[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

The Dimerization of 2-Amino-5-nitrobenzonitrile

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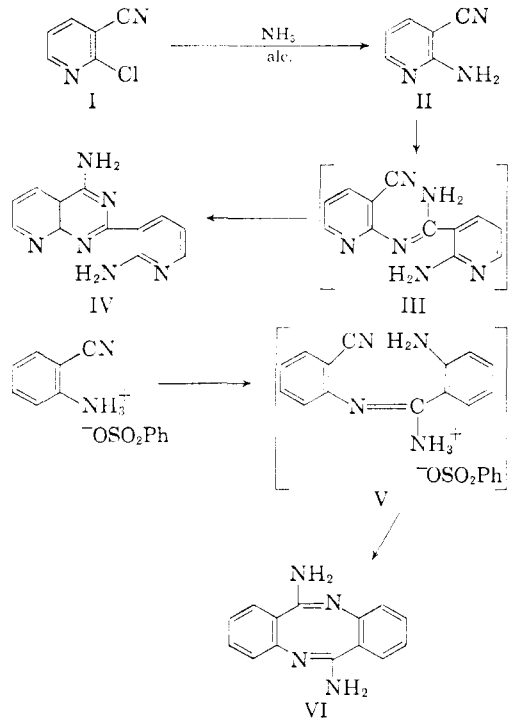
2-Amino-5-nitrobenzonitrile dimerizes readily at 180° in alcoholic ammonia to give 2-(2-amino-5-nitrophenyl)-4-amino-6-nitroquinazoline (VII). The structure of VII was rigorously confirmed by conversion to 2-(3-nitrophenyl)-6-nitro-4(3*H*)-quinazolone (IX) and to 2-(2-amino-5-nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XII), both of which were then synthesized independently. It was found that aromatic *o*-aminonitriles undergo intermolecular condensations with aromatic nitriles in basic media to give 2-aryl-4-aminoquinazolines in good yield, and that this reaction constitutes a general method for the synthesis of fused 4-aminopyrimidine heterocycles. The mechanisms of these condensation and dimerization reactions are discussed.

Recently Taylor, Crovetti and Knopf[1] showed that the action of alcoholic ammonia on 2-chloronicotinonitrile (I) yielded, in addition to 2-aminonicotinonitrile (II), a high melting, insoluble yellow solid. The amount of this material formed in the reaction increased as the severity of the reaction conditions was increased until, at 180°, it became the major product of the reaction. Furthermore, 2-aminonicotinonitrile (II) was shown to be an intermediate in its formation, and, indeed, II could be converted to the yellow solid not only by heating with ammonia, but also by refluxing with sodium ethoxide in ethanol or even by simple pyrolysis. Analysis and a molecular weight determination indicated that the yellow solid was a dimer of II, and degradation studies established its structure as 2-(3-(2-aminopyridyl))-4-aminopyrido[2,3-d]pyrimidine (IV). The dimerization was considered to have taken place *via* the intermediate formation of an amidine (III) which then underwent intramolecular cyclization. It was pointed out that the previously reported pyrolytic conversion of the *p*-toluenesulfonic acid salt of 2-aminobenzonitrile to 6,12-diaminophenhomazine (VI)[2] yielded an 8- rather than a 6-membered dimer because protonation of the presumed intermediate V must involve the more basic amidine function, leaving only the aromatic amino group for intramolecular ring closure.

Further investigation showed that 2-aminobenzonitrile failed to dimerize under basic conditions, and it thus became of considerable interest to study the factors responsible for the facile dimerization of the closely related 2-aminonicotinonitrile. The present paper is concerned with our study of the dimerization of 2-amino-5-nitrobenzonitrile, an aromatic analog of 2-aminonicotinonitrile, and describes a new, general synthesis of 4-aminoquinazolines.

2-Amino-5-nitrobenzonitrile is a known compound, having been prepared by Baudet[3] by the action of alcoholic ammonia on 2-chloro-5-nitrobenzonitrile in a sealed tube at 150°. The latter compound was in turn prepared by nitration with fuming nitric acid of 2-chlorobenzonitrile, which resulted from the Sandmeyer reaction with cuprous cyanide on 2-chloroaniline. In the course of repeating this reaction sequence, the temperature of the final amination step was unintentionally allowed to reach 170°. In addition to an excellent yield of 2-amino-5-nitrobenzonitrile, we also obtained an extremely insoluble, high-melting, yellow by-product which did not contain a nitrile group (infrared) but which was isomeric with 2-amino-5-nitrobenzonitrile. The same product could be obtained in good yield simply by treatment of the latter compound with alcoholic ammonia at 180°.



This product was shown to be 2-(2-amino-5-nitrophenyl)-4-amino-6-nitroquinazoline (VII) and not the 8-membered ring isomer, VIII as follows: Treatment of the product with two equivalents of nitrous acid, followed by vigorous boiling of the reaction mixture, resulted in the formation of 2-(3-nitrophenyl)-6-nitro-4(3*H*)quinazolone (IX). This unexpected result was confirmed by an independent synthesis of IX by condensation of 3-nitrobenzoyl chloride with 2-amino-5-nitrobenzonitrile to give the anilide XI, followed by cyclization with basic hydrogen peroxide. The product of this reaction sequence proved to be identical in every respect with the diazotization product obtained from VII. The unexpected reductive diazotization of the aromatic amino group of VII was confirmed by a stepwise conversion of VII to

(1) E. C. Taylor, A. J. Crovetti and R. J. Knopf, THIS JOURNAL, **80**, 427 (1958).

