[CONTRIBUTION FROM THE MCARDLE MEMORIAL LABORATORY, THE MEDICAL SCHOOL, UNIVERSITY OF WISCONSIN]

Synthesis of Nitro and Amino Derivatives of Benzo[c]cinnoline

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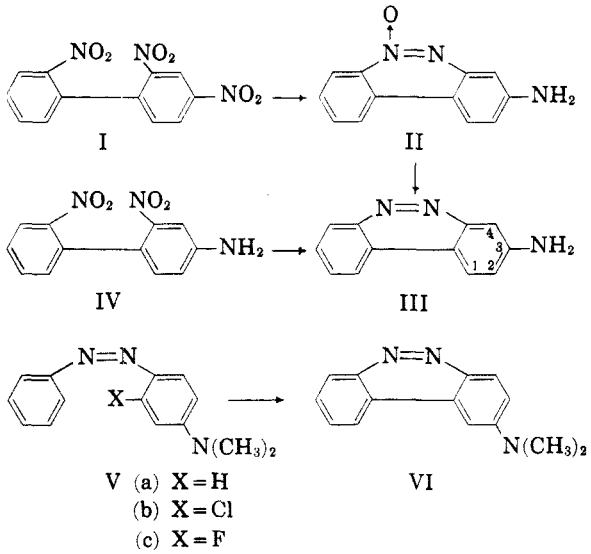
3-Aminobenzo[c]cinnoline has been prepared from 2,2',4-trinitrobiphenyl and from 2,2'-dinitro-4-aminobiphenyl. The preparations of 1-nitrobenzo[c]cinnoline, x-nitrobenzo[c]cinnoline, and their corresponding amines as reported by Smith and Ruby have been confirmed. The fourth possible isomer, y-nitrobenzo[c]cinnoline, and the corresponding amine have been prepared from benzo[c]cinnoline. In attempts to assign positions to these isomers it was found that 4-dimethylaminoazobenzene could be cyclized to 2-dimethylaminobenzo[c]cinnoline in an eutectic melt of aluminum trichloride.

Two amino derivatives of benzo[c]cinnoline, a phenanthrene analog containing two nitrogen atoms in place of the 9,10 CH groups, were synthesized for metabolic and carcinogenicity tests because of their similarity to several known carcinogens. The first, 3-aminobenzo[c]cinnoline (III), may be regarded as an analog of the carcinogens 3-aminophenanthrene[1] and 2-aminofluorene.[1] The second, 2-dimethylaminobenzo[c]cinnoline (VI), can be considered as a cyclized derivative of the hepatocarcinogen 4-dimethylaminoazobenzene.[2]



Of the four possible monoaminobenzo[c]cinnolines the structure of only one has been given with sufficient proof. Smith and Ruby[3] nitrated benzo[c]cinnoline in an excess of a mixture of nitric and sulfuric acids at 0° and obtained a mononitro derivative which upon reduction gave an amine melting at 167°. The benzenesulfonamido derivative of this amine was reduced at the azo linkage and then was deaminated to give a compound identical with authentic 2-benzenesulfonamidobiphenyl. Thus the initial nitro and amino derivatives were the 1-isomers.

