acid. Methoxyl group: calcd. for $C_{10}H_{11}O\cdot OCH_3$, 17.4%; found 17.4%. Mixed melting point determination with dehydroperillic acid supplied by Dr. A. B. Anderson, m. p. 88°.

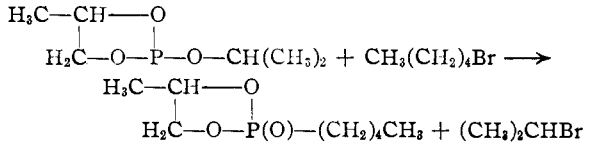
OREGON FOREST PRODUCTS LABORATORY AND
DEPARTMENT OF CHEMISTRY
OREGON STATE COLLEGE
CORVALLIS, OREGON RECEIVED JULY 31, 1950

Phosphonation with a Phosphite Ester of Propanediol

BY F. W. MITCHELL, JR.,[1] AND H. J. LUCAS

The reaction of trialkyl phosphites with alkyl halides in the Arbuzov reaction[2] is essentially a phosphonation reaction, since one product is an ester of a phosphonic acid. The reaction has been studied fairly extensively with acyclic esters,[3] and has been extended recently to cyclic trialkyl phosphites derived from ethanediol and 3-methoxy-1,2-propanediol.[4] Whereas ethyl ethylene phosphite (2-ethoxy-1,3,2-dioxaphospholane)[5] underwent the isomerization reaction with ring opening, the presence of the methoxymethyl side chain in esters of 3-methoxy-1,2-propanediol led to ring stabilization. When the isomerization reagent was ethyl bromide the reaction product was the cyclic ester of ethanephosphonic acid.

In connection with work on cyclic trialkyl phosphites derived from glycols[5] it has been found



that 2-isopropoxy-4-methyl-1,3,2-dioxaphospholane (isopropyl propylene phosphite) when heated with 1-bromopentane undergoes the Arbuzov reaction without ring opening. The product is propylene pentane-1-phosphonate (2-oxo-2-*n*-amyl-4-methyl-1,3,2-dioxaphospholane). The recovery of isopropyl bromide in 78% yield indicates this is essentially the sole reaction. The stability of the ring system in this case is in agreement with the conclusions of the Russian workers,[4] that a side chain in the glycol residue increases the stability of the ring system.

Experimental

In a boiler attached to a distillation column held at 60°, 0.2 mole (32.8 g.) of 2-isopropoxy-1,3,2-dioxaphospholane and 0.2 mole (30.2 g.) of 1-bromopentane, b. p., 127–127.5°, were heated at refluxing temperature for a period of 9 hours, during which time isopropyl bromide slowly distilled; weight 19 g. (78% yield); b. p. 57.5–58.5° at 745 mm.; $n^{25}D$ 1.4216 (literature value 1.4251). Distillation of the residue gave 28 g. (73% yield) of propylene pentane-1-phosphonate, a slightly viscous liquid, b. p. 131–132° at 1.5 mm., $n^{25}D$ 1.4481, d^{25}_4 1.1052.

Hydrolysis of 10 g. (0.052 mole) with 6 *N* HCl[6] for 30 minutes, followed by slow distillation with paraformaldehyde according to the procedure of Senkus[7] gave 3 ml. (63% yield) of formal. Excess formaldehyde was removed from the boiler liquid as methalal, b. p. 44–45°, and the water was evaporated at reduced pressure, leaving a dark brown oil which partly solidified overnight. Oil and solid were separated from each other by flotation with a carbon tetrachloride–ligroin mixture. Crystallization of the solid from hot ligroin gave 2.3 g. (25% yield) of thin colorless plates, m. p. 120–122°, believed to be pentane-1-phosphonic acid.[8]

(1) National Aniline and Film Corporation, Easton, Pa.

(2) A. E. Arbuzov and A. A. Dunin, *J. Russ. Phys.-Chem. Soc.*, **46**, 295 (1914); A. E. Arbuzov, "On the Structure of Phosphorous Acid," N. Alexandria, 1905.

(3) G. M. Kosolapoff, THIS JOURNAL, **66**, 109 (1944). A number of references to earlier work are given.

(4) A. E. Arbuzov, V. M. Zoroaster and N. T. Rizpolozhenskil, *Bull. acad. sci. U. R. S. S. Classe sci. chim.*, 208, 1948; *cf. C. A.*, **42**, 4932 (1948).

