covered material amounted to 1.04 g. (91% yield of bibenzyl). Recrystallization from ethanol gave a product, m.p. 52.5° (reported[10] 52°). A mixed melting point with an authentic sample of bibenzyl showed no depression. The infrared spectra were identical.

Alkaline Degradation of Dihydrostreptomycin

W. J. POLGLASE

Department of Biochemistry, University of British Columbia, Vancouver, Canada

Received January 2, 1962

Wolfrom and Polglase[1] have reported the preparation of a compound, designated decaacetyldideguanyldihydrostreptomycin, from dihydrostreptomycin, by alkaline degradation followed by acetylation. The intermediate compound, herein designated dideguanyldihydrostreptomycin was not isolated.[1] This latter compound was required for studies on the mechanism of action of streptomycin,[2] and the present note is concerned with its preparation and characterization.

Experimental

Dideguanyldihydrostreptomycin Bicarbonate.—Dihydrostreptomycin sesquisulfate (5.0 g.) was dissolved in 20 ml. of water and 250 ml. of a saturated solution of barium hydroxide was added. The barium sulfate precipitate was filtered off and the filtrate was refluxed for 28 hr. (until evolution of ammonia had ceased). The straw-colored reaction mixture was cooled and the excess of barium hydroxide was neutralized with carbon dioxide gas. The solution was filtered and concentrated to a sirup. The sirup was dissolved in warm methanol and filtered. Crystalline material (2.48 g., 68%) was obtained following cooling overnight. A portion of the product was recrystallized from hot methanol and dried at room temperature for analysis. M.p., 156°; $[\alpha]^{24}\text{D}$ −121° (*c* 1, water).

Anal. Calcd. for $(C_{19}H_{37}O_{12}N_3)_2 \cdot H_2CO_3$: C, 44.2; H, 7.2; N, 7.9. Found: C, 44.4; H, 7.4; N, 7.9.

Acetylation with acetic anhydride and pyridine gave the decaacetate (m.p. 259–260°) described previously.[1]

Dideguanyldihydrostreptomycin.—This was obtained from the bicarbonate derivative described above following heating for 2 hr. at 110° *in vacuo*.

Anal. Calcd. for $C_{19}H_{37}O_{12}N_3$: C, 45.6; H, 7.5; N, 8.3; N—CH_3, 5.8; C—CH_3, 5.4. Found: C, 45.3; H, 7.7; N, 8.1; N—CH_3, 5.0; C—CH_3, 5.6.

Dideguanyldihydrostreptomycin Trihydrochloride.—Dideguanyldihydrostreptomycin bicarbonate (2.0 g.) was suspended in 25 ml. of cold methanol and concd. hydrochloric acid was added dropwise until the mixture was acidic to Congo Red paper. The solution was filtered and seven volumes of acetone were added. The amorphous precipitate was collected by centrifuging, washed with acetone, and dried; yield 2.1 g. (91%).

Anal. Calcd. for $C_{19}H_{37}O_{12}N_3 \cdot 3HCl$: N, 6.9. Found: N, 6.9.

In a potentiometric titration of dideguanyldihydrostreptomycin trihydrochloride, 3 equivalents of sodium hydroxide were consumed. The two primary amino groups are weakly basic (*pK* about 6.3). The secondary amino group had a *pK* of 7.9, corresponding to the *pK* of this group in streptomycin.[3]

Acknowledgment.—This work was supported by a grant (M.T. 750) from the National Research Council of Canada.

---

[1] M. L. Wolfrom and W. J. Polglase, *J. Am. Chem. Soc.*, **70**, 2835 (1949).

[2] W. J. Polglase, to be published.

[3] J. Fried and O. Wintersteiner, *J. Am. Chem. Soc.*, **69**, 79 (1947).

The Nitration of 2′,5′-Dialkoxyacetophenones[1]

C. A. HOWE, V. J. PECORE, AND P. M. CLINTON

Department of Chemistry, Clarkson College of Technology, Potsdam, N. Y.