(2) F. W. Cooper and M. W. Partridge, *J. Chem. Soc.*, 3429 (1954).

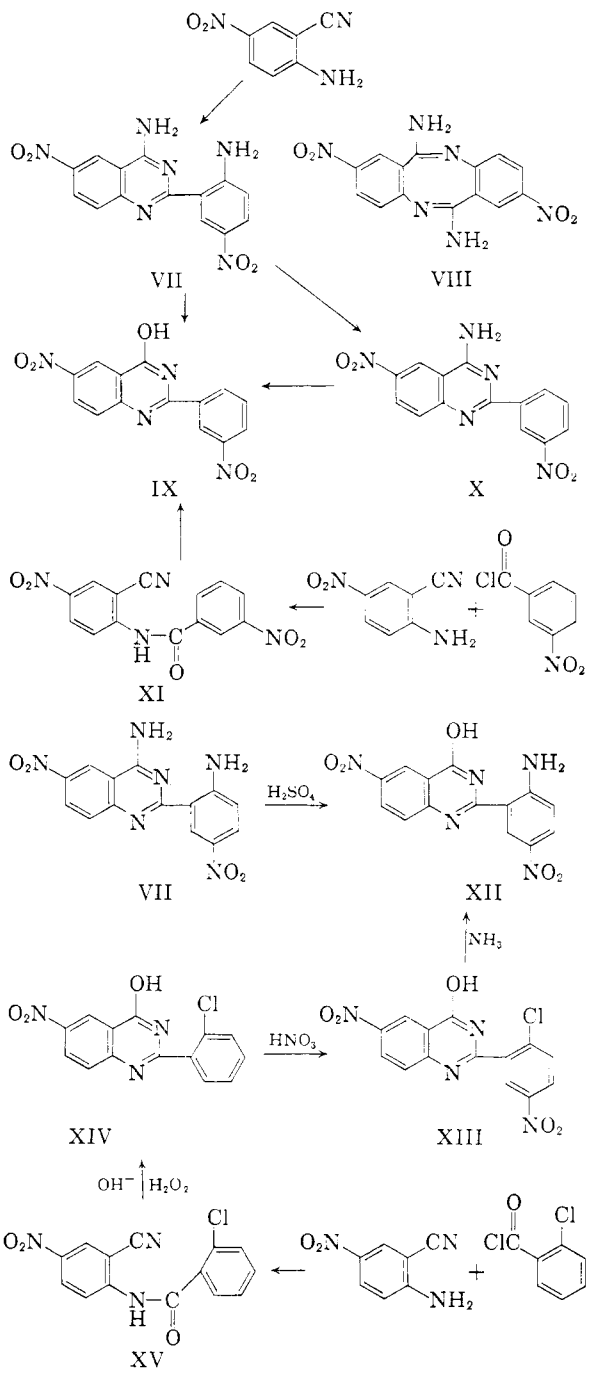
(3) H. Ph. Baudet, *Rec. trav. chim.*, **43**, 707 (1924).

IX. Thus, treatment of VII with one mole of nitrous acid in ethanol resulted in the evolution of acetaldehyde and the formation of 2-(3-nitrophenyl)-4-amino-6-nitroquinazoline (X), which, upon subsequent treatment with aqueous nitrous acid, was converted to IX.

Further proof for the assigned structure VII to the dimer of 2-amino-5-nitrobenzonitrile was obtained as follows: Vigorous treatment of VII with 50% sulfuric acid resulted in hydrolysis of the 4-amino group with the formation of a product formulated as 2-(2-amino-5-nitrophenyl)-6-nitro-4(3*H*)-quinazolone(XII), which was then synthesized independently. Condensation of 2-amino-5-nitrobenzonitrile with 2-chlorobenzoyl chloride in refluxing pyridine yielded N-(2-cyano-4-nitrophenyl)-2-chlorobenzamide (XV), which cyclized in alkaline hydrogen peroxide to 2-(2-chlorophenyl)-6-nitro-4(3*H*)-quinazolone(XIV). This compound was unaffected by fuming nitric acid, but underwent almost quantitative mononitration upon treatment with potassium nitrate in concentrated sulfuric acid. Since 4(3*H*)-quinazolones are nitrated first in the 6-position[4] (which, however, already carries a nitro group in XIV), nitration presumably must have taken place in the 2-phenyl substituent, a conclusion which was confirmed by subsequent treatment of the dinitro derivative XIII with alcoholic ammonia. The product of this reaction proved to be identical in all respects with the hydrolysis product of VII and therefore must be 2-(2 - amino - 5 - nitrophenyl) - 6 - nitro - 4(3*H*)-quinazolone (XII). Thus, the dimerization product of 2-amino-5-nitrobenzonitrile is conclusively shown to be 2-(2-amino-5-nitrophenyl)-4-amino-6-nitroquinazoline (VII).