King and King[4] have reported the formation of two monoaminobenzo[c]cinnolines by reduction of the appropriate mononitrobenzo[c]cinnoline-N-oxides. However, certain aspects of this work now appear doubtful. The nitro oxides were prepared by the nitration of benzo[c]cinnoline-N-oxide with fuming nitric acid at 80–90° for 3 hours. It seemed unlikely to us that nitration under these relatively drastic conditions would yield a mononitro derivative as the major product. On repeating the synthesis we obtained a major nitro compound which initially had the melting point of 269° given by King and King; on further purification it melted at 275–277°. However, its nitrogen analysis corresponded to that of a *di*nitrobenzo[c]cinnoline-N,N'-*di*oxide (Calc'd N for a mononitrobenzo[c]cinnoline-N-oxide: 17.42%; Calc'd N for a dinitrobenzo[c]cinnoline-N,N'-dioxide: 19.55%; Found: N, 19.20, 19.44). Considerable doubt also exists concerning the structure proofs given for the amines derived from the mononitro compounds obtained by King and King. These proofs involved the reduction of the azo linkage and cyclization to known aminocarbazoles. The major amino compound melted at 243° and gave an orange-red color in acid; it was considered to be the 2-amino isomer since it yielded an aminocarbazole melting at 243°. King and King attributed to this compound the formula of the previously known 3-aminocarbazole for which they quote a melting point of 243° from the paper by Ullmann.[5] However, it has been pointed out[6] that Ullmann actually states that 3-aminocarbazole melts at 254°, and that dipole measurements[6] on the major nitro compound are not consistent with the structure assigned to it. The minor amino derivative obtained by King and King melted at 194–195°. It was described as 3-aminobenzo[c]cinnoline since on degradation it yielded what was presumably 2-aminocarbazole. Mixture melting points of the carbazoles derived from these cinnolines with the authentic carbazoles were not

(1) Miller, Sandin, Miller, and Rusch, *Cancer Research*, **15**, 188 (1955).

(2) Miller and Miller in *Advances in Cancer Research*, edited by Greenstein and Haddow, Vol. I, Academic Press, Inc., New York, N. Y., 1953, p. 339.

(3) Smith and Ruby, *J. Am. Chem. Soc.*, **76**, 5807 (1954).

(4) King and King, *J. Chem. Soc.*, 824 (1945).

(5) Ullmann, *Ann.*, **332**, 99 (1904).

(6) Calderbank and LeFevre, *J. Chem. Soc.*, 649 (1951).

reported. These discrepancies led King and King to state in a footnote to the paper by Calderbank and LeFevre,[6] after a defense of their work, that "Nevertheless, in view of the dipole determination by Calderbank and LeFevre, our conclusions as to the orientation of the nitrocinnoline-6-oxides cannot be maintained."

We have prepared 3-aminobenzo[*c*]cinnoline by two relatively unequivocal syntheses from biphenyl derivatives in which nitro and amino groups are fixed in the 2,2′-, and 4 positions. The first method employed 2,2′,4-trinitrobiphenyl (I) which has a well established constitution since its synthesis starts from 2,2′-dinitrobiphenyl and since on further nitration it gives 2,2′,4,4′-tetranitrobiphenyl identical with the compound obtained from 2,4-dinitro-1-chlorobenzene[7] by the Ullmann reaction. Reduction of I by sodium polysulfide gave a compound that was presumably 3-aminobenzo[*c*]cinnoline-N-oxide (II). Further reduction of II with sodium amalgam in ethanol or by hydrogenation over Raney nickel gave 3-aminobenzo[*c*]cinnoline (III). III was also obtained when II was reduced with zinc dust in acetic acid; this method was used to reduce benzo[*c*]cinnoline-N-oxide to benzo[*c*]-cinnoline.[8] Direct treatment of I with sodium amalgam did not give III. The second method involved the direct ring closure of 2,2′-dinitro-4-aminobiphenyl with sodium amalgam to form III in low yield. The structure of this starting material is well established since it is formed by the nitration of 2-nitro-4′-aminobiphenyl and upon deamination it gives 2,2′-dinitrobiphenyl identical with the compound obtained from 1-nitro-2-chlorobenzene[9] by the Ullmann reaction. The identity of the two samples of III was shown by their mixture melting point. III melts at 163–165° and gives a violet color in alcoholic hydrochloric acid.