(5) H. J. Lucas, F. W. Mitchell, Jr., and C. N. Scully, THIS JOURNAL, **72**, 5491 (1950).

(6) Similar to the procedure of G. M. Kosolapoff, *ibid.*, **67**, 1180 (1945), who used concentrated acid, however.

(7) M. Senkus, *Ind. Eng. Chem.*, **38**, 913 (1946), recovered 2,3-butanediol from dilute aqueous solutions by conversion to the formal.

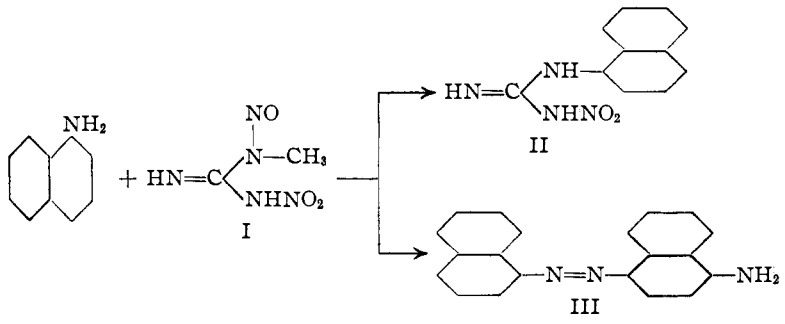
(8) Of the fourteen normal alkanephosphonic acids listed by Kosolapoff[6] none of the others melted higher than 106°.

CONTRIBUTION NO. 1437 FROM THE
GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA 4, CALIF. RECEIVED JULY 20, 1950

A New Method for the Preparation of Azo Dyes[1]

BY EUGENE LIEBER AND KONRAD PARKER[2]

While investigating the formation of N-Ar-N′-nitroguanidines, where Ar is a polycyclic aromatic hydrocarbon radical, by the method of McKay and Wright[3] using N-methyl-N-nitroso-N′-nitroguanidine (I), it was observed that α-naphthylamine gave rise to two products; N-(α-naphthyl)-N′-nitroguanidine (II), the expected product, in 25% yield (based on the nitroso compound used), and a large amount of dark brownish-green needles having dye properties. This was subsequently identified as 4-amino-α,α-azo-naphthalene[4] and amounted to 50% of the theoretical yield



(1) Studies in the Guanidine Series. VIII.

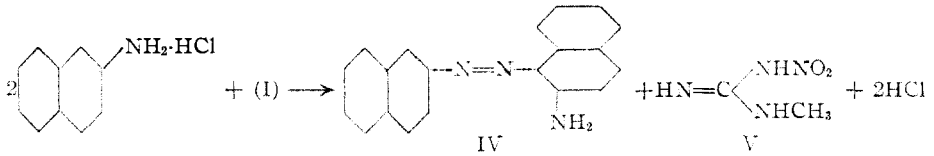
(2) Abstracted from a portion of the thesis submitted by Konrad Parker to the Graduate School of Illinois Institute of Technology in partial fulfillment of the requirements for the degree, Master of Science.

(3) A. F. McKay and G. F Wright, THIS JOURNAL, **69**, 3028 (1947).

(4) Michaelis and Erdmann, *Ber.*, **28**, 2198 (1895).

and hence comprised the major product. β-Naphthylamine, on the other hand, gave only one product, the expected N-(β-naphthyl)-N′-nitroguanidine in 65% yield.

The unexpected formation of the azo dye in large yield in the reaction of α-naphthylamine with N-methyl-N-nitroso-N′-nitroguanidine prompted a study of the reaction of aromatic amine hydrochlorides with the same reagent. With both the hydrochlorides of α- and β-naphthylamine, only one product was obtained, namely, the azo dye in practically quantitative yield. For example, β-naphthylamine hydrochloride gave a 95% yield of 2-amino-α,β-azonaphthalene (IV). An examination of the by-product indicated that the reaction may be represented as



N-methyl-N′-nitroguanidine[5] (V) was isolated in 60% yield.

Further study has indicated that the reaction is a general one for similarly constituted nitrosamines. Nitrosoguanidine (VI) with β-naphthylamine hydrochloride gave a 90% yield of the azo dye (IV) and guanidine was isolated from the reaction mixture. In a similar manner methylnitrosourea (VII) also gave a 90% yield of the azo dye (IV).