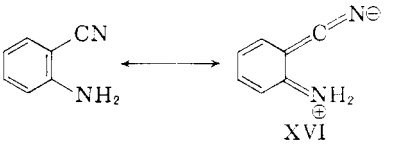
In recalling that the dimerization (under acidic conditions) of 2-aminobenzonitrile gives a phenhomazine, it should be pointed out that a number of factors undoubtedly influence the course of the dimerization of the corresponding 5-nitro derivative to give a quinazoline derivative. Although the relative importance of these various factors is not known, they certainly include the facts that: (a) the reaction, since it is carried out in alkaline solution, yields an intermediate amidine which, being a stronger base than the aromatic amino group, participates exclusively in the subsequent intramolecular ring closure; (b) the observed product is more aromatic and less strained than the isomeric phenhomazine; and (c) the intermediate amidine, by assuming the less strained *trans* relationship of the two bulky aromatic rings, favors quinazoline formation by placing the amidine–NH_2 and –CN groups close to each other.

In view of the successful dimerization of 2-amino-5-nitrobenzonitrile described above, it was of considerable interest that neither 2-amino-5-methylbenzonitrile nor 2-amino-5-bromobenzonitrile could be induced to dimerize, even under rather drastic conditions. A clue to the unreactivity of these compounds was found in the observation that 2-amino-5-nitrobenzonitrile, although it dimerized readily in alcoholic ammonia, failed to dimerize in the presence of a molar excess of sodium ethoxide in ethanol. Under these conditions a bright red, high melting solid was obtained which possessed all the properties expected of a sodium salt and which regenerated 2-amino-5-nitrobenzonitrile when dissolved in water. The failure of this compound to dimerize under these conditions must be attributed to inactivation of the cyano group as a site for nucleophilic attack by resonance delocalization of the negative charge produced by anion formation. The failure of 2-amino-5-methyl- and 5-bromobenzonitrile and of 2-aminobenzonitrile itself to dimerize under alkaline conditions may therefore be rationalized on the assumption that contributions to the resonance hybrid of structures such as XVI

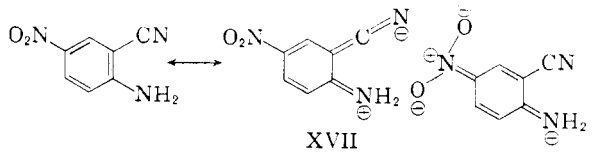


(4) O. Yu Magidson and E. S. Golovchinskaya, *J. Gen. Chem. (U.S.S.R.)*, **8**, 1797 (1938).

must deactivate the nitrile group sufficiently to prevent initial nucleophilic attack. Since 2-amino-5-nitrobenzonitrile does not form a red solution in alcoholic ammonia, complete conversion to the above anion, with resulting inactivation of the nitrile group, would not appear to be taking place. The contribution of structures such as XVII to the resonance hybrid would be expected to be less important in view of predominant contributions of structures involving the nitro group (XVIII). Furthermore, the nitrile group of 2-amino-5-nitrobenzonitrile is activated by the *m*-nitro substituent.

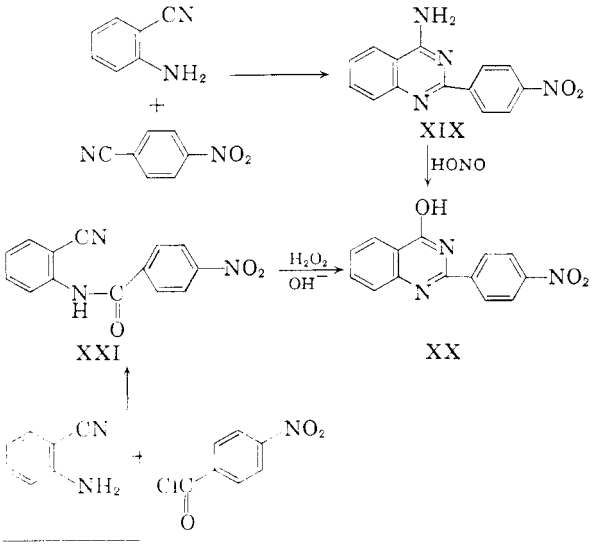


XVI

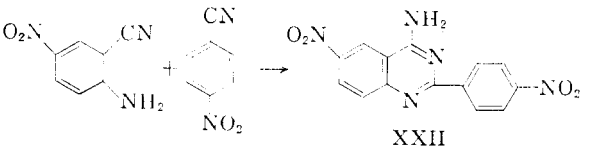


XVII XVIII

If the assumption is correct that the dominant factor determining dimerization is the ability of the nitrile grouping to undergo nucleophilic attack rather than the basicity of the attacking amino group, then the above *o*-aminonitriles should undergo *intermolecular* condensations with compounds possessing more reactive nitrile groupings. This proved to be the case, for 2-aminobenzonitrile reacted readily with 4-nitrobenzonitrile to give 2-(4-nitrophenyl)-4-aminoquinazoline (XIX) in good yield. The structure of XIX was established by treatment with nitrous acid to give 2-(4-nitrophenyl)-4(3*H*)-quinazolone (XX).[5] The identity of XX was further checked by an independent synthesis involving alkaline hydrogen peroxide cyclization of the anilide XXI formed from 2-aminobenzonitrile and 4-nitrobenzoyl chloride. Moreover, the prediction that 2-amino-5-nitrobenzonitrile should react preferentially with a second, more reactive nitrile rather than undergo dimerization was also realized, for treatment of 2-amino-5-nitrobenzonitrile in alcoholic ammonia with 4-nitrobenzonitrile resulted in the exclusive formation of 2-(4-nitrophenyl)-4-amino-6-nitroquinazoline (XXII); no dimer could be found in the reaction mixture. The structure of XXII was similarly established by diazotization to 2-(4-nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XXIII) followed by an independent synthesis of XXIII by the alkaline hydrogen peroxide cyclization of N-(2-cyano-4-nitrophenyl)-4-nitrobenzamide.



