before[1] for the first three lines of the table and more precise than those in the literature for the third[5] and the fourth[6] line of the table.

Since the carbon skeleton of the molecules is not altered during the rearrangements, as shown before[1] by hydrogenation to known paraffins, the problem of identification is merely one of locating the double bonds. This was done by oxidation with ozone, using experimental procedures described previously.[7,8] The ozonization results are listed in the notes of the table.

Comments

For double bond displacement chromia-coated alumina proved considerably more efficient than plain alumina. It permitted operation at 250° instead of 365° and reduced polymer formation.

The ease of rearrangement was found contradictory. In agreement with our first experiments, terminal bonds shifted inwards with ease, particularly well over a methyl group in *iso*-position; yet two compounds so constructed failed to rearrange.

(5) Bjelouss, *Ber.*, **43**, 2332 (1910).
(6) v. Auwers and Westermann, *ibid.*, **54**, 2993 (1921).
(7) Henne and Hill, THIS JOURNAL, **65**, 752 (1943).
(8) Henne and Perilstein, *ibid.*, **65**, 2183 (1943).

Summary

Twelve dienes of the 1,5-type were subjected to double bond shift at 250° over a catalyst made of chromia and alumina. Seven were transformed to conjugated diolefins, while five failed to rearrange to any appreciable extent.

COLUMBUS, OHIO RECEIVED OCTOBER 20, 1943

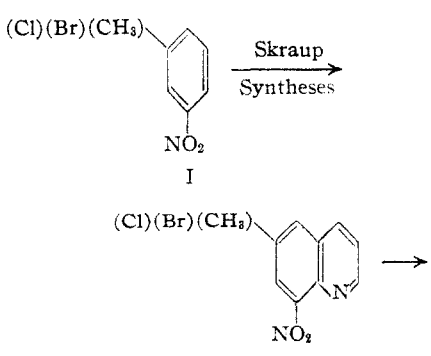
[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

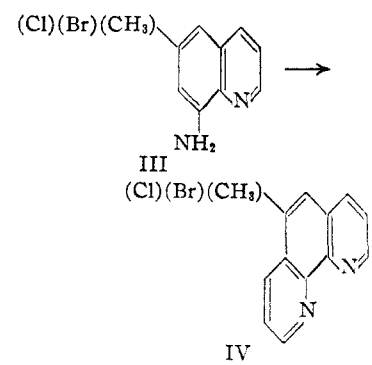
Derivatives of 1,10-Phenanthroline[1]

BY FREDERIC RICHTER[2] AND G. FREDERICK SMITH

Although the application of the Skraup synthesis to 1,2-phenylenediamines suitably substituted in either the 4- and/or 5-positions might appear to be the most direct approach to the synthesis of 5 (6)-substituted (1,10)-phenthrolines the side reactions encountered practically preclude the isolation of the desired products.[3]

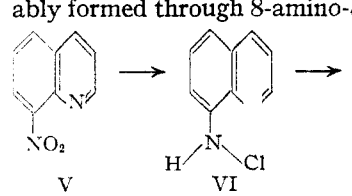
Application of the method of Skraup to suitably substituted 2-nitroanilines (I) followed by reduction of the resulting 8-nitroquinolines (II) and application of a second Skraup synthesis to the 8-aminoquinolines so formed (III) is the basis for the present series of syntheses





From the standpoint of orientation, at least, 2-nitroanilines substituted in either the 4- or 5-positions could be employed because these positions later become equivalent in 1,10-phenanthroline. In the present series only 4-substituted 2-nitroanilines were employed.

The 5(6)-chloro(1,10)-phenanthroline obtained by the above scheme agreed in melting point and other properties with that obtained by Kuczynski and Sucharda[4] who heated a mixture of 8-nitroquinoline, glycerol and hydrochloric acid under pressure. It is of interest to note that the chlorophenanthroline obtained by this method is probably formed through 8-amino-5-chloroquinoline

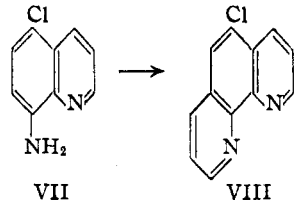


(1) This contribution contains material from a dissertation presented by Frederic Richter to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1941.

(2) Aeration Processes Corporation Fellow in chemistry during the years 1939–1941. Present address: United States Rubber Company, Hogansville, Georgia.