A study of the literature including that of a recent monograph[6] has indicated that the reaction discussed above is a unique method for the preparation of azo dyes.

Experimental[7]

N-Methyl-N-nitroso-N′-nitroguanidine (I).—This was prepared from N-methyl-N′-nitroguanidine by the method of McKay.[5] The product had a m. p. of 116–118° and was obtained in 80% yield.

Reaction of (I) with α-Naphthylamine. Formation of N-(α-Naphthyl)-N′-nitroguanidine (II) and 4-Amino-α,α-azo-naphthalene (III).—To 5 g. (0.035 mole) of α-naphthylamine (m. p. 48°, test for chloride was negative) dissolved in 100 ml. of 80% ethanol, 2 g. (0.013 mole) of N-methyl-N-nitroso-N′-nitroguanidine was added with mechanical agitation. The orange-red solution turned deep red on standing at room temperature for 48 hours. After cooling, a mixed whitish-brown precipitate appeared which, after repeated recrystallization from ethanol, gave 0.3 g. (25%) of a white crystalline product, m. p. 214–216°. This was identified as N-(α-naphthyl)-N′-nitroguanidine.

Anal.[8] Calcd. for $C_{11}H_{10}N_4O_2$: C, 57.38; H, 4.38; N, 24.34. Found: C, 57.50; H, 4.47; N, 24.27.

The filtrate on dilution with water precipitated a greenish-brown powder which after recrystallization from ethanol gave 2.0 g. (50%) of brownish-green needles which were identified as 4-amino-α,α-azonaphthalene (III), m. p. 173–175°.

Anal. Calcd. for $C_{20}H_{15}N_3$: C, 80.78; H, 5.09; N, 14.13. Found: C, 80.58; H, 5.12; N, 14.17.

N-(β-Naphthyl)-N′-nitroguanidine.—To 6.5 g. (0.045 mole) of β-naphthylamine (m. p. 107°, test for chloride negative) dissolved in 150 ml. ethanol and cooled to 25°, was added 2.5 g. (0.017 mole) of N-methyl-N-nitroso-N′-nitroguanidine. The solution was allowed to stand at room temperature for 48 hours. The precipitate of coarse yellow crystals was collected on a Buchner funnel and washed with a small amount of alcohol. A yield of 2.8 g. (72%) of crude product was obtained. For purification the product was recrystallized twice from alcohol using decolorizing carbon. The purified product, m. p. 195–196°, was obtained in 65% yield.

Anal. Calcd. for $C_{11}H_{10}N_4O_2$: C, 57.38; H, 4.38; N, 24.34. Found: C, 57.70; H, 4.47; N, 24.50.

Reaction of N-Methyl-N-nitroso-N′-nitroguanidine with Naphthylamine Hydrochlorides. A. α-Naphthylamine Hydrochloride.—To 2.5 g. of N-methyl-N-nitroso-N′-nitroguanidine (0.017 mole) was added a solution of 8 g. (0.045 mole) of α-naphthylamine hydrochloride in 120 cc. 60% ethanol. After standing at room temperature for 48 hours followed by cooling, the deep purple solution deposited a precipitate of brown needles. A yield of 1.7 g. of product was collected by filtration and an additional 1.4 g. was obtained after diluting the filtrate to twice its volume with water. The total yield was quantitative. Recrystallization from 75% ethanol yielded a product having a m. p. 170–172°. It was found to be identical to the 4-amino-α,α-azo-naphthalene (III) isolated in the corresponding reaction with α-naphthylamine.

Anal. Calcd. for $C_{20}H_{15}N_3$: N, 14.13. Found: N, 14.62.

B. **β-Naphthylamine Hydrochloride. 2-Amino-α,β-azo-naphthalene (IV) and N-Methyl-N′-nitroguanide (V).**—To 2.5 g. (0.017 mole) of N-methyl-N-nitroso-N′-nitroguanidine was added a solution of 8 g. (0.045 mole) of β-naphthylamine hydrochloride in 120 ml. of 50% ethanol. After standing at room temperature for 48 hours followed by cooling, the deep purple solution deposited an orange powder. A total yield of 4.5 g. (93%) was obtained. Purification for analysis was effected by recrystallization from ethanol; orange crystals, m. p. 148°, identified as 2-amino-α,β-azo-naphthalene (IV).