XIX XXI XX



XXII

We consider that all of the above reactions leading to 4-aminoquinazolines involve an initial nucleophilic attack of the amino group of the *o*-aminonitrile upon the nitrile of a second molecule (leading to dimerization) or a second, more reactive nitrile (leading to mixed condensation), with subsequent ring closure of the resulting intermediate amidine. Although amidine formation from amines and nitriles generally is not favored under basic conditions, it would appear that cyclization to a low energy, highly aromatic 4-aminoquinazoline in the terminal step provides sufficient driving force to displace an otherwise unfavorable initial equilibrium. The basic medium (alcoholic ammonia) appears to be necessary for the proton transfers required both in the formation of the intermediate amidine and in the final cyclization. Amidine formation between a nitrile and ammonia (which is a much stronger base than the *o*-aminonitrile) may also lead to product *via* alternate pathways of addition and cyclization,[5a] but this cannot represent the only pathway to product, since triethylamine may successfully be substituted for ammonia in the dimerization of 2-amino-5-nitrobenzonitrile.

It would thus appear that the reaction of *o*-aminonitriles with nitriles under alkaline conditions constitutes a general method for the preparation of condensed 2-substituted 4-aminopyrimidine systems. The application of this method to the synthesis of other quinazolines, purines, pyrazolo-(3,4-d)pyrimidines and pteridines will form the subject of subsequent communications.

Experimental[6]

2-(2-Amino-5-nitrophenyl)-4-amino-6-nitroquinazoline (VII). Method A.—A mixture of 3.6 g. of 2-chloro-5-nitrobenzonitrile[7] and 20 ml. of methanol saturated with ammonia was heated in a steel bomb at 150–170° for 3 hours. The bomb was allowed to cool and was vented in the hood. In addition to 2.1 g. of 2-amino-5-nitrobenzonitrile,[3] 0.25 g. (7.8%) of an orange crystalline solid, m.p. > 360°, was obtained. The material was purified by recrystallization from aqueous dimethylformamide followed by vacuum sublimation at 300° (0.05 mm.).

(5) D. T. Zentmyer and E. C. Wagner, *J. Org. Chem.*, **14**, 967 (1949).

(5a) For example, the reaction of benzamidine with 2-ethylthio-4-amino-5-cyanopyrimidine in ethanol solution yields 2-ethylthio-5-amino-7-phenylpyrimido[4,5-d]pyrimidine (E. C. Taylor, R. J. Knopf, R. F. Meyer, A. Holmes and M. L. Hoefle, THIS JOURNAL, in press).

(6) We are indebted for the microanalyses to Dr. Joseph F. Alicino, Metuchen, N. J. All melting points are corrected.

(7) W. Borsche, *Ber.*, **54**, 660 (1921).

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA

Compound	Solvent	max, mμ				Log			
2-(2-Amino-5-nitrophenyl)-4-amino-quinazoline (VII)	Dioxane	270	376			4.43	4.33		
2-(3-Nitrophenyl)-4-amino-6-nitro-quinazoline (X)	Dioxane	270–367				4.49	4.02		
2-(3-Nitrophenyl)-6-nitro-4(3*H*)-quinazolone (IX)	0.1 *N* NaOH	262	366			4.53	4.10		
2-(2-Chlorophenyl)-6-nitro-4(3*H*)-quinazolone (XIV)	EtOH	318				4.03			
2-(2-Chloro-5-nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XIII)	Dioxane	318				4.16			
	0.1 *N* NaOH	259	366			4.38	4.04		
2-(2-Amino-5-nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XII)	0.1 *N* NaOH	259	380			4.38	4.27		
2-(4-Nitrophenyl)-4-aminoquinazoline (XIX)	Dioxane	229	281	318	330	4.39	4.33	4.17	4.14
2-(4-Nitrophenyl)-4(3*H*)-quinazolone (XX)	0.1 *N* NaOH	225	277			4.45	4.23		
		304–308 (sh)				4.13			
		315–320 (sh)				4.10			
2-(4-Nitrophenyl)-4-amino-6-nitro-quinazolone (XXII)	Dioxane	281	372			4.45	4.18		
2-(4-Nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XXIII)	0.1 *N* NaOH	278	372			4.41	4.06		

Anal. Calcd. for $C_{14}H_{10}N_6O_4$: C, 51.5; H, 3.1. Found: C, 51.5; H, 3.2.

When the above reaction was carried out at 190° for 3.5 hours, the yield of 2-amino-5-nitrobenzonitrile was lowered to 29% and the yield of the dimer was raised to 71%.