Received January 2, 1962

The compositions of the products obtained from nitration of 2′, 5′-diethoxy- and 2′, 5′-di-*n*-butoxyacetophenone have been determined and compared with those previously reported for 2′,5′-dimethoxyacetophenone.[2]

For nitrations carried out at −20° in concentrated nitric acid, all three 2′,5′-dialkoxyacetophenones yielded mixtures of the 4′- and 6′-nitro derivatives, with the latter accounting for approximately three fourths of the product (see Table I). A small increase in the proportion of 4′-substitution with increasing alkoxy group size was observed. The results are consistent with a direct attack on the ring by the electrophilic reagent[3] and with preferential stabilization of the transition state involved in 6′-substitution through conjugation of the 2′-alkoxy group with the carbonyl side chain.[2]

TABLE I

NITRATION[a] OF 2′,5′-DIALKOXYACETOPHENONES IN CONCENTRATED NITRIC ACID

Run	Alkoxy Groups	%, 4′Nitro[b]	%, 6′-Nitro[b]
A[c]	Dimethoxy	21	79
B[c,d]	Dimethoxy	23	77
1	Diethoxy	28 (26)	72 (74)
2[e]	Diethoxy	27	73
3	Di-*n*-butoxy	26	74
4	Di-*n*-butoxy	31 (31)	69 (69)

[a] At −20°; addition and aging periods 4 and 2.5 hr., respectively, unless otherwise noted. [b] Data in parentheses based on infrared analyses; all others based on alumina chromatography. [c] Ref. 2. [d] Addition and aging periods 90 and 15 min., respectively. [e] Addition and aging periods 1 and 2.5 hr., respectively.

Product compositions for nitrations carried out at −20° in a mixture of nitric and sulfuric acids

---

[1] Research supported in part by National Science Foundation Grant G7358.

[2] C. A. Howe, C. R. Hamel, E. D. Stedman, and F. Hyman, *J. Org. Chem.*, **25**, 1245 (1960).

[3] An increase in the proportion of 6′ substitution with increasing alkoxy group size would have provided evidence for attack *via* the carbonyl side chain [see K. L. Nelson, *J. Org. Chem.*, **21**, 153 (1956)].

TABLE II

NITRATION[a] OF 2′,5′-DIALKOXYACETOPHENONES IN NITRIC ACID–SULFURIC ACID MIXTURE

Run	Alkoxy Groups	%, Dinitro	%, 4′-Nitro	%, 6′-Nitro
C[b]	Dimethoxy	23[c,d]	27[d]	50[e]
5	Diethoxy	66[c,d]	15[d]	19[e]
6[f]	Diethoxy	79[c,d]	11[d]	10[e]
7	Di-*n*-butoxy	80[h]	7[g]	11[g]

[a] At −20° in 2:1 mixture (by volume) of concd. HNO_3 and concd. H_2SO_4; addition and aging periods 90 and 15 min., respectively, unless otherwise noted. [b] Ref. 2. [c] Analyzed as 1,4-dialkoxy-2,3-dinitrobenzene (it is possible that small amounts of isomeric dinitro compounds may have escaped detection). [d] Based on infrared analysis of band 1 material from alumina chromatography. [e] Based on alumina chromatography. [f] Addition and aging periods 90 and 60 min., respectively. [g] Based on infrared analysis of crude product. [h] Estimated from nitrogen analysis of crude product.

differed markedly from those obtained in nitric acid alone (see Table II). In each case where sulfuric acid was used, partial replacement of the acetyl group by a nitro group[4] was found to have occurred, probably because of the greater acidity of the medium. The results previously reported[2] for the mixed acid nitration of 2′,5′-dimethoxyacetophenone have been found to be in error; the analytical procedure employed did not differentiate between the 4′-nitro derivative and the 1,4-dimethoxy-2,3-dinitrobenzene produced from the acetyl-nitro replacement. Since the tendency for replacement increases with increasing alkoxy group size and the 6′-nitro derivatives (in which the acetyl group is *ortho* to two substituents) appear to undergo the replacement reaction more readily than the 4′-nitro derivatives, steric factors are evidently important. The following reaction sequence is consistent with the experimental results: mononitration of the 2′,5′-dialkoxyacetophenone, replacement of the acetyl group by hydrogen[5] (most readily in the case of the 6′-nitro derivative), and nitration of the resulting 1,4-dialkoxy-2-nitrobenzene to yield chiefly the 1,4-dialkoxy-2,3-dinitrobenzene.[6,7]

Experimental[8]

Nitration Reactions.—2′,5′-Diethoxyacetophenone[9] and 2′, 5′-di-*n*-butoxyacetophenone[9] were nitrated at −20° both in concd. nitric acid and in a 2:1 mixture (by volume) of concd. nitric acid and concd. sulfuric acid.[10] Runs 1 and 2 involved the nitration of the diethoxy compound in concd. nitric acid, runs 3 and 4 the nitration of the di-*n*-butoxy compound in concd. nitric acid, runs 5 and 6 the nitration of the diethoxy compound in the mixed acid medium, and run 7 the nitration of the di-*n*-butoxy compound in the mixed acid medium.[11] The nitrations of the di-*n*-butoxy compound were performed in the dark in a nitrogen atmosphere since the reaction mixture appeared to darken in air and light; no such tendency was observed in the case of the dimethoxy[2] or diethoxy compounds.