We have confirmed the data given by Smith and Ruby[3] for the nitration of benzo[*c*]cinnoline. They report that the minor nitration product or x-nitro compound melts at 230°; after two further crystallizations from nitrobenzene we found that this compound melts at 238–240°. Their major amine, 1-aminobenzo[*c*]cinnoline, melts at 167–169° but it is not identical with III, melting point 163–165°, since the melting point of a mixture shows a 30° depression. Thus the identities of the 1- and 3-aminobenzo[*c*]cinnolines appear to be established.

The nitration of benzo[*c*]cinnoline-N-oxide in sulfuric acid with the theoretical amounts of nitric acid or ethyl nitrate at 70–80° gave x-nitrobenzo-[*c*]cinnoline-N-oxide which melted at 208–210° after several recrystallizations. Reduction of this nitro compound with stannous chloride gave an aminobenzo[*c*]cinnoline which melted at 197–199° and gave no depression of the melting point when mixed with the minor x-aminobenzo[*c*]cinnoline, melting point 198–200°, reported by Smith and Ruby.[3] However, several recrystallizations of this amine raised the melting point to 206–207°. It is possible that the minor amine reported by King and King[4] as "3-aminobenzocinnoline," m.p. 194–195°, is the x-isomer.

We have obtained the fourth possible mononitro derivative of benzo[*c*]cinnoline by nitration of this compound in sulfuric acid with the theoretical amount of nitric acid at 80–90°. This new y-nitrobenzo[*c*]cinnoline melts at 141–142°. Reduction with stannous chloride gives y-aminobenzo[*c*]cinnoline which melts after several crystallizations at 144–145°. Benzo[*c*]cinnoline was not nitrated by an excess of nitrous acid.

Thus x-amino- and y-aminobenzo[*c*]cinnoline correspond to the remaining 2 (or 4)- and 4 (or 2)-amino isomers.

It is worthy of note that the 1- and 3-amino and also 3,8-diaminobenzo[*c*]cinnoline[10] are violet, while the 2- and 4-amino isomers (respectively *p*- and *o*- to the azo linkage) are dark blue in acid solution. The deeper color of the latter isomers is probably due to resonance of the amino group with the strongly chromophoric azo linkage.

In an attempt to decide which of the two unidentified amino isomers was 2-aminobenzo[*c*]cinnoline we tried to prepare it from 2,2′-dinitro-5-aminobiphenyl.[11] However, this compound gave no cinnoline derivative when reduced with sodium amalgam, sodium sulfide, or by catalytic hydrogenation of the free or acetylated base. The constitution of the starting compound is well established[11] and we obtained upon deamination in ethanol a high yield of 2,2′-dinitrobiphenyl giving no depression of the melting point upon mixing with an authentic sample. The identity of the reduction product that was obtained requires further investigation; its acetyl derivative melted at 221–223° after crystallization from aqueous ethanol and gave a strong green fluorescence in this solvent.

Another route to substituted benzo[*c*]cinnolines is the oxidative cyclization of azobenzene derivatives in an eutectic melt of aluminum trichloride.[12] An attempt was made to prepare 2-aminobenzo[*c*]-cinnoline from 4-aminoazobenzene in this way. However, no cinnoline derivative could be isolated and up to 70 per cent of the starting product was recovered unchanged. On the other hand, 4-dimethylaminoazobenzene (Va) was relatively easily dehydrogenated by this method to give 2-dimethylaminobenzo[*c*]cinnoline (VI); this compound has

(7) Gull and Turner, *J. Chem. Soc.*, 491 (1929).
(8) Badger, Seidler, and Thomson, *J. Chem. Soc.*, 3207 (1951).
(9) Finzi and Bellavita, *Gazz. chim. ital.*, **68**, 77 (1938).
(10) Täuber, *Ber.*, **24**, 3081 (1891).
(11) Case, *J. Am. Chem. Soc.*, **67**, 116 (1945).
(12) Wolfram, *Chem. Zentr.*, **I**, 1361 (1931); Hausdörfer and Schörnig, German Patent 513,206 (1930), I. G. Farbenindustrie.

the surprisingly high melting point of 369–371° and gives a deep blue-violet color in ethanolic hydrochloric acid. 2-Chloro- and 2-fluoro-4-dimethylaminoazobenzene (Vb and Vc) also give VI in this way although less rigorous conditions (70–80° for 1 hour) are required. The identity of these different samples of VI was established by their mixture melting points. 4-Diethylaminoazobenzene was cyclized by this method to 2-diethylaminobenzo[c]cinnoline.

