(with a Glas-col heating mantle), with stirring, to reflux for 24 hours. The first extraction resulted in *vigorous* reaction and an efficient condenser was needed.

As the hydride reacted, the reaction mixture turned decidedly green in color. After several extractions the aluminum–polymer complex began to precipitate, finally covering the entire surface of the solution with a semi-transparent, gelatinous mass.

After 24 hours, the reaction mixture was allowed to cool to room temperature. The Soxhlet extractor was removed and 1 *N* sulfuric acid added cautiously until the reaction tested distinctly acid to congo red paper (*ca.* 350 ml.). The precipitate gradually redissolved (8–10 hours) leaving a small white, gelatinous residue. The polymer solution was then decanted, placed in a stoppered filter-flask and evaporated under vacuum to approximately one-half volume. Since the butadiene–allyl alcohol copolymer became insoluble when dried, it was customarily stored in this solution.

Viscosities were run on benzene solutions of this polymer which were obtained by precipitating the polymer from the tetrahydrofuran solution, pressing the methanol from the sample and immediately redissolving the polymer in dry benzene. Under these conditions the polymer was soluble and gave inherent viscosities of 1.8 to 2.4. Elemental analyses were run on these polymers.

Anal. Calcd. (on the basis of complete reduction): C, 86.81; H, 11.09. Found: C, 86.95; H, 11.19.

This analysis corresponds to a copolymer of butadiene and allyl alcohol which contained 7.87%, by weight, of allyl alcohol.

p-Nitrophenyl Isocyanate Derivative of the Copolymer.—A sample of 1.16 g. of butadiene–allyl alcohol copolymer, which had been reprecipitated from benzene, was dissolved in 50 ml. of dry benzene. Five grams of *p*-nitrophenyl isocyanate was added and the mixture refluxed for eight hours. The product was isolated by pouring the reaction mixture into a fourfold excess of methyl alcohol and purified by seven reprecipitations from benzene. The product was a pale yellow, rubbery solid.

Anal. Calcd. for N: 3.08. Found: N, 3.20.

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Creatine Ethyl Ether

BY JAMES D. MOLD, ROBERT C. GORE, JOSEPH M. LYNCH AND E. J. SCHANTZ

RECEIVED AUGUST 27, 1954

The preparation of esters of creatine was reported first by Dox and Yoder.[1] This was accomplished by passing dry hydrogen chloride into absolute alcoholic solutions of creatine at room temperature, a procedure which has been used successfully for the preparation of esters of amino acids. Analyses for nitrogen and chloride were in agreement with the expected values for creatine ester hydrochlorides, and recrystallization from another alcohol did not alter the analyses, indicating that the compounds were not solvates. Dox and Yoder also noted that upon melting, the esters decomposed with gas evolution, and the residue solidified to form a compound with properties similar to those of creatinine hydrochloride. Kapfhammer[2] repeated the preparation of several of these compounds and confirmed the findings of Dox and Yoder. He pointed out, however, that the behavior of these compounds in chemical reactions and toward colorimetric tests and precipitating agents was typical of creatinine and not of creatine. Since the conditions used for many of these reactions did not correspond to conditions favorable for the rapid conversion of creatine to creatinine, Kapfhammer concluded that these compounds were, in reality, derivatives of creatinine in which the alcohol was not bound as a solvate nor by an ester linkage but in "some uncertain manner." Hynd and MacFarlane[3] later showed that the behavior of creatine methyl ester hydrochloride toward nitrous acid was more consistent with that of a true derivative of creatine since 65% of its total nitrogen was evolved in the Van Slyke procedure compared to 68% for creatine, while only 39% of the total nitrogen of creatinine is liberated under similar conditions; however, when the chloride was removed by shaking creatine methyl ester hydrochloride with silver carbonate prior to the nitrous acid treatment, only 41% of the total nitrogen was evolved, suggesting that a conversion to creatinine may have occurred. Failey and Brand,[4] upon careful electrometric titration of creatine methyl ester hydrochloride with sodium hydroxide, noted an irreversible conversion during the titration, with the end-product giving a titration curve identical to creatinine.