Alumina Chromatography.—The crude nitration products from runs 1–4 were separated by the previously described alumina chromatography technique.[2] In each case two well resolved yellow bands were obtained, the more mobile band (band 1) representing 2′,5′-dialkoxy-4′-nitroacetophenone, the less mobile band (band 2) 2′,5′-dialkoxy-6′-nitroacetophenone. On evaporation of the benzene solvent, the eluted components were recovered in an essentially pure state as evidenced by melting points and quantitative ultraviolet analyses. The crude products from runs 5 and 6 were partially separated by chromatography, two well resolved yellow bands being obtained. The band 1 material melted over a wide range and consisted of a mixture of 2′,5′-diethoxy-4′-nitroacetophenone and 1,4-diethoxy-2,3-dinitrobenzene; The composition of these mixtures was determined by infrared analysis. Band 2 represented essentially pure 2′,5′-diethoxy-6′-nitroacetophenone. The crude product from the mixed acid nitration of 2′,5′-dimethoxyacetophenone (run C, ref. 2) was reanalyzed in view of the above results. Separation into two bands was again achieved band 1 representing a mixture of 2′,5′-dimethoxy-4′-nitroacetophenone ($\lambda_{max}^{C_2H_5OH}$ 365 mμ) and 1,4-dimethoxy-2,3-dinitrobenzene ($\lambda_{max}^{C_2H_5OH}$ 360 mμ) and band 2 representing 2′,5′-dimethoxy-6′-nitroacetophenone. The ultraviolet spectrophotometric method previously employed[2] did not differentiate between the two unresolved band 1 components. The composition of band 1 was determined by infrared analysis. The crude product from run 7 could not be separated by chromatography.

Material balances, based on the weights of material charged and resolved components, varied from 97 to 103%. The results (reported as the average of two to four determinations agreeing within 3% and expressed as weight per cent of material in bands 1 and 2, respectively) were as follows:

Run 1: 28, 72; run 2: 27, 73; run 3: 26, 74; run 4: 31, 69; run 5: 81, 19; run 6: 90, 10; run C (ref. 2): 50, 50.

Infrared Analyses.[8]—The crude products from runs 1 and 4 and the band 1 samples from the chromatography of the products from runs 5, 6, and C (ref. 2) were analyzed quantitatively as two component systems. In each case, comparison of the spectrum of the mixture with the spectra of the reference samples of the two components showed no extraneous absorption bands, and it was possible to select a wave length for the determination of each component relatively free from interference. Calculations were made based on Beer's law using the base line approximation method[12] with appropriate corrections for interference. Material balances, based on the actual sample weights and the amounts of the two components as determined spectrophotometrically, varied from 97 to 101%. A partial infrared analysis of the product from run 7 was also made.

The results (expressed as weight per cent of each component, together with the solvents and wave lengths used) were as follows:

Product, run 1 (CCl_4): 2′,5′-diethoxy-4′-nitroacetophenone (5.91 μ), 26; 2′,5′-diethoxy-6′-nitroacetophenone (5.82 μ); 74. Product, run 4 (CCl_4): 2′,5′-di-*n*-butoxy-4′-nitroacetophenone (5.91 μ), 31; 2′,5′-di-*n*-butoxy-6′-nitroacetophenone (5.82 μ), 69. Product, Run 7 (CCl_4): 2′,5′-di-*n*-butoxy-4′-nitroacetophenone (5.91μ), 7; 2′, 5′-di-*n*-butoxy-

---

(4) D. V. Nightingale, *Chem. Revs.*, **40**, 117 (1947).

(5) R. T. Arnold and E. Rondestvedt, *J. Am. Chem. Soc.*, **68**, 2176 (1946); A. Klages and G. Lickroth, *Ber.*, **32**, 1549 (1899); W. M. Schubert and H. K. Latourette, *J. Am. Chem. Soc.*, **74**, 1829 (1952).

(6) R. Nietzki and F. Rechberg, *Ber.*, **23**, 1212 (1890).