Method B.—A suspension of 5.0 g. of 2-amino-5-nitrobenzonitrile in 50 ml. of liquid ammonia was heated for 3 hours in a steel bomb at 180°. The ammonia was vented off and the reaction residue extracted repeatedly with boiling water to remove unreacted starting material. Recrystallization of the insoluble residue from aqueous dimethylformamide afforded 3.5 g. (70%) of the dimer, identical in all respects with the product obtained by method A.

2-(3-Nitrophenyl)-4-amino-6-nitroquinazoline (X).—A suspension of 0.5 g. of 2-(2-amino-5-nitrophenyl)-4-amino-6-nitroquinazoline in a mixture of 8.5 ml. of 95% ethanol and 3 ml. of concentrated sulfuric acid was cooled to 0–5° by means of an ice-bath and treated with 0.25 g. of sodium nitrite with efficient stirring. After all the sodium nitrite had been added, the ice-bath was removed and stirring continued. The reaction mixture was allowed to warm up to room temperature and was then heated vigorously under reflux for 30 minutes. Acetaldehyde was evolved during this time, and an orange solid separated from solution. The reaction mixture was cooled and filtered and the collected solid washed well with water and dried at 100° to give 0.43 g. (90%) of crude product, m.p. 265–280° dec. Recrystallization from aqueous dimethylformamide followed by vacuum sublimation at 260° (0.05 mm.) yielded fine orange needles, m.p. 315° dec.

Anal. Calcd. for $C_{14}H_9N_5O_4$: C, 54.0; H, 2.9; N, 22.5. Found: C, 54.0; H, 3.2; N, 22.5.

N-(2-Cyano-4-nitrophenyl)-3-nitrobenzamide (XI).—A mixture of 1.8 g. of 2-amino-5-nitrobenzonitrile, 1.9 g. of 3-nitrobenzoyl chloride and 10 ml. of dry pyridine was heated under reflux for 2 hours, cooled and poured into 60 ml. of water. The orange solid which separated was collected by filtration, washed well with water and dried; yield 2.4 g. (69%), m.p. 206–211°. Recrystallization from aqueous dimethylformamide yielded fine buff-colored needles, m.p. 216–217°.

Anal. Calcd. for $C_{14}H_8N_4O_5$: C, 53.9; H, 2.6; N, 17.95. Found: C, 54.0; H, 2.8; N, 18.2.

2-(3-Nitrophenyl)-6-nitro-4(3H)-quinazolone (IX). Method A.—A well-stirred suspension of 0.6 g. of 2-(2-amino-5-nitrophenyl)-4-amino-6-nitroquinazoline in 8 ml. of 50% sulfuric acid was cooled to 0–5° and treated with a solution of 0.28 g. of sodium nitrite in 3 ml. of water. The mixture was allowed to warm to room temperature and was then heated slowly to boiling. Nitrogen was evolved and an orange solid separated. The mixture was boiled for 15 minutes, cooled, diluted with water and filtered to give 0.5 g. (87%) of crude product, m.p. 320–332° dec. Recrystallization from aqueous dimethylformamide followed by vacuum sublimation at 280° (0.05 mm.) yielded a pale yellow solid, m.p. 345–346° dec.

Anal. Calcd. for $C_{14}H_8N_4O_5$: C, 53.9; H, 2.6; N, 17.95. Found: C, 53.9; H, 2.5; N, 17.4.

Method B.—To a well-stirred suspension of 0.2 g. of 2-(3-nitrophenyl)-4-amino-6-nitroquinazoline in 6 ml. of 50% sulfuric acid was added 0.1 g. of sodium nitrite and the mixture stirred for 5 minutes and then heated under reflux for 2 hours. The orange starting material dissolved and a light yellow solid separated. The cooled mixture was diluted with water, filtered and the collected solid washed thoroughly with water and dried to give 0.18 g. (88%) of crude product, m.p. 335–340° dec. Vacuum sublimation at 280° (0.05 mm.) raised the decomposition point to 345–346°. The product was identical in all respects with the product obtained by method A.

Method C.—A mixture of 0.5 g. of N-(2-cyano-4-nitrophenyl)-3-nitrobenzamide, 10 ml. of 16% sodium hydroxide and 20 ml. of 3% hydrogen peroxide was heated cautiously until the initial vigorous reaction subsided and then refluxed gently for 1 hour. The mixture was cooled, an additional 5 ml. of 3% hydrogen peroxide added and refluxing continued for 30 minutes. The cooled mixture was then filtered and the collected yellow solid was suspended in 5% sulfuric acid and stirred for several minutes. Filtration then yielded 0.46 g. (92%) of crude product, m.p. 340–345° dec. Recrystallization from aqueous dimethylformamide followed by sublimation yielded pure IX, m.p. 345–346° dec., identical in all respects with the products formed by methods A and B above.

N-(2-Cyano-4-nitrophenyl)-2-chlorobenzamide (XV).—A suspension of 1.4 g. of 2-amino-5-nitrobenzonitrile and 1.6 g. of 2-chlorobenzoyl chloride in 15 ml. of dry pyridine was heated under reflux for 3 hours, cooled and poured into 150 ml. of cold water. The resulting mixture was made alkaline by the addition of *N* sodium hydroxide and refrigerated overnight. Filtration then gave 2.45 g. (95%) of crude tan product, m.p. 170–180°. Recrystallization from ethanol with the use of charcoal afforded fine colorless needles, m.p. 186–187°.