In order to establish clearly whether these compounds are derivatives of creatine or of creatinine, it was felt that only the mildest conditions could be applied due to the extreme lability of these compounds. For this reason a spectrophotometric approach was investigated. Stuckey[5] has shown that compounds of the glycocyamidine or of the hydantoin type, with a hydrogen atom on the lactam nitrogen, have characteristic absorption maxima in the 220–240 mμ region when the spectra are observed in alkaline solution. Open chain structures such as creatine or hydantoic acid do not possess an absorption maximum in this region. Gaede and Grüttner[6] have shown that creatinine has a maximum at 234 mμ with a molecular extinction of 7420 when the spectrum is observed in an aqueous phosphate buffer solution at *p*H 7.38. Creatine under similar conditions was shown to absorb very little in the same region.

The preparation of the ethyl ester of creatine hydrochloride was found to be accomplished easily according to the directions of Dox and Yoder.[1] It was not found possible to prepare this compound by passage of dry hydrogen chloride into an anhydrous ethanol solution of creatinine,[7] indicating that the ester is not formed through the intermediate production of creatinine. At *p*H 7 the ethyl ester of creatine exhibited a rather strong absorption maximum at 235 mμ. However, the intensity of absorption was increasing at a very rapid rate and it approached a value equal to that for an equimolar amount of creatinine within about 30 minutes. A study of the absorption spectrum for creatinine in aqueous buffer solutions of *p*H less than 7 indicated that it was possible to observe the characteristic maximum at *p*H values as low as about 5, although at a somewhat diminished

(1) A. W. Dox and L. Yoder, *J. Biol. Chem.*, **54**, 671 (1922).

(2) J. Kapfhammer, *Biochem. Z.*, **156**, 182 (1925); A. Hunter, "Creatine and Creatinine," Longmans, Green and Co., Ltd., London, 1928, pp. 38–39.

(3) A. Hynd and M. G. MacFarlane, *Biochem. J.*, **20**, 1264 (1927).

(4) C. F. Failey and E. Brand, *J. Biol. Chem.*, **102**, 767 (1933).

(5) R. E. Stuckey, *J. Chem. Soc.*, 331 (1947).

(6) K. Gaede and R. Grüttner, *Naturwissenschaften*, **39**, 63 (1952).

(7) These experiments were performed by Dr. John M. Ladino.