Attempts to methylate either x-amino or y-aminobenzo[c]cinnoline to give VI did not give any identifiable products. Unfortunately this work had to be terminated before an unequivocal structure assignment of these isomers could be worked out.

The visible and ultraviolet spectra of 4-dimethylaminoazobenzene[13] and 2-dimethylaminobenzo[c]cinnoline in acid ethanol show an over-all similarity except that the whole spectrum of the latter compound is shifted approximately 60 mμ to longer wave lengths. Maximal extinction coefficients in ethanolic hydrochloric acid[13] of 4.06 x 10^4 at 580 mμ, 4.44 x 10^3 at 360 mμ, and 7.50 x 10^3 at 284 mμ were observed for the cinnoline derivative. The similarity of these spectra is somewhat unexpected and may be related to the suppression in acid solution of the limit formulae of this cinnoline involving the biphenyl linkage.

EXPERIMENTAL

Melting points are corrected and were taken with a Maquenne block.

3-Aminobenzo[c]cinnoline (III). (*a*) *From 2,2',4-trinitrobiphenyl* (I). Compound I (2 g.) was suspended in 120 ml. of boiling ethanol and a solution of 0.84 g. of sulfur flowers and 8 g. of sodium sulfide nonahydrate in 12 ml. of water was added dropwise. The mixture was refluxed 10 min. more and then was poured into 450 ml. of water. The amorphous gray precipitate was filtered after 1 to 1½ hours and the filtrate was allowed to stand overnight. A compound, presumably 3-aminobenzo[c]cinnoline-N-oxide (II), separated in the form of short, dark orange needles; yield 0.35 g. (24%). It crystallized from nitrobenzene as cubes and as thick platelets from acetic acid, m.p. 238–239°; it was soluble in nitrobenzene and acetic acid, less soluble in ethanol, and insoluble in ether and petroleum ether. The hydrochloride crystallized from acetic acid as colorless tiny needles, m.p. 205–207°.

Anal. Calc'd for $C_{12}H_9N_3O$: C, 68.40; H, 4.26; N, 19.90. Found: C, 67.67; H, 4.24; N, 20.10.

Repeated purification did not raise the percentage of carbon found in this compound.

II (0.320 g.) was reduced with 3 to 4 g. of fresh 3% sodium amalgam by boiling in ethanol for a few minutes. The alcoholic solution was decanted, water was added, and the ethanol was evaporated *in vacuo*. Upon cooling long orange-yellow needles of III separated; yield 0.20 g. (68%). It crystallized from aqueous methanol in long fine needles and from benzene in short thick needles, m.p. 163–165°; it was soluble in alcohol, acetone, less soluble in water and benzene, and insoluble in petroleum ether. III gives a violet color in ethanolic hydrochloric acid.

Anal. Calc'd for $C_{12}H_9N_3$: C, 73.83; H, 4.64; N, 21.53. Found: C, 73.70; H, 4.64; N, 21.64.

(*b*) *From 2,2'-dinitro-4-aminobiphenyl* (IV). The nitration of 2-nitro-4'-aminobiphenyl was carried out as described by Finzi and Bellavita[9] but the resulting isomers were separated by chromatography on alumina with a mixture of cyclohexane and benzene. Two grams of crude nitration product gave 1.1 g. of 2,2'-dinitro-4-aminobiphenyl (IV), m.p. 138–139° (lit.[9] 138–139°) and 0.5 g. of 2,4-dinitro-4'-aminobiphenyl, m.p. 137–139° (lit.[9] 138–139°). The mixture melting point of the two isomers was 103–106°.