(3) C. R. Smith, THIS JOURNAL, **52**, 397 (1930).

(4) Kuczynski and Sucharda, *Roczniki Chem.*, **16**, 513 (1936).



VII VIII

The first step is postulated as involving N-chlorination of (V). This is followed by rearrangement to (VII) and finally conversion to the phenanthroline. Under no condition would one expect the chlorine atom of (VI) to migrate to the 6-position (meta rearrangement). In the present series the chloro phenanthroline is obtained through 8-amino-6-chloroquinoline (III) and the fact that both products appear to be identical is good evidence for the equivalence of the 5- and 6-positions in 1,10-phenanthroline. This in turn points to a symmetrical structure for the base.

The synthesis of a 5(6)-bromo(1,10)-phenanthroline by a Skraup reaction on 4-bromo-1,2-phenylenediamine was previously claimed[5] and the melting point reported as 215° whereas the bromo derivative (IV) was found to melt at 119°. The analysis of our product and the correlation between the absorption spectra of the ferrous complexes of (IV) and the parent base, together with the difficulties encountered in attempting a synthesis from a 1,2-phenylenediamine indicate that the previously claimed[5] synthesis of 5(6)-bromo-(1,10)-phenanthroline is incorrect.

Attempts to obtain a 5(6) carboxylic acid by oxidation of the methyl derivative (IV) failed even under the mildest conditions, presumably because of the rupture of the 5,6-double bond. This is entirely analogous to the case of the corresponding methylphenanthrene where oxidation readily takes place at the 9,10-double bond to produce 2,2′-biphenyldicarboxylic acid.

The 5(6)-methyl derivative was found to undergo nitration with greater ease than the unsubstituted base. Under the same conditions, *viz.*, nitrating mixture, temperature and time, the methylphenanthroline gave a 20–25% higher yield of its nitration product than did the parent compound. This result was to be expected in view of the analogous case of toluene and benzene, the former undergoing the usual substitution reactions more readily than the latter. Nitration of either the methyl derivative or the parent compound undoubtedly takes place in the 5- or 6-position since one would hardly expect substitution to occur in the pyridine rings under the conditions employed.

Excellent correlation was found between the absorption spectra of the ferrous complexes of (IV) and that of the parent base over the wave length region of 400–670 millimicrons.

Experimental

Because of the difficulties encountered in the preliminary stages of this investigation in controlling the Skraup reactions a general procedure was developed. It was found that the directions usually given[6] include a ratio of sulfuric acid to glycerol which is too high and which usually results in excessive carbonization with consequent loss of yield. The best mass relations found were: one mole of the aromatic amine, four moles of glycerol (dried by passing dry air through the liquid for three hours at 170–180°), three-fourths mole of arsenic pentoxide and a weight of sulfuric acid (sp. gr. 1.84) equal to about 50–60% of the weight of glycerol employed. The use of stronger sulfuric acid, *e. g.*, 10–20% oleum, did not present any advantage.

In general, the amine, glycerol and arsenic oxide were weighed into a three-necked flask. This was then equipped with a glycerol-sealed stirrer, thermometer dipping into the liquid and a Pyrex water-cooled condenser with a calcium chloride tube for eliminating atmospheric moisture. The sulfuric acid was added through the condenser with vigorous stirring, and at such a rate as to maintain the temperature below 130°. The temperature was then held at 130–135° for one hour and finally the mixture was kept in active reflux for six additional hours. Longer heating did not improve the yields.

After cooling, the tarry reaction mixture was poured into water, neutralized in the cold with aqueous ammonia and the quinoline or phenanthroline recovered by filtration or extraction with boiling benzene. The nitroquinolines were employed without further purification. The reduction of the 6-chloro- and 6-bromo-nitroquinolines was accomplished with stannous chloride in hydrochloric acid solution; the nitromethylquinoline was reduced with ammonium sulfide formed *in situ* by passing hydrogen sulfide into an ammoniacal ethanol solution of the base.

8-Nitro-6-chloroquinoline.—Yield of dry, crude product 47–48 g. from 43.1 g. (0.25 mole) of 4-chloro-2-nitroaniline. A portion was recrystallized from ethanol, m. p. 159°.