(7) Other possibilities include a one-step displacement of acetyl by nitro.

(8) All melting points uncorrected; ultraviolet spectra measured in 95% ethanol with a Beckman DU spectrophotometer; infrared spectra measured by Analytical Research Associates, Cleveland, Ohio, with a Beckman IR-4 spectrophotometer; elemental microanalyses by Geller Laboratories, Bardonia, N. Y. The authors are indebted to P. M. Robisch for technical assistance.

(9) R. W. Bost and C. A. Howe, *J. Am. Chem. Soc.*, **74**, 257 (1952).

(10) For details, see procedures for runs A and C, ref. 2.

(11) See footnotes a and e, Table I and footnotes a and f, Table II.

(12) J. J. Heigl, M. F. Bell, and J. U. White, *Anal. Chem.*, **19**, 293 (1947).

6′-nitroacetophenone (5.82 μ), 11. Band 1, run 5 (C_6H_6): 2′,5′-diethoxy-4′-nitroacetophenone (8.22 μ), 19; 1,4-diethoxy-2,3-dinitrobenzene (7.82 μ), 81 (*analysis of an artificial mixture.* 4′-Nitro cpd.: actual, 20; found, 17. Dinitro cpd.: actual 80, found 83). Band 1, run 6 (C_6H_6): 2′,5′-diethoxy-4′-nitroacetophenone (8.22 μ), 12; 1,4-diethoxy-2,3-dinitrobenzene (7.82 μ), 88. Band 1, run C, (ref. 2) (C_6H_6): 2′,5′-dimethoxy-4′-nitroacetophenone (8.20 μ), 54; 1,4-dimethoxy-2,3-dinitrobenzene, (12.40 μ), 46 (*analysis of an artificial mixture.* 4′-Nitro cpd.: actual, 55; found, 52. Dinitro cpd.: actual, 45; found, 48).

Reference Samples and Structure Determinations.—2′,5′-Diethoxy-4′-nitroacetophenone was isolated by fractional crystallization (methanol) of run 1 product as yellow needles, m.p. 132–133°, $\lambda_{max}^{C_2H_5OH}$ 362 mμ.

Anal. Calcd. for $C_{12}H_{15}O_5N$: C, 56.9; H, 6.0. Found: C, 57.1; H, 6.0.

Treatment of this material with hypohalite yielded 2,5-diethoxy-4-nitrobenzoic acid, yellow-green needles from methanol, m.p. 175–176°.

Anal. Calcd. for $C_{11}H_{13}O_6N$: C, 51.8; H, 5.1; N, 5.5. Found: C, 52.3; H, 5.3; N, 5.6.

Dealkylation of this compound with hydrobromic acid[2] yielded 2,5-dihydroxy-4-nitrobenzoic acid, m.p. 242° dec. (no depression with an authentic sample prepared by an unambiguous procedure[13,14]).

2′,5′-Diethoxy-6′-nitroacetophenone was isolated by fractional crystallization of run 1 product as yellow needles, m.p. 83–84°, $\lambda_{max}^{C_2H_5OH}$ 336 mμ.

Anal. Calcd. for $C_{12}H_{15}O_5N$: C, 56.9; H, 6.0. Found: C, 57.1; H, 6.0.

This compound was brominated in chloroform solution (ultraviolet light) and the crude product treated with yellow ammonium sulfide solution.[15] The resulting lustrous, dark blue product had typical indigotin properties: blue-green color in nitrobenzene; ethanol solution decolorized by sodium hydrosulfite, color returning on air oxidation.[15]

Reference samples of 2′,5′-di-*n*-butoxy-4′-nitroacetophenone and 2′,5′-di-*n*-butoxy-6′-nitroacetophenone were prepared by recrystallization (aqueous ethanol) of samples obtained from chromatography of run 3 product. Recrystallization of band 1 material (m.p. 56–57.5°) yielded yellow needles of the 4′-nitro derivative, m.p. 58–58.5°, $\lambda_{max}^{C_2H_5OH}$ 366 mμ.

Anal. Calcd. for $C_{16}H_{23}O_5N$: C, 62.1; H, 7.5; N, 4.5. Found: C, 62.5; H, 7.7; N, 4.8.

Recrystallization of band 2 material (m.p. 36–37°) yielded pale yellow, waxy crystals of the 6′-nitro derivative, m.p. 36–36.5°, $\lambda_{max}^{C_2H_5OH}$ 343 mμ.