Anal. Calcd. for $C_{14}H_8N_3O_3Cl$: C, 55.7; H, 2.7; N, 13.9. Found: C, 55.6; H, 2.8; N, 13.6.

2-(2-Chlorophenyl)-6-nitro-4(3*H*)-quinazolone (XIV).—A mixture of 1.0 g. of N-(2-cyano-4-nitrophenyl)-2-chlorobenzamide, 18 ml. of 17% sodium hydroxide and 35 ml. of 3% hydrogen peroxide was heated gently under reflux for 1 hour, an additional 10 ml. of 3% hydrogen peroxide added and refluxing continued for 45 minutes. The resulting red solu-

tion was acidified with glacial acetic acid and readjusted to *p*H 8 with ammonium hydroxide. The flocculent yellow solid which separated was collected by filtration, washed thoroughly with water and dried at 100°; yield 0.9 g. (90%), m.p. 272–280°. Recrystallization from ethanol yielded fine yellow needles, m.p. 278–279°.

Anal. Calcd. for $C_{14}H_8N_3O_3Cl$: C, 55.7; H, 2.7; N, 13.9. Found: C, 55.7; H, 2.7; N, 13.8.

2-(2-Chloro-5-nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XIII).—To a solution of 0.61 g. of potassium nitrate in 10 ml. of concentrated sulfuric acid was added 1.6 g. of 2-(2-chlorophenyl)-6-nitro-4(3*H*)-quinazolone and the well-stirred mixture was heated for 45 minutes in an oil-bath at 90°. The resulting viscous solution was cooled to room temperature and poured over 50 g. of crushed ice. The light pink solid which separated was collected by filtration, washed well with water and dried to give 1.8 g. (98%) of crude product, m.p. 320–325° dec. (with initial softening at 312°). Vacuum sublimation at 270° (0.05 mm.) yielded small lemon-yellow needles, m.p. 324–325° dec.

Anal. Calcd. for $C_{14}H_7N_4O_5Cl$: C, 48.7; H, 2.0; N, 16.2. Found: C, 48.5; H, 1.8; N, 15.9.

2-(2-Amino-5-nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XII). Method A.—A suspension of 0.5 g. of 2-(2-amino-5-nitrophenyl)-4-amino-6-nitroquinazoline in 8 ml. of 50% sulfuric acid was heated under reflux for 2.5 hours. During this time the starting material dissolved and a light orange solid started to separate. The mixture was chilled to 0°, diluted with 20 ml. of water and then neutralized with concentrated ammonium hydroxide. The precipitated orange solid was collected by filtration, washed with water and dried; yield 0.3 g. (60%), m.p. > 360°. The material was purified for analysis by recrystallization from aqueous dimethylformamide followed by vacuum sublimation at 300° (0.05 mm.).

Anal. Calcd. for $C_{14}H_9N_5O_5$: C, 51.4; H, 2.8; N, 21.4. Found: C, 51.7; H, 2.5; N, 22.0.

Method B.—A suspension of 0.25 g. of 2-(2-chloro-5-nitrophenyl)-6-nitro-4(3*H*)quinazolone in 10 ml. of concentrated ammonium hydroxide was heated in a steel bomb for 15 hours at 150°. The bomb was then cooled, vented in the hood and the reaction mixture diluted with 15 ml. of water and filtered; yield 0.22 g. (93%), m.p. >360°. The orange product was purified by recrystallization from aqueous dimethylformamide followed by vacuum sublimation at 290° (0.05 mm.). Examination of its infrared and ultraviolet spectra showed it to be identical with the product obtained by method A.

2-Amino-5-methylbenzonitrile was prepared by reduction of 2-nitro-5-methylbenzonitrile with tin and hydrochloric acid according to the method described by Findeklee.[8]

2-Nitro-5-bromobenzonitrile.—To a well-stirred solution of 7.6 g. of potassium nitrate in 50 ml. of concentrated sulfuric acid was added in small portions and at room temperature 13.05 g. of 3-bromobenzonitrile.[9] The resulting mixture was warmed on a steam-bath to 70°, allowed to cool slowly to room temperature and poured into 500 g. of crushed ice. The resulting suspension of light tan solid was stirred for 30 minutes and filtered, and the collected solid washed thoroughly with water and dried: yield 12.7 g. (78%), m.p. 95–103°. Recrystallization from ethanol with the use of charcoal afforded stout yellow needles, m.p. 115–117°.

Anal. Calcd. for $C_7H_3NO_2Br$: C, 37.1; H, 1.3. Found: C, 36.9; H, 1.3.

2-Amino-5-bromobenzonitrile.—To a well-stirred suspension of 20 g. of mossy tin in 22.4 ml. of 25% hydrochloric acid was added in small portions 7.7 g. of 2-nitro-5-bromobenzonitrile. The flask was cooled occasionally in an ice-bath to prevent the temperature of the reaction mixture from rising above 40°. After 3.5 hours of stirring, the mixture was diluted with 30 ml. of water, filtered to remove unreacted tin, and the filtrate treated with 120 ml. of 15% sodium hydroxide. It was then extracted with three 50-ml. portions of ether, the combined ether extracts dried over anhydrous potassium carbonate and the ether removed by evaporation to give 4.45 g. (66%) of crude yellow amine, m.p. 89–95°. Recrystallization from carbon tetrachloride followed by vacuum sublimation at 90° (0.1 mm.) gave glistening white needles, m.p. 96–97°.