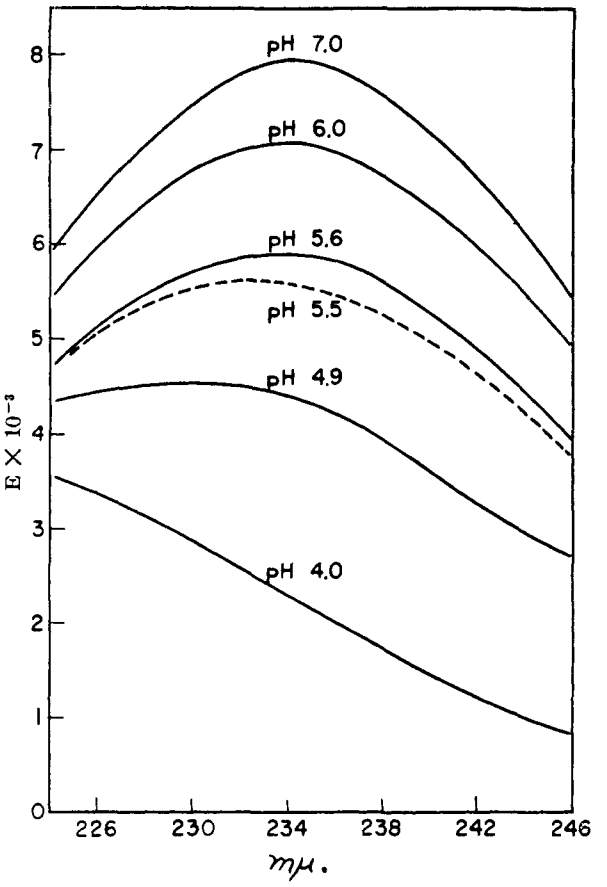

Fig. 1.—Ultraviolet absorption spectra of creatinine in aqueous buffer solutions: creatinine, ———; creatine ethyl ester (after 24 hr. at *p*H 5.5) - - - -. Creatine gave general absorption in this region with molecular extinction less than 0.04×10^{-3} at *p*H 5.5. The *p*H 4.0 solution was prepared with 0.01 *M* sodium acetate buffer; the other solutions were prepared with 0.001 *M* phosphate buffers. All spectra were determined with a Beckman model DU spectrophotometer *versus* blank solutions prepared similarly.

extinction (Fig. 1). Creatine gave negligible absorption at *p*H 5 or 7. When the spectrum of creatine ethyl ester was observed at *p*H 5.5, only a relatively small absorption was noted at 235 mμ, 85 seconds after mixing with the buffer. However, this increased steadily to a value equal to that for creatinine within 24 hours (Fig. 2). This behavior suggests that the structure of the ester is originally not that of a creatinine compound but that it is very rapidly transformed into such a compound at *p*H 5.5 or greater, the rate of the reaction increasing with the *p*H. If the solutions were observed for longer periods, the maxima for creatinine and "creatine ethyl ester" both decreased in value, apparently due to the establishment of the creatine–creatinine equilibrium.[8]

Although the extreme lability of the creatine esters at *p*H values as low as 5.5 might be somewhat unexpected, reactions of this type, in which an ester is condensed with a guanidine compound under basic conditions, in some cases are reported to

(8) R. K. Cannan and A. Shore, *Biochem. J.*, **22**, 920 (1928); G. Edgar and H. E. Shiver, THIS JOURNAL, **47**, 1179 (1925).

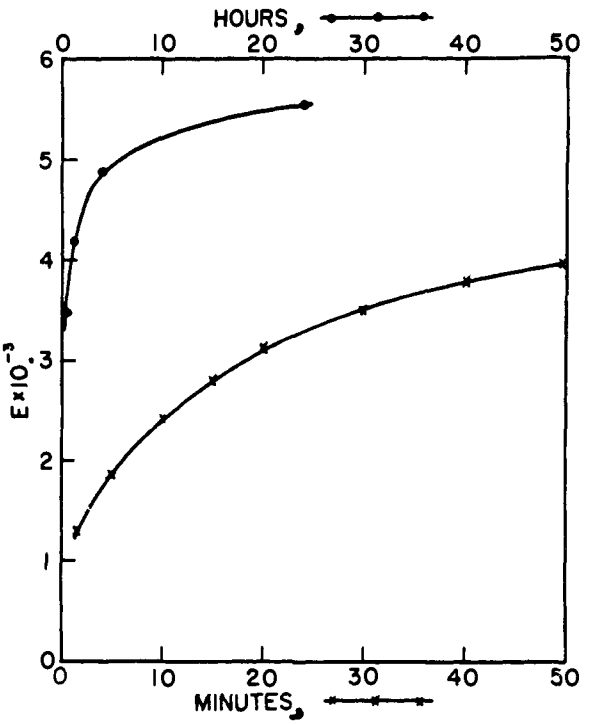

Fig. 2.—Intensity of the ultraviolet absorption at 235 mμ for a solution of creatine ethyl ester in aqueous buffer at *p*H 5.5. The solution was prepared with 0.01 *M* phosphate buffer and the absorption determined with a Beckman model DU spectrophotometer *versus* a blank solution prepared similarly.