One gram of IV was dissolved in 70 ml. of methanol and was treated during 10 min. with 70 g. of 3% sodium amalgam at 30°. The solution was decanted, diluted with water, the methanol evaporated *in vacuo*, and the crude product allowed to crystallize in the refrigerator; yield 0.60 g. This material contained a large proportion of a still unidentified compound crystallizing from ethanol in brilliant pale yellow platelets, m.p. 122–124°. By fractionating from benzene 0.020 g. of III was isolated, m.p. 162–164°; no depression of melting point occurred when it was mixed with the sample from (*a*).

x-Nitrobenzo[c]cinnoline-N-oxide. Benzo[c]cinnoline-N-oxide (8 g.) was dissolved in 80 ml. of concentrated sulfuric acid and nitrated by adding dropwise a mixture of 2.6 ml. of nitric acid (*d.* 1.42) and 30 ml. of concentrated sulfuric acid. After the addition the mixture was heated to 70–80° and stirred for 2 more hours. After cooling it was poured on ice; yield 7.8 g. (79%) of crude x-nitrobenzo[c]cinnoline-N-oxide. It was crystallized once from nitrobenzene and ethanol and three times from pyridine and ethanol to give small, pale yellow needles melting at 208–210° (highest m.p.). The nitration with ethyl nitrate was carried out in a similar way.

x-Aminobenzo[c]cinnoline. To 0.63 g. of x-nitrobenzo[c]cinnoline-N-oxide suspended in 5 ml. of concentrated hydrochloric acid, 2.4 g. of stannous chloride dihydrate in 10 ml. of concentrated hydrochloric acid was added dropwise with shaking. Immediately a colorless crystalline precipitate formed. This suspension was heated at 50–60° for ½ hour, filtered on a sintered glass filter, and washed with a little concentrated hydrochloric acid. The free base was obtained by treatment with cold 10% sodium hydroxide; yield 0.23 g. (45%). On crystallization from a mixture of benzene and petroleum ether it gave thick orange platelets, m.p. 197–199°. No depression of m.p. occurred when it was mixed with the x-amino isomer prepared according to Smith and Ruby,[3] m.p. 198–200°. After four successive crystallizations from ethanol the melting point was raised to 206–207° (platelets).

y-Nitrobenzo[c]cinnoline. Benzo[c]cinnoline (8 g.) was dissolved in 100 ml. of concentrated sulfuric acid and nitrated by adding dropwise a mixture of 3 ml. of nitric acid (*d.* 1.42) and 20 ml. of concentrated sulfuric acid. After the addition the mixture was heated to 80–90° and stirred for 2 hours more. After pouring on ice 7 g. (70%) of crude product was collected. Crystallization from ethanol gave long pale yellow needles, m.p. 141–142°; it was soluble in acetone, acetic acid, concentrated hydrochloric acid, less soluble in ether, benzene, ethanol, and insoluble in petroleum ether.

Anal. Calc'd for $C_{12}H_7N_3O_2$: C, 63.99; H, 3.13; N, 18.67. Found: C, 63.83; H, 3.09; N, 18.77.

y-Aminobenzo[c]cinnoline. To 0.42 g. of the y-nitro compound 1.15 g. of stannous chloride dihydrate was added as described for the x-nitro derivative. A yield of 0.17 g. (46%) was obtained; after three crystallizations from 50% aqueous ethanol orange needles melting at 144–145° separated. The melting point did not change after two further crystallizations. When mixed with 25–30% of the x-amino derivative it gave a m.p. of 139–140°. It was soluble in ethanol, acetone, acetic acid, dilute hydrochloric acid and insoluble in petroleum ether.