Anal. Calcd. for $C_{20}H_{15}N_3$: C, 80.78; H, 5.09; N, 14.13. Found: C, 80.75; H, 5.25; N, 14.00.

The filtrate was evaporated under vacuum to 15 ml. and cooled. The resulting precipitate was filtered and recrystallized from hot water and twice from ethanol; yield 0.9 g. (62%), m. p. 160°. A mixed melting point with an authentic sample of N-methyl-N′-nitroguanidine[5] showed no depression.

Anal. Calcd. for $C_2H_6N_4O_2$: N, 47.42. Found: N, 47.40.

Reaction of Methylnitrosourea with β-Naphthylamine Hydrochloride.—To 1.1 g. (0.01 mole) of recrystallized methylnitrosourea[9] (m. p. 122°) was added a solution of

(5) A. F. McKay, THIS JOURNAL, **71**, 1968 (1948).

(6) K. H. Saunders, "The Aromatic Diazo Compounds," Longmans, Green and Co., New York, N. Y., 1949.

(7) All melting points are uncorrected.

(8) Micro-analyses by Micro-Tech Laboratories, Skokie, Illinois.

(9) F. Arndt, "Organic Syntheses," Coll. Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 461.

3.6 g. (0.02 mole) of β-naphthylamine hydrochloride in 50 ml. of 75% ethanol. After standing at room temperature for 48 hours, 10 ml. of water was added and the solution cooled. A yield of 2.65 g. (90%) of crude orange crystals was obtained which after recrystallization from ether–ethanol melted at 157°. The product was identified as 2-amino-α,β-azo-naphthalene (IV).

Anal. Calcd. for $C_{20}H_{15}N_3$: N, 14.13. Found: N, 14.10.

The filtrate was evaporated under vacuum in an attempt to recover methylurea. However, no residue was obtained.

Reaction of Nitrosoguanidine with β-Naphthylamine Hydrochloride.—To 1 g. (0.011 mole) of nitrosoguanidine[10] was added a solution of 4.0 g. (0.022 mole) of β-naphthylamine hydrochloride in 80 ml. of 50% ethanol. After standing at room temperature for 72 hours, the solution was diluted with 40 ml. of water and the orange-red precipitate filtered. A yield of 3 g. (90%) of 2-amino-α,β-azo-naphthalene was obtained.

Anal. Calcd. for $C_{20}H_{15}N_3$: N, 14.13. Found: N, 14.01.

To the filtrate was added 125 ml. of a 1% solution of ammonium picrate. The resulting precipitate was filtered off and weighed 1.5 g. (47%) and melted at 315° (dec.). This was identified as guanidine picrate by analysis.

Anal. Calcd. for $C_7H_8N_6O_7$: N, 29.11. Found: N, 28.93.

(10) E. Lieber and G. B. L. Smith, THIS JOURNAL, **57**, 2479 (1935).

DEPARTMENT OF CHEMISTRY
ILLINOIS INSTITUTE OF TECHNOLOGY
CHICAGO 16, ILLINOIS RECEIVED JUNE 26, 1950

Preparation of 6,7-d_2-Estrone Acetate

BY W. H. PEARLMAN AND M. R. J. PEARLMAN

Estrogens stably labeled with deuterium should prove very useful in metabolism experiments. A route for the preparation of such compounds is indicated in this report starting with Δ^6-dehydroestrone, a substance first prepared by Pearlman and Wintersteiner[1] from equilin and now obtainable in about 40% yield by aromatization of $\Delta^{1,4,6}$-androstatrienedione-3,17 by a procedure recently described by Rosenkranz, *et al.*[2] The conditions for the catalytic reduction of the equilin isomer to estrone were somewhat modified in the present study and deuterium gas was employed; the yield of 6,7-d_2-estrone acetate from Δ^6-dehydroestrone acetate was practically quantitative and the content of stably bound deuterium almost theoretical. Inasmuch as the partial synthesis of estradiol[3] and recently of estriol from estrone has been achieved, the preparation of these estrogens with deuterium in ring B seems feasible.