proceed with exceeding rapidity. The reaction of certain esters with guanidine as the free base is exothermic and the reaction mixture must be cooled to prevent it from proceeding too vigorously.[9] With the esters of α-amino or α-hydroxy acids, a similar reaction occurs spontaneously and is followed by a further rapid transformation in which ammonia is split out and a cyclic guanidine amide or oxazole is formed.[10] The general reaction is, of course, well known[11] and has been used extensively in the preparation of the pyrimidines.[12]

To establish further the structure of the hydrochloride of creatine ethyl ester, the infrared absorption spectrum was observed. Since this was done with the mulled crystals, little opportunity existed for chemical change. The spectrum was compared with the spectra of the hydrochlorides of creatine and of creatinine (Fig. 3). The general similarity of the spectrum of the ester to that of creatine hydrochloride and its lack of similarity to that of creatinine hydrochloride may be noted. The presence of strong absorption bands at 1730 and 1220 cm.$^{-1}$ in the spectrum for the hydrochloride of creatine ethyl ester is confirmation that the alcohol is bound to the molecule in a typical ester linkage.[13]

(9) W. Traube, *Ber.*, **43**, 3586 (1910).

(10) W. Traube and R. Ascher, *ibid.*, **46**, 2077 (1913); E. Abderhalden and H. Sickel, *Z. physiol. Chem.*, **175**, 68 (1928); L. Zervas and M. Bergmann, *Ber.*, **61**, 1195 (1928).

(11) T. B. Johnson and B. H. Nicolet, THIS JOURNAL, **37**, 2416 (1915); H. Schotte, H. Priewe and H. Roescheisen, *Z. physiol. Chem.*, **174**, 119 (1928).

(12) T. B. Johnson and D. A. Hahn, *Chem. Revs.*, **13**, 212 (1933).

(13) H. W. Thompson and P. Torkington, *J. Chem. Soc.*, 640 (1945).