Anal. Calc'd for $C_{12}H_9N_3$: C, 73.83; H, 4.64; N, 21.53. Found: C, 73.90; H, 4.60; N, 21.53.

2,2'-Dinitro-5-aminobiphenyl. The partial reduction of

(13) Miller, Sapp, and Miller, *J. Am. Chem. Soc.*, **70**, 3458 (1948).

2,3′-dinitrobiphenyl to 2-nitro-3′-aminobiphenyl with sodium polysulfide was patterned after the method Guglialmelli and Franco[14] used for the partial reduction of 2,4′-dinitrobiphenyl. This method gave twice the yield obtained by Case[11] for this compound.

2,3′-Dinitrobiphenyl (2 g.) (crystallized from cyclohexane) was suspended in 120 ml. of boiling 50% aqueous ethanol and reduced by adding a solution of 0.84 g. of sulfur flowers and 8 g. of sodium sulfide nonahydrate in 12 ml. of water dropwise during 5 minutes. The refluxing was continued for 10 min. or until complete solution of the dinitro compound occurred; the mixture then was poured into water and allowed to stand overnight. The crystalline free base was filtered off; yield 0.9 g. (51%). The yield dropped when the refluxing time was extended.

The nitration of the base was carried out after Case[11] to give 2,2′-dinitro-5-aminobiphenyl, m.p. 130–131° (lit.[11] 130–131°).

2-Dimethylaminobenzo[c]cinnoline (VI). In a 500-ml. three neck flask fitted with a stainless steel paddle stirrer, thermometer, gas evacuation tube, and electric heating mantle, 10 g. of dry 4-dimethylaminoazobenzene (Va) was introduced portionwise into an eutectic melt of 157 g. of anhydrous aluminum trichloride, 9.3 g. of sodium chloride, 6.7 g. of potassium chloride, and 2.6 g. of sodium fluoride without external heating at an internal temperature between 85–90°. After the addition the temperature was maintained between 88–93° by external heating and the stirring was continued at this temperature for 3½ hours more. Then the reaction mixture was carefully poured onto ice, boiled, and filtered after cooling. The precipitate was treated with 10% sodium carbonate, filtered, washed with water and thoroughly dried. The brown mass was powdered and extracted with xylene in a Soxhlet extractor until the solution in the upper chamber became nearly colorless (about four days). Three to four volumes of petroleum ether were added to the xylene extract (from which part of the product usually crystallized). The brick-red precipitate was filtered after standing and extracted with boiling ethanol. The insoluble precipitate was filtered to give 3.0 to 3.2 g. of product (30–32%). After four successive crystallizations from nitrobenzene dark orange wing-like needles melting at 369–371° were obtained. The melting point did not change with further crystallization. It was soluble in nitrobenzene, quinoline, less soluble in xylene and toluene, and insoluble in ethanol and petroleum ether. It gave a deep blue-violet color in ethanolic hydrochloric acid and a cherry-red solution in concentrated sulfuric acid.

Anal. Calc'd for $C_{14}H_{13}N_3$: C, 75.31; H, 5.87; N, 18.82. Found: C, 75.05; H, 6.16; N, 18.78.

2-Diethylaminobenzo[c]cinnoline was obtained in the same way from 4-diethylaminoazobenzene. It was more soluble than the dimethyl derivative and after three crystallizations from chlorobenzene it gave tiny dark orange oval platelets with a melting point of 315–317°.

Spectra. Five times recrystallized samples of 4-dimethylaminoazobenzene (ethanol) and 2-dimethylaminobenzo[c]cinnoline (nitrobenzene) were dissolved in ethanolic hydrochloric acid[13] to give 1.5 to 3 × 10^{-5} *molar* solutions. The spectra were taken with a Beckman DK recording spectrophotometer.

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(14) Guglialmelli and Franco, *Anales asoc. quim. argentina*, **18**, 190 (1930); *Chem. Zentr.*, **II**, 1136 (1931).