysuccinimide esters of a number of N-protected amino acids. These are colorless crystalline derivatives with good stability (Tables I and II, Experimental examples 2–5). Exposure to the atmosphere for weeks or months resulted in slight lowering of the melting points in some cases, but storage of the pure compounds in a desiccator gave no change in melting points in 2 months.

(1) Preliminary communication: *J. Am. Chem. Soc.*, **85**, 3039 (1963).
(2) M. Bodanszky, *Ann. N. Y. Acad. Sci.*, **88**, 655 (1960).
(3) R. Schwyzer, M. Feurer, and B. Iselin, *Helv. Chim. Acta*, **38**, 84 (1955).
(4) (a) G. H. L. Nefkens and G. I. Tesser, *J. Am. Chem. Soc.*, **83**, 1263 (1961); (b) G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. trav. chim.*, **81**, 683 (1962).
(5) "Beilstein," Vol. 21, 4th Ed., 1935, p. 380.
(6) D. E. Ames and T. F. Grey, *J. Chem. Soc.*, 631 (1955).
(7) R. Wegler, F. Grewe, and K. Mehlhose, U. S. Patent 2,816,111 (1957).
(8) D. F. Elliot and D. W. Russell, *Biochem. J.*, **66**, 49P (1957).
(9) M. Rothe and F. W. Kunitz, *Ann. Chem.*, **609**, 88 (1957).

Acylpeptide esters were conveniently prepared by the reaction of N-hydroxysuccinimide derivatives with equivalent amounts of amino acid or peptide esters in organic solvents at room temperature. Usually a 40-min. reaction time was used (a longer period may improve yields in some cases) and the peptide product was precipitated by the addition of water. Examples 6 and 7 (Experimental), illustrate the procedure used.

It was of interest to try reactions under aqueous conditions, since older methods of peptide synthesis usually give poorer yields in the presence of water. Illustrative results are given in examples 8–14. Several experiments in the synthesis of Z·gly-L-try·OH (example 11) showed that the use of 2 equivalents of sodium bicarbonate is desirable. Examples 8B and 9B indicate that the analogous use of esters of N-hydroxyphthalimide gave poor results largely because of the water insolubility of N-hydroxyphthalimide.

The results with the aqueous reactions are promising for the development of conditions for the lengthening of peptide chains by the reaction of esters of N-hydroxysuccinimide with salts of peptides or proteins. Further work in this direction is projected.

Experimental

Melting points were determined on a calibrated Fisher-Johns block.

1. N-Hydroxysuccinimide.—Succinic anhydride (100 g., 1.0 mole) and hydroxylamine hydrochloride (70 g., 1.0 mole) were combined in a 1-l. flask. The flask was placed on a rotary evaporator and the contents heated by a silicone oil bath. The volatile products which formed were removed under vacuum provided by a water aspirator and, after passage through a cold water condenser, were caught in a Dry Ice trap. The contents of the flask were rapidly heated to about 125° where fusion occurred with evolution of gases. Over the next hour the temperature was increased slowly up to 160°, at which point the formation of water had virtually ceased. The heating was discontinued. When the temperature had dropped to 125°, the amber liquid was poured into a beaker containing 400 ml. of ether which was vigorously stirred. The ether layer was decanted after the product solidified and the residue heated to boiling with 400 ml. of dry 1-butanol. The mixture was filtered and the filtrate rapidly chilled to 0°. After a 1-hr. period, the crystalline material was collected by filtration and the residue was washed carefully with 1-butanol, then ether. The crude product, melting at 93 to 95°, amounted to 75 g. and had a light tan color. This material was treated briefly with 450 ml. of hot ethyl acetate (6 ml./g.) and the mixture was filtered. Cooling and chilling of the filtrate yielded 46 g. of white crystalline material, m.p. 99–100°. The material which had not dissolved in ethyl acetate was retreated with an additional 75 ml. of hot ethyl ace-