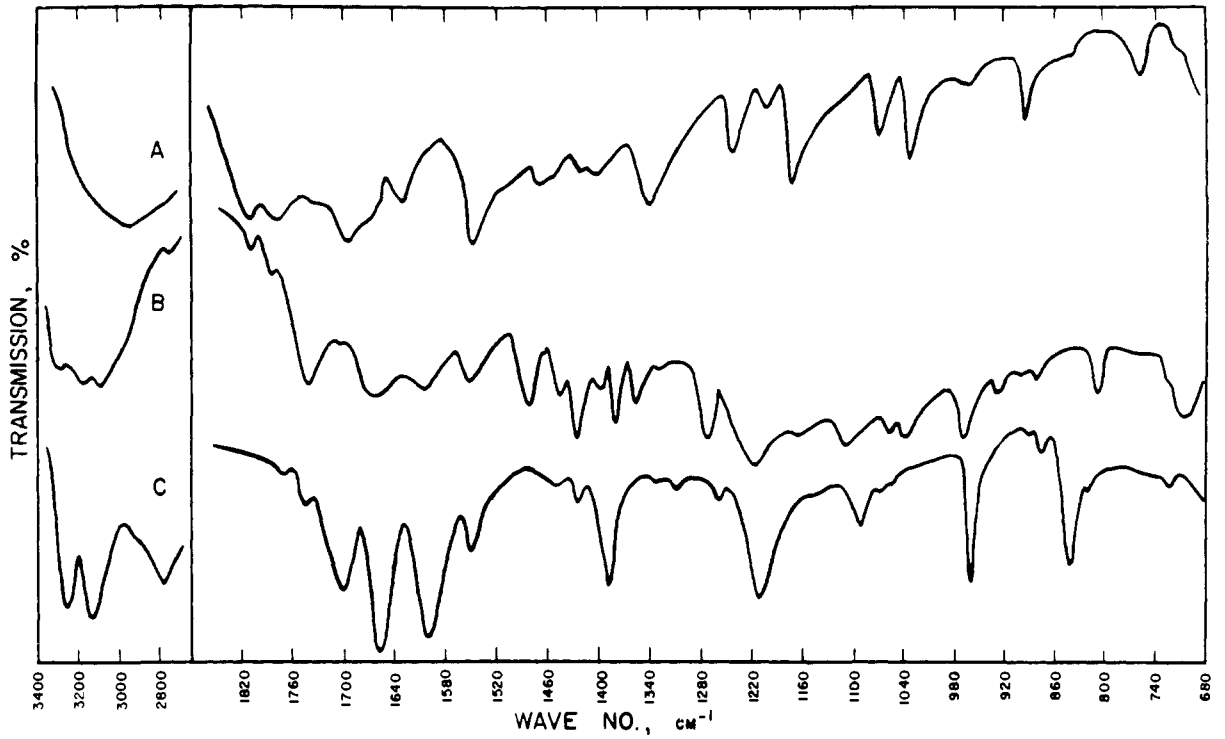

Fig. 3.—Infrared absorption spectra of creatinine hydrochloride (A), creatine ethyl ester hydrochloride (B), and creatine hydrochloride (C). The spectra were measured by E. Wenek with a model 12C Perkin–Elmer spectrophotometer. The solid samples were mulled with hexachlorobutadiene for the region from 2.0–6.0 μ and 6.6–8.0 μ and with Nujol for the region from 6.0–6.6 μ and 8.0–15.0 μ to avoid background absorption. The mulls were supported on sodium chloride plates.

Experimental

Creatine Ethyl Ester Hydrochloride.[7]—This compound was prepared according to the directions of Dox and Yoder.[1] The product melted at 161° (Dox and Yoder, m.p. 163°) when dropped on a preheated melting point block; otherwise, a transformation occurred to give a higher melting compound.

Anal.[14] Calcd. for $C_6H_{14}N_2O_2Cl$: N, 21.48; Cl, 18.12. Found: N, 21.73; Cl, 18.05.

(14) Analyses were performed by the Microanalytical Laboratory, Squibb Institute for Medical Research, New Brunswick, N. J., under the direction of Mr. J. F. Alicino.

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The S_N Mechanism in Aromatic Compounds. XII

BY JOSEPH MILLER[1]

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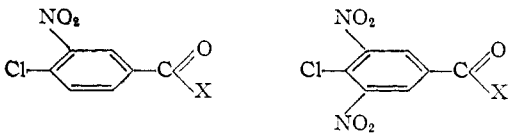
The activating power of a series of carbonyl 1-substituents in 4-chloro 3-nitro compounds (I)[1] has already been investigated by the author[2] and found to be in the theoretical order, *viz.*, COPh > COMe > CO_2Me > $CONH_2$ > CO_2^- > H. The corresponding 4-chloro-3,5-dinitro compounds (II), with the exception of the acetophenone, have now been investigated, though some react inconveniently fast, and show the same order.

(1) Fulbright Visiting Lecturer/Research Scholar, University of California, Berkeley, for Spring semester 1954.

(2) J. Miller, THIS JOURNAL, **76**, 448 (1954).



I II

The results obtained in the present work for replacement of Cl by OMe^- in methanol are given as Table I, which also compares the S.R.F.'s[3] of carbonyl substituents in the mono- and dinitro series. Owing to a combination of fast rates and short temperature range of rate measurements, the Arrhenius parameters for the CO_2Me and COPh compounds are not considered to be very accurate.

Discussion

The approximate independence of aromatic substituent effects is well known, and has also been confirmed for aromatic S_N reactions by earlier results of the author, and various co-workers (quoted in several previous communications). The only real difference here between mono- and dinitro series is a slightly smaller total range of S.R.F.'s in the latter, which is readily explicable on the basis that the over-all –I–T effect should be less effective in the more highly electron deficient ring. The larger value for the S.R.F. of the carboxylate ion and amide in the dinitro series is a consequence of comparisons at different temperatures.

In both series the carbonyl substituents cause a drop in activation energy, as compared with the

(3) J. Miller, *J. Chem. Soc.*, 3550 (1952).