(8) W. Findeklee, *Ber.*, **38**, 3542 (1905).

(9) T. Sandmeyer, *ibid.*, **18**, 1492 (1885).

Anal. Calcd. for $C_7H_5N_2Br$: C, 42.7; H, 2.5. Found: C, 43.0; H, 2.7.

The structure of this material was confirmed by alkaline hydrolysis to 2-amino-5-bromobenzoic acid, m.p. 217–218°, which was shown by a mixture melting point determination and by comparison of infrared spectra to be identical with an authentic sample of 2-amino-5-bromobenzoic acid prepared by direct bromination of anthranilic acid by the method of Wheeler.[10]

2-(4-Nitrophenyl)-4-aminoquinazoline (XIX).—A mixture of 2.36 g. of 2-aminobenzonitrile, 3.7 g. of 4-nitrobenzonitrile and 20 ml. of methanolic ammonia was heated in a steel bomb at 180–190° for 3.5 hours. The bomb was allowed to cool and was vented in a hood. The reaction mixture was filtered, the filtrate cooled and refiltered, and the combined solids repeatedly extracted with boiling water to give 2.5 g. (48%) of insoluble yellow crystals, m.p. 211–214°. Recrystallization from acetone raised the melting point to 220–221°.

Anal. Calcd. for $C_{14}H_{10}N_4O_2$: C, 63.15; H, 3.8; N, 21.0. Found: C, 63.3; H, 3.6; N, 21.2.

N-(2-Cyanophenyl)-4-nitrobenzamide (XXI).—A mixture of 2.0 g. of 2-aminobenzonitrile, 3.12 g. of 4-nitrobenzoyl chloride and 17 ml. of dry pyridine was heated gently until it boiled, and then refluxed for 2 hours. It was then poured into 70 ml. of water and the precipitated white platelets collected by filtration and dried; yield 4.2 g. (93%), m.p. 229–230°. Recrystallization from ethanol did not alter the melting point.

Anal. Calcd. for $C_{14}H_9N_3O_3$: C, 62.9; H, 3.4; N, 15.7. Found: C, 63.2; H, 3.4; N, 15.8.

2-(4-Nitrophenyl)-4(3*H*)-quinazolone (XX). Method A.—To a well-stirred suspension of 0.1 g. of 2-(4-nitrophenyl)-4-aminoquinazoline in 4 ml. of 50% sulfuric acid was added 0.05 g. of solid sodium nitrite and the mixture was stirred at room temperature for 5 minutes, heated under reflux for 2 hours and then cooled and diluted with water. Filtration yielded 0.075 g. (75%) of a cream-colored solid, m.p. >360°, which was recrystallized from dimethylformamide to give a yellow solid. This compound is reported to melt at 351–352° (uncor.).[5]

Anal. Calcd. for $C_{14}H_9N_3O_3$: C, 62.9; H, 3.4; N, 15.7. Found: C, 63.1; H, 3.3; N, 15.6.

Method B.—To a suspension of 0.5 g. of N-(2-cyanophenyl)-4-nitrobenzamide in 10 ml. of 16% sodium hydroxide was added 20 ml. of 3% hydrogen peroxide and the mixture was heated cautiously until the initial vigorous reaction had subsided. It was then heated under reflux for 1 hour, cooled, treated with 5 ml. of 3% hydrogen peroxide and heated under reflux for an additional 45 minutes. Cooling and filtering yielded 0.25 g. (50%) of a yellow solid, m.p. >360°, which was recrystallized from dimethylformamide. Comparison of its infrared and ultraviolet absorption spectra with those given by the product obtained by method A showed the two compounds to be identical.

2-(4-Nitrophenyl)-4-amino-6-nitroquinazoline (XXII).—A mixture of 1.6 g. of 2-amino-5-nitrobenzonitrile, 2.4 g. of 4-nitrobenzonitrile and 20 ml. of methanolic ammonia was heated in a steel bomb at 180–190° for 3.5 hours. The bomb was cooled, vented in a hood, and the contents filtered. The collected solid was dried, pulverized and repeatedly extracted with boiling water. The insoluble material (2.65 g. (87%), m.p. 286–288°) was recrystallized from dimethylformamide to give yellow crystals, m.p. 303–304°.

Anal. Calcd. for $C_{14}H_9N_5O_5$: C, 54.0; H, 2.9; N, 22.5. Found: C, 54.0; H, 3.0; N, 22.9.

N-(2-Cyano-4-nitrophenyl)-4-nitrobenzamide.—A mixture of 1.3 g. of 2-amino-5-nitrobenzonitrile, 1.5 g. of 4-nitrobenzoyl chloride and 8 ml. of dry pyridine was heated gently until refluxing commenced, and refluxed for 2 hours. The reaction mixture was poured into 60 ml. of cold water and the brown solid which separated collected by filtration and washed with water; yield 1.9 g. (77%), m.p. 194–199°. Recrystallization from ethanol–acetone (1:1) raised the melting point to 199–199.5°.