5(6)-Chloro-(1,10)-phenanthroline.—Needles from benzene–petroleum ether, m. p. 123°; yield 24.0 g. (56%) from 35.7 g. (0.2 mole) of 8-amino-6-chloroquinoline.

Anal. Calcd. for $C_{12}H_7N_2Cl$: C, 67.13; H, 3.29; N, 13.06. Found: C, 67.17; H, 3.39; N, 12.92.

8-Nitro-6-bromoquinoline.—Yield of dry, crude product 60 g. from 54.3 g. (0.25 mole) of 4-bromo-2-nitroaniline, m. p. 170° (from ethanol).

8-Amino-6-bromoquinoline.—Recrystallized from petr. ether, m. p. 78°; yield 34.0 g. or 61% based on the 4-bromo-2-nitroaniline taken.

5(6)-Bromo-(1,10)-phenanthroline.—The monohydrate crystallized as white needles from moist benzene, m. p. 86°.

Anal. Calcd. for $C_{12}H_7N_2BrH_2O$: H_2O, 6.51. Found: H_2O, 6.93.

The anhydrous compound was obtained either by drying the monohydrate in a pistol (ethanol 1/2 mm.) or by recrystallizing from chloroform after distilling out the water of hydration, m. p. 119°; yield 11.8 g. or 46% based on the 8-amino-6-bromoquinoline (0.1 mole) taken.

Anal. Calcd. for $C_{12}H_7N_2Br$: C, 55.61; H, 2.72; N, 10.81. Found: C, 55.73; H, 2.85; N, 10.56.

5(6)-Bromo-(1,10)-phenanthroline Monopicrate.—Yellow needles from 95% ethanol, m. p. 228°.

Anal. Calcd. for $C_{18}H_{10}N_5O_7Br$: N, 14.34. Found: N, 14.28.

8-Nitro-6-methylquinoline.—Yield of dry, crude product 38-40 g. from 38.0 g. (0.25 mole) of 3-nitro-4-aminotoluene, m. p. 121–122° (from ethanol).

8-Amino-6-chloroquinoline.—Recrystallized from petroleum ether, m. p. 73°; yield 24.3 g. or 54% based on the 4-chloro-2-nitroaniline.

8-Amino-6-methylquinoline.—Recrystallized from petroleum ether, m. p. 60–62°; yield 19.2 g. or 49% based on the 3-nitro-4-aminotoluene.

5(6)-Methyl-(1,10)-phenanthroline.—The residue from the benzene extract was distilled *in vacuo* and the fraction,

(5) N. V. Nederlandsche Kininefabriek, French Patent 804,454.

(6) E. G. Houben-Weyl, "Die Methoden der organischen Chemie," Part 2, Vol. I, p. 369.

b. p. 280–282° (13 mm.), recrystallized from benzene-petroleum ether, m. p. 114°; yield 38.2 g. or 66% based on the 8-amino-6-methylquinoline (0.3 mole) taken.

Anal. Calcd. for $C_{18}H_{10}N_2$: C, 80.38; H, 5.19; N, 14.43. Found: C, 80.58; H, 5.28; N, 14.60.

5(6)-Methyl-(1,10)-phenanthroline Monopicrate.—Fine yellow needles from 95% ethanol; m. p. 203–204°.

Anal. Calcd. for $C_{19}H_{13}N_5O_7$: C, 53.89; H, 3.10; N, 16.54. Found: C, 53.96; H, 3.27; N, 16.67.

5-Nitro-6-methyl-(1,10)-phenanthroline.—A mixture of 1.00 g. of 5(6)-methyl-(1,10)-phenanthroline (m. p. 114°), 5 cc. of sulfuric acid (sp. gr. 1.84) and 3 cc. of nitric acid (sp. gr. 1.42) was held at 120° for two hours. The yellow nitration mixture was poured onto 50 g. of ice and the cold solution neutralized with 30% sodium hydroxide. The precipitate was filtered off and dried: fine yellow needles from 95% ethanol; m. p. 268–270°; yield 0.98 g. (80%).

Anal. Calcd. for $C_{13}H_9N_3O_2$: N, 17.57. Found: N, 17.69.

Acknowledgment.—The authors gratefully acknowledge the valuable suggestions offered by Professor C. S. Marvel in the course of this work.