Anal. Calcd. for $C_{16}H_{23}O_5N$: C, 62.1; H, 7.5; N, 4.5. Found: C, 62.2; H, 7.4; N, 4.6.

Assignment of structures for the above two compounds was based on a comparison of their melting points, mobility on alumina, and ultraviolet and visible absorption spectra with those of the 4′- and 6′-nitro derivatives of 2′,5′-dimethoxy- and 2′,5′-diethoxyacetophenone.

Fractional crystallization (methanol) of band 1 material obtained on chromatography of the product from run C (ref. 2) yielded 1,4-dimethoxy-2,3-dinitrobenzene as yellow needles, m.p. 188–189°, $\lambda_{max}^{C_2H_5OH}$ 358 mμ.

Anal. Calcd. for $C_8H_8O_6N_2$: C, 42.2; H, 3.5; N, 12.3. Found: C, 42.5; H, 3.6; N, 12.2.

Assignment of structure was based on the melting point. 1,4-Dimethoxy-2,3-dinitrobenzene has been reported to melt at 186°,[16] the 2,5-dinitro isomer at 200–202°,[17] and the 2,6-dinitro isomer at 109–111.°[18]

Fractional crystallization (methanol) of band 1 material from the chromatography of run 5 product yielded 1,4-diethoxy-2,3-dinitrobenzene[6] as yellow needles, m.p. 141–142°, $\lambda_{max}^{C_2H_5OH}$ 358 mμ.

Anal. Calcd. for $C_{10}H_{12}O_6N_2$: C, 46.9; H, 4.7; N, 10.9. Found: C, 46.7; H, 4.7; N, 11.0.

This compound was hydrogenated in ethanol at room temperature with palladium-charcoal catalyst at a pressure of 40 p.s.i. An equimolar amount of benzil was added after removal of the catalyst and the mixture refluxed for 45 min. The solids obtained on concentration were recrystallized from ethanol to yield 5,8-diethoxy-2,3-diphenylquinoxaline as pale yellow needles, m.p. 165–165.5° (lit., m.p. 163°,[6] 165°[19]).

Anal. Calcd. for $C_{24}H_{24}O_2N_2$: N, 7.5. Found: N, 7.4.

Fractional crystallization (methanol) of the run 7 product yielded small amounts of two di-*n*-butoxydinitrobenzenes. One of these was obtained as yellow cubes, m.p. 73–74°, $\lambda_{max}^{C_2H_5OH}$ 360 mμ.

Anal. Calcd. for $C_{14}H_{20}O_6N_2$: C, 53.8; H, 6.5; N, 9.0. Found: C, 54.0; H, 6.4; N, 9.0.

The other was obtained as yellow needles, m.p. 136–137°, $\lambda_{max}^{C_2H_5OH}$ 366 mμ.

Anal. Calcd. for $C_{14}H_{20}O_6N_2$: C, 53.8; H, 6.5; N, 9.0. Found: C, 54.1; H, 6.5; N, 9.1.

The run 7 product exhibited strong nitro absorption (6.4 and 7.3 μ), relatively weak carbonyl absorption (5.8 μ) and gave a negative ferric chloride test. Elemental analysis indicated the sample to consist chiefly of dinitro material.

Anal. Calcd. for $C_{16}H_{23}O_5N$ (di-*n*-butoxynitroacetophenone): N, 4.5. Calcd. for $C_{14}H_{20}O_6N_2$(di-*n*-butoxydinitrobenzene): N, 9.0. Found: N, 8.0.

---

[13] S. C. Bhattacharyya and D. E. Seymour, *J. Chem. Soc.*, 1139 (1950).

[14] The authors are grateful to Smith & Nephew Research, Ltd., Hunsdon, England for this sample.

[15] R. W. Bost and C. A. Howe, *J. Am. Chem. Soc.*, **73**, 5864 (1951).

[16] S. Kawai, J. Kosaka, and M. Hatano, *Proc. Japan. Acad.*, **30**, 774 (1954).

[17] R. Nietzki and F. Rechberg, *Ber.*, **11**, 1037 (1878); G. M. Robinson, *J. Chem. Soc.*, **109**, 1087 (1916).

[18] J. F. Bunnett, H. Moe, and D. Knutson, *J. Am. Chem. Soc.*, **76**, 3936 (1954); A. Burger and F. T. Fitchett, *J. Am. Chem. Soc.*, **75**, 1359 (1953).

[19] E. S. Lane and C. Williams, *J. Chem. Soc.*, 2983 (1956).