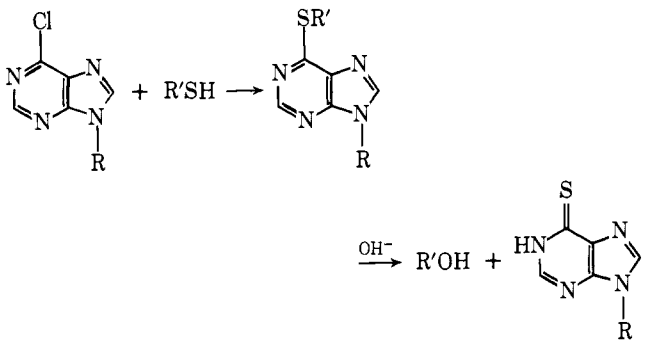
Communications to the Editor

Transmutation of Natural Amino Acid Residues: Replacement of Sulfur by Oxygen in Derivatives of Cysteine and Other Mercaptans

Sir:

Derivatives of cysteine are readily alkylated in aqueous solution by 1-fluoro-2,4-dinitrobenzene[1,2] or by 6-chloropurine ribonucleoside.[3] Treatment of the products with alkali results in the abstraction of sulfur.[1-3] Patchornik et al.[1,2] found that, when the leaving group is 2,4-dinitrothiophenol, alkaline cleavage occurs with β-elimination to yield a dehydroalanine residue in place of the original cysteine residue. We find that, when the leaving group is 6-mercaptopurine ribonucleoside, alkaline cleavage is hydrolytic, yielding serine in place of the original cysteine. This affords a technique for "chemical mutation", complementary to methods described earlier by Polgar and Bender[4] and by Neet and Koshland[5] for converting serine to cysteine residues. During this writing, Clark and Lowe[6] reported their achievement of the same objective by an entirely different route involving derivatization of cysteine with a halo ketone, conversion to an aldehyde by photolysis, and reduction of the photoproduct to yield a serine residue.



6-Chloropurine ribonucleoside reacts rapidly with thiolate anions in water at room temperature.[3] These reactions, monitored by observing the purine chromophore (at pH 10, 6-chloropurine ribonucleoside, λ_{max} 265 nm (ϵ_{265} 9.1×10^3); 6-ethylmercaptopurine, λ_{max} 290 nm (ϵ_{290} 1.94×10^4)), proceed with rate constants in the neighborhood of 8 M^{-1} min^{-1} at 25 °C and are essentially complete within 20 min when either or both the reactants are present at a concentration of 0.05 M.

When the glutathione product was treated with 1 N KOH at 25 °C, 6-mercaptopurine ribonucleoside (λ_{max} 312 nm (ϵ_{312} 1.96×10^4) at pH 14) was rapidly formed, in accord with earlier findings.[3] The apparent first-order rate constant for this reaction was 0.13 min^{-1} in 1 N KOH, and this rate was found to vary in proportion to the concentration of KOH present when the latter