Summary

1. A procedure for the synthesis of 5(6) derivatives of 1,10-phenanthroline has been described.

2. The previously reported synthesis of 5(6)-bromo-1,10-phenanthroline has been proved erroneous.

3. Evidence for the equivalence of the 5- and 6-positions in 1,10-phenanthroline has been presented. This in turn indicates a symmetrical structure for 1,10-phenanthroline.

HOGANSVILLE, GEORGIA RECEIVED NOVEMBER 15, 1943
URBANA, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, MEDICAL RESEARCH DIVISION, SHARP & DOHME, INC.]

Tryptic Digestion of Bovine Serum and Other Proteins in the Presence of Ethyl Alcohol

BY EDWIN A. RISLEY, ANN C. BUFFINGTON AND L. EARLE ARNOW

In connection with some problems under investigation in this Laboratory, it became desirable to prepare tryptic digests of bovine serum. The chief difficulties encountered revolved around the slow digestion of relatively concentrated serum solutions and the development of putrefaction after a few days of digestion. It was found that both of these difficulties could be overcome if suitable amounts of ethyl alcohol were included in the digestive mixtures.

The effect of alcohol on tryptic digestion has not been investigated extensively. In 1896 Chittenden and Mendel[1] found that the digestion of fibrin by pancreatic extracts was inhibited by increasing concentrations of alcohol. However, appreciable digestion still occurred in 20% alcoholic solution. Vernon[2] stated that extracts of pancreas prepared with 25% alcohol were slightly less active in digesting fibrin than were similar extracts prepared with saline solution. Gizelt,[3] using egg white in Mett tubes as a substrate and pancreatic juice as a source of enzyme, found that alcohol in concentrations less than 20% inhibited enzymatic activity. Concentrations of 20% or higher abolished all enzymatic activity. Bayliss[4] suspended crude trypsin in 80% alcohol. This suspension was reported to digest gliadin slowly. Since most of the tryptic activity could be removed from the suspension by simple filtration, Bayliss concluded that trypsin in an insoluble form must still be active. Edie[5] concluded that the action of crude trypsin on fibrin was inhibited to some degree by concentrations of alcohol higher than 3%. Some digestion took place in 25% alcohol, but none was detected at 50%. On the other hand, no appreciable inhibition of digestion of casein was noted until the alcohol concentration was raised to 10% and, indeed, digestion in 25% alcohol was almost as efficient as in non-alcoholic media. Some digestion of casein occurred even in the presence of 50% alcohol. Abderhalden and Reich[6] studied the influence of various alcohols on the rate of tryptic digestion of *d,l*-leucylglycyl-*d,l*-leucine. No significant inhibition of digestion in concentrations of ethyl alcohol of 10% or lower was detected. Twenty per cent. alcohol markedly inhibited digestion of the peptide.

Experimental

The bovine serum used in these experiments was supplied to us in spray dried form through the courtesy of Dr. David Klein of the Wilson Laboratories. Difco trypsin, 1:250, was used as a source of enzyme. The alcohol employed was 95% ethyl alcohol. A micro-Kjeldahl method was used for nitrogen determinations. Amino nitrogen determinations were done with the Koch[7] modification of the Van Slyke[8] micro-apparatus.

Van Slyke[9] pointed out many years ago that the presence of alcohol causes the formation of excess gas in the amino nitrogen apparatus. Provided the concentration of alcohol is not too high, the excess gas can be removed by increasing the shaking time during absorption. In all the analyses reported in this paper, the solutions were diluted and, in the great majority of instances, contained approxi-

(1) R. H. Chittenden and L. B. Mendel, *Am. J. Med. Sci.*, **111**, 181 (1896).
(2) H. M. Vernon, *J. Physiol.*, **26**, 405 (1901).
(3) A. Gizelt, *Arch. ges. Physiol.*, **111**, 620 (1906).
(4) W. M. Bayliss, *J. Physiol.*, **50**, 85 (1915).
(5) E. S. Edie, *Biochem. J.*, **13**, 219 (1919).
(6) E. Abderhalden and F. Reich, *Fermentforsch.*, **11**, 64 (1929).
(7) F. C. Koch, *J. Biol. Chem.*, **84**, 601 (1929).
(8) D. D. Van Slyke, *ibid.*, **16**, 121 (1913).
(9) D. D. Van Slyke, *ibid.*, **12**, 275 (1912).