(4 H, multiplet). Desulfurization of VIb with W-2 Raney nickel gave a quantitative yield of the methyl ketone VIc, mp 122–123°, nmr, δ 2.14 (3 H, singlet). Catalytic reduction of the diketone in the presence of platinum afforded the diol VIIa as a two-component mixture of epimers at C_{15}. The final steps parallel those used in the synthesis of (−)-sandaracopimaradiene.[11] Thus, the diols VIIa without separation were benzoylated and the dibenzoate mixture VIIb was pyrolyzed at 440°. There was obtained a 55% yield of methyl sandaracopimarate (Ib). Ester cleavage of Ib with lithium iodide in collidine[12] gave (−)-sandaracopimaric acid (Ia), mp 165–168°, undepressed on admixture with authentic[13] Ia, $[\alpha]_D$ −19.8° (*c* 0.2, ethanol) [lit.[1a] $[\alpha]_D$ −20°]. The infrared spectra and mobilities on thin-layer chromatography of the natural and synthetic Ia were identical.

(11) P. Johnston, R. C. Sheppard, C. E. Stehr, and S. Turner, *J. Chem. Soc., C*, 1847 (1966).
(12) F. Elsinger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, **43**, 113 (1960).
(13) The author thanks Dr. O. E. Edwards for a sample of natural sandaracopimaric acid.

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Relative Reactivities of *p*-Nitrophenyl Phosphate and Phosphorothioate toward Alkaline Phosphatase and in Aqueous Hydrolysis

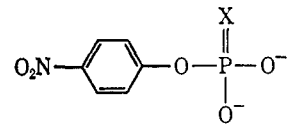
Sir:

There have been several reports of enzymatic studies on sulfur analogs of normal phosphate ester substrates. Thus Neumann, *et al.*,[1] find that S esters of phosphorothioic acid are rapidly cleaved by *E. coli* alkaline phosphatase. However, Eckstein has reported that nucleoside 5′-phosphorothioates are inert to both alkaline phosphatases[2] and some,[3] but not all,[4] acid phosphonoesterases; in contrast to these P=S compounds, the P=O derivatives are of course rapidly hydrolyzed. Recently[4] Neumann has reported that *p*-nitrophenyl phosphorothioate (I) is inert to *E. coli* alkaline phosphatase.

We have also investigated the hydrolysis of I by *E. coli* alkaline phosphatase[5] and find that while I is hydrolyzed slowly relative to the normal phosphate substrate II, I is by no means enzymatically inert. In fact the rate ratio for the sulfur derivative II and oxygen analog I can be explained chiefly on a straightforward chemical basis and furnishes a new type of evidence on the enzyme mechanism.

(1) H. Neumann, L. Boross, and E. Katchalski, *J. Biol. Chem.*, **242**, 3142 (1967).
(2) F. Eckstein, *J. Am. Chem. Soc.*, **88**, 4292 (1966).
(3) F. Eckstein and H. Sternbach, *Biochim. Biophys. Acta*, **146**, 618 (1967); *cf.* also H. Matzura and F. Eckstein, *European J. Biochem.*, **3**, 448 (1968).
(4) H. Neumann, *J. Biol. Chem.*, **243**, 4671 (1968).
(5) Chromatographically purified *E. coli* alkaline phosphatase, Worthington Biochemical BAPC7 JB, was further purified by a procedure developed by Professor Wilmer Fife. The enzyme was centrifuged at 3600 rpm with 65% saturated ammonium sulfate solution at 0° for 30 min, 10 mg of the solid was dissolved in 1 ml of 0.1 *M* N-ethylmorpholine buffer pH 8.0–0.1 *M* NaCl, and the residue was removed at 5000 rpm, 0°, 30 min. This stock solution was diluted tenfold in buffer to produce the working solution, *ca.* 1 mg/ml. Enzyme concentration in each reaction is approximately 3×10^{-2} mg/ml.

Solutions of I were prepared by dissolving *p*-nitrophenylthiophoshoryl dichloridate[6] in dioxane and hydrolyzing in a pH-stat at pH 8.0 with 1 *N* aqueous NaOH. Base uptake ceased after 4.0 equiv and spectroscopic assay indicated *ca.* 2% *p*-nitrophenol. Kinetic studies (*vide infra*) indicated minor contamination by II. I was stable in solution, but decomposed on attempted isolation under a variety of conditions, so the solution of I was utilized in hydrolysis studies.



I, X=S
II, X=O

The hydrolysis of I in 0.1 *N* N-ethylmorpholine buffer–1 *N* NaCl, pH 8.0, 25°, was followed with a Cary spectrophotometer at 410 mμ.

Contamination of I by a small amount of II led to a rapid initial rate and interfered with the normal K_m determination; however, hydrolysis of I is competitively inhibited by inorganic phosphate, so K_m for I was determined to be 1.3×10^{-4} *M* from the observed rate effect of added phosphate and its known inhibition constant. V_{max} was determined in the usual fashion to be 5.9×10^{-8} *M* sec^{-1}; V_{max} for II under the same conditions is 6.3×10^{-6} *M* sec^{-1} and $K_{mII} = 1.5 \times 10^{-5}$ *M*. Thus K_m for the thiophosphate ester is *ca.* eight times greater than for the oxygen ester, as has been observed in another case.[3] The major difference between I and II is that k_{catII} is $100k_{catI}$.

By contrast, in nonenzymatic aqueous solution I hydrolyzes much more rapidly than II. Both hydrolyses[7] are first order in H^+, and the two compounds have different pK_a's, so near the pK_a the rate ratio is pH dependent. At pH 8.0, 70°, k_I/k_{II} is 48, while at pH 7.0, 70°, $k_I/k_{II} = 63$. Ketelaar[8] has reported relative rates of alkaline hydrolysis for a series of phosphate and thiophosphate triesters and found a rate ratio k_S/k_O of *ca.* 0.03. The inversion in the S/O rate ratio in phosphate monoester *vs.* triester aqueous hydrolyses is reasonable. It is well established that triesters follow an addition–elimination mechanism, in which the P=O (P=S) bond order decreases in the transition state and the oxygen (sulfur) increases in charge, while the monoesters use an elimination (to metaphosphate)–addition sequence, in which the P=O (P=S) bond order increases in the transition state and the charge on oxygen (sulfur) diminishes. The effects themselves probably reflect mainly the lesser electronegativity of sulfur compared with oxygen.

The S/O rate ratio for the enzymatic hydrolysis of I and II resembles that for alkaline hydrolysis of triesters and is the inverse of that for nonenzymatic hydrolysis of I and II. This points strongly to an addition–elimination sequence for alkaline phosphatase. While there is some difference in binding of I and II as well, it seems unlikely that this could so drastically invert the

(6) H. Tolkmith, *J. Org. Chem.*, **23**, 1685 (1858).
(7) We confirm the data of K. Holbrook and L. Ouellet, *Can. J. Chem.*, **36**, 686 (1958), on the hydrolysis of II.
(8) J. Ketelaar, H. Gersmann, and K. Koopmans, *Rec. Trav. Chim.*, **71**, 1253 (1952).

k_S/k_O expected if the enzyme used the metaphosphate mechanism.[9]

(9) Support of this work by the National Institutes of Health is gratefully acknowledged.

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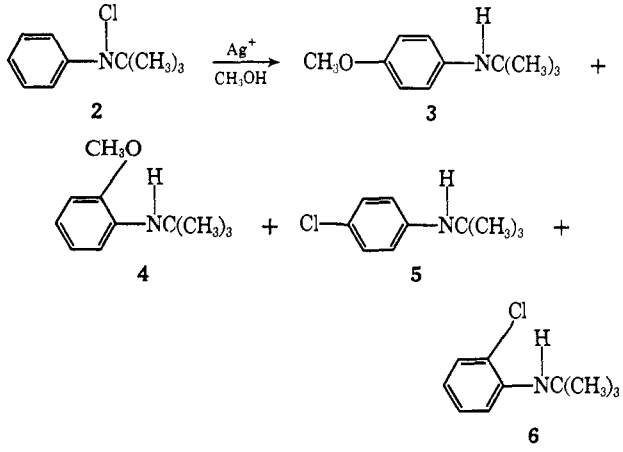
Anilenium Ions. Intermediates in the Nucleophilic Substitution of Anilines[1]

Sir:

In general, nucleophilic aromatic substitution is found to occur on aromatic systems abundantly substituted with electron-withdrawing groups and in the special case of diazonium salts.[2] We wish to report at this time a simple method which makes possible the nucleophilic substitution of anilines under extremely mild conditions.

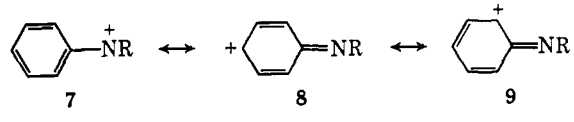
Recently, it was proposed that the chlorination of the aromatic ring of anilines[3] and the rearrangement of N-chloroanilides[2b,4] to *o*- and *p*-chloroaniline derivatives occur *via* N-chlorination,[5] followed by heterolysis of the N–Cl bond to yield an amide anion and positive chlorine, which electrophilically attacks the aromatic ring. Our studies on the reactions of nitrenium ions[1] suggested to us that in the presence of silver ion the N–Cl bond might be heterolytically cleaved to yield divalent electron-deficient nitrogen and chloride anion.[6] In order to test this hypothesis we prepared N-chloro-N-methylaniline (**1**)[5,7] and N-chloro-N-*t*-butylaniline (**2**).[7,8] When **2** was treated with methanolic silver perchlorate or methanolic silver trifluoroacetate at −8°, we obtained a mixture of **3**, **4**, **5**, and **6** in yields of 39, 6, 6, and 28%,[9] respectively, for a total yield of 79% based on unrecovered N-*t*-butylaniline (which generally accounted for *ca.* 5–10% of **2**). The formation of **3** as the major product clearly demonstrated the nucleophilic character of this reaction.



Undoubtedly the formation of both **3** and **4** required nucleophilic attack by methanol (a relatively poor nucleophile) on the aromatic ring.[10] When the reaction was carried out at −8° in the absence of silver ion no trace of either **3** or **4** was found. This non silver ion catalyzed reaction, which gave 8% N-*t*-butylaniline, 52% **6**, and 14% **5**, was considerably slower than the corresponding silver ion catalyzed reaction. In addition the *o*- to *p*-chloro-N-*t*-butylaniline ratio was 4.6 in the silver ion catalyzed reaction and 3.7 in the non silver ion catalyzed reaction.[11]

N-Chloro-N-methylaniline (**1**) gave results very similar to those obtained with **2**. Under silver ion catalyzed conditions at −20° we obtained N-methyl-*p*-anisidine (30%), N-methyl-*o*-anisidine (1%), *p*-chloro-N-methylaniline (6%), *o*-chloro-N-methylaniline (9%), and N-methylaniline (5%). The over-all yield (51%) from **1** was considerably lower than that from **2** due to the slow oxidation of the reaction products by silver ion.

Several mechanistic questions remain to be answered in relation to these substitution reactions. It is not clear whether the reaction is SN2′ or SN1′; that is, whether the reaction is a silver ion assisted displacement or whether a discrete "anilenium" ion such as **7** is formed. Since carbon should be able to bear a positive



charge more readily than nitrogen, it would be anticipated that the charge on **7** would be predominantly delocalized into the ring with **8** and **9** being the principal resonance contributors. This would explain the formation of the observed anisole derivatives.

One question which cannot be answered on the basis of the data presently available is whether the formation of the rearranged chloroanilines occurs in a separate reaction involving reverse heterolytic cleavage of the N–Cl bond to yield positive chlorine which then electrophilically attacks the ring or whether the *o*- and *p*-chloroanilines are arising *via* a tight ion pair involving **7**. The second possibility has the advantage that a

(1) Paper V in a series on the chemistry of nitrenium ions. For previous papers in this series see (a) P. G. Gassman and B. L. Fox, *Chem. Commun.*, 153 (1966); (b) P. G. Gassman and B. L. Fox, *J. Am. Chem. Soc.*, **89**, 338 (1967); (c) P. G. Gassman and R. L. Cryberg, *ibid.*, **90**, 1355 (1968); (d) P. G. Gassman, F. Hoyda, and J. Dygos, *ibid.*, **90**, 2716 (1968).

(2) For reviews of nucleophilic aromatic substitution see: (a) J. F. Bunnett and R. E. Zahler, *Chem. Rev.*, **49**, 273 (1951); (b) E. D. Hughes and C. K. Ingold, *Quart. Rev.* (London), **6**, 34 (1952); (c) J. F. Bunnett, *ibid.*, **12**, 1 (1958); (d) R. Sauer and R. Huisgen, *Angew. Chem.*, 72, 294 (1960); (e) S. D. Ross, *Progr. Phys. Org. Chem.*, **1**, 31 (1963).

(3) R. S. Neale, R. G. Schepers, and M. R. Walsh, *J. Org. Chem.*, **29**, 3390 (1964).

(4) K. M. Johnston, G. H. Williams, and H. J. Williams, *Chem. Ind.* (London), 991 (1966).

(5) P. Haberfield and D. Paul, *J. Am. Chem. Soc.*, **87**, 5502 (1965).

(6) This appeared even more likely in view of the acid-catalyzed rearrangement of arylhydroxylamines: H. E. Heller, E. D. Hughes, and C. K. Ingold, *Nature*, **168**, 909 (1951). See also R. A. Abramovitch and B. A. Davis, *Chem. Rev.*, **64**, 176 (1964).

(7) N-Chloro-N-methylaniline (**1**) and N-chloro-N-*t*-butylaniline (**2**) were prepared *via* reaction of N-methylaniline and N-*t*-butylaniline with sodium hypochlorite at −8°. Compounds **1** and **2** routinely titrated for greater than 95% active chlorine. Reductive removal of the active chlorine showed that only traces of material with a chlorinated aromatic ring were formed under the conditions used to prepare **1** and **2**.

(8) N-*t*-Butylaniline was prepared according to the procedure of A. Bell and M. B. Knowles, U. S. Patent 2,692,287 (1956); *Chem. Abstr.*, 50, 2666*e* (1956).

(9) In refluxing methanol in the presence of excess silver trifluoroacetate the yields of **3**, **4**, **5**, and **6** were 35, 5, 6, and 22%, respectively. This experiment was carried out by dripping a solution of **2** into a refluxing solution of silver trifluoroacetate in methanol.

(10) Under the reaction conditions **5** and **6** were not converted to either **3** or **4**.

(11) An *o*- to *p*-chloro-N-*t*-butylaniline ratio of 1.85 has been reported[3] for the reaction of N-*t*-butylaniline with N-chlorosuccinimide in refluxing benzene.