Anal. Calcd. for $C_{14}H_8N_4O_5$: C, 53.85; H, 2.6; N, 17.95. Found: C, 54.0; H, 2.6; N, 17.6.

2-(4-Nitrophenyl)-6-nitro-4(3*H*)-quinazolone (XXIII). Method A.—To a well-stirred solution of 0.1 g. of 2-(4-nitro-

(10) A. S. Wheeler, THIS JOURNAL, **31**, 565 (1909).

phenyl)-4-amino-6-nitroquinazoline in 4 ml. of 50% sulfuric acid was added 0.05 g. of sodium nitrite and the mixture was allowed to stir for 5 minutes at room temperature. It was then heated under reflux for 2 hours, cooled, diluted with water and filtered; yield 0.078 g. (78%). Recrystallization from dimethylformamide gave a tan solid, m.p. 317–318° dec.

Anal. Calcd. for $C_{14}H_8N_4O_5$: C, 53.85; H, 2.6; N, 17.95. Found: C, 53.6; H, 2.75; N, 18.0.

Method B.—A suspension of 0.5 g. of N-(2-cyano-4-nitrophenyl)-4-nitrobenzamide in 10 ml. of 16% sodium hydroxide was treated with 20 ml. of 3% hydrogen peroxide and the mixture heated cautiously until the initial vigorous reaction had subsided. It was then heated under reflux for 1 hour, treated with an additional 5 ml. of 3% hydrogen peroxide, and heated again for 45 minutes. The cooled reaction mixture was filtered and the brown solid which was collected was stirred for 10 minutes with 5% sulfuric acid. Filtration yielded 0.24 g. (48%) of crude product which was recrystallized from dimethylformamide to give a tan product, m.p. 315–316° dec. The compound was identical with the product obtained by method A above, as judged by a comparison of both infrared and ultraviolet absorption spectra.

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CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONN., AND THE EASTERN REGIONAL RESEARCH LABORATORY,[1] PHILADELPHIA 18, PENNA.]

Molecular Interactions in β-Lactoglobulin. I. The Electrophoretic Heterogeneity of β-Lactoglobulin Close to its Isoelectric Point

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An electrophoretic study of β-lactoglobulin was carried out close to its isoelectric point. It was shown that the heterogeneity observed at *p*H 5.3–5.6 is due primarily to the presence of the two genetic species. Calibration curves for composition analysis have been prepared and the various nomenclatures found in the literature have been reconciled in terms of the Aschaffenburg β-lactoglobulins A and B.

Introduction

The electrophoretic heterogeneity of crystalline preparations of β-lactoglobulin has been observed by a number of investigators.[2–9] Depending on the conditions, two, three or even four electrophoretic components can be observed, especially if the experiment is permitted to proceed to maximal resolution, which in some cases requires as long as 24 hours.[8] A certain amount of disagreement exists among the various authors as to whether β-lactoglobulin is heterogeneous electrophoretically at any given set of conditions, especially above its isoelectric point (*p*H 5.1–5.3). Thus, Li[2] reported that, while at the *p*H's of 5.3 and 5.6, this protein was homogeneous, at *p*H 4.8 and 6.5 it resolved into three components, the composition being different at the two *p*H's. Polis and coworkers,[5] working at *p*H 4.8 in a 0.1 ionic strength acetate buffer, concluded that β-lactoglobulin consists of two components with mobilities of 1.9 and 3.0 × 10^{-5} cm.^{2}/v. sec. under those conditions. They assigned the name of β_1-lactoglobulin to the slow component and β_2-lactoglobulin to the rapid component. Furthermore, they reported the isolation of β_1-lactoglobulin in pure form and its identification as a true molecular entity. These authors, however, did not observe any heterogeneity in the *p*H region alkaline to the isoelectric point, as Li had reported.[2] Smithies[7] reported that, contrary to the findings of Li and of Polis, this protein was heterogeneous electrophoretically in the *p*H region of 5.3–5.5 but concluded that the patterns were too complicated to determine from the composition of the protein. Ogston and Tilley[9] have carried out electrophoretic experiments as a function of temperature at *p*H 4.66 and correlated these with some ultracentrifuge experiments. They concluded that at least part of the heterogeneity of β-lactoglobulin observed at that *p*H is the result of reversible association favored by low temperature, low *p*H and high protein concentration. They concluded further that the situation is complicated by the presence of probably two species of β-lactoglobulin, only one of which is capable of associating. Working as a function of *p*H, the present authors[10] have found that the association observed by Ogston and Tilley[9] is limited to the *p*H region between 3.5 and 5.2, thus eliminating the possibility of intermolecular interactions as the explanation for electrophoretic heterogeneity in the *p*H region below 3.5 or above 5.2.

In 1955, Aschaffenburg and Drewry,[11] working with milk obtained from individual cows, showed by paper electrophoresis (*p*H 8.6, 0.05 ionic strength veronal buffer) that β-lactoglobulin consists of two genetically different proteins and that furthermore different cows may produce either one of the two species of this protein or a mixture of the two. These authors initially assigned the names of β_1-lactoglobulin to the rapidly migrating protein and β_2-lactoglobulin to the slowly migrating one but later changed the nomenclature to β-lactoglobulin A (β-A) and β-lactoglobulin B (β-B), respectively.[12] Each one of these components, however, was still

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