Experimental[4]

Ninety-eight milligrams of the acetyl derivative, m.p. 139–140°, of Δ^6-dehydroestrone, m.p. 260–262° (kindly furnished by Syntex, S. A., Mexico City, D. F., through the courtesy of Dr. G. Rosenkranz) was dissolved in 35 ml. of cyclohexane and shaken in deuterium at atmospheric pressure at 25° in the presence of 98 mg. of 5% palladium-on-charcoal catalyst (previously treated with deuterium); the uptake of gas ceased in about 20 minutes. The deuterated product was recovered and crystallized from alcohol to give 91 mg., m.p. 125–126°, which did not depress the melting point on admixture with estrone acetate, m.p. 125–126°. This product was refluxed for 1.5 hours with 5% potassium hydroxide in 90% methanol and then allowed to remain at room temperature for 48 hours. The estrogenic material was recovered, treated with acetic anhydride in pyridine for 24 hours and the acetate chromatographed over 2 g. of aluminum oxide (Harshaw Chemical Co.) and eluted with petroleum ether:ethyl ether (1:1) to yield 63.5 mg. of colorless material. It yielded, on crystallization from alcohol, a product, m.p. 125–126°, $[\alpha]^{29}D$ +152° ± 6° (abs. ethanol), ε 796, $\lambda^{alc.}_{max.}$ 270 mμ; ε 438, $\lambda^{alc.}_{min.}$ 250 mμ; *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 77.02; H, 7.70. Found: C, 76.55; H, 7.58. Isotope analysis[5]: found 8.23 atom % excess deuterium (theoretical value, 8.33, based on the introduction of 2 atoms of deuterium). This product did not depress the melting point on admixture with estrone acetate, m.p. 125–126°, $[\alpha]^{29}D$ +155 ± 6° (abs. ethanol), ε 798, $\lambda^{alc.}_{max.}$ 270 mμ; ε 441, $\lambda^{alc.}_{min.}$ 250 mμ.

Acknowledgment.—This investigation was supported by a grant-in-aid from the United States Public Health Service, under the National Cancer Institute Act.

(1) Pearlman and Wintersteiner, *J. Biol. Chem.*, **132**, 605 (1940); Pearlman and Wintersteiner, *Nature*, **165**, 815 (1950).

(2) Rosenkranz, Djerassi, Kaufman, Pataki and Romo, *ibid.*, **165**, 815 (1950).

(3) Estradiol may likewise be obtained by aromatization of $\Delta^{1,4,6}$-androstatrienol-17-one-3,17 acetate.[2]

(4) All melting points are corrected. The carbon and hydrogen analyses were performed by Mr. James Rigas.

(5) The "falling-drop method" was employed as described by Keston, Rittenberg and Schoenheimer, *J. Biol. Chem.*, **122**, 227 (1937–1938), and modified by M. Cohn in "Preparation and Measurement of Isotopic Tracers," J. W. Edwards, Ann Arbor, Mich., 1947.

DEPARTMENT OF BIOCHEMISTRY
JEFFERSON MEDICAL COLLEGE
PHILADELPHIA 7, PA. RECEIVED JUNE 30, 1950

The Use of Silver Nitrate and Sodium Dichromate in the Detection of Purines by Paper Partition Chromatography[1]

BY ROSE M. REGUERA AND ISAAC ASIMOV

The technique of paper partition chromatography has been frequently applied in the last few years to the determination of the nature and quantity of the purine and pyrimidine bases present in nucleic acids.[2–10] Vischer and Chargaff[3] have introduced a "sulfide-spot" technique to render the separated bases visible prior to quantitative photometric determination. This technique involves the precipitation of the bases as mercury salts, with subsequent conversion to mercuric sulfide.

(1) The work described in this paper was done with the aid of a grant from the United States Public Health Service.

(2) Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

(3) Vischer and Chargaff, *ibid.*, **176**, 703 (1948).

(4) Vischer and Chargaff, *ibid.*, **176**, 715 (1948).

(5) Chargaff, Vischer, Doniger, Green and Misani, *ibid.*, **177**, 405 (1948).

(6) Vischer, Zamenhof and Chargaff, *ibid.*, **177**, 429 (1948).

(7) Chargaff, Magasanik, Doniger and Vischer, THIS JOURNAL, **71**, 1513 (1949).

(8) Holiday and Johnson, *Nature*, **163**, 216 (1949).

(9) Markham and Smith, *Biochem. J.*, **45**, 294 (1949).

(10) Chargaff, Zamenhof and Green, *Nature*, **165**, 756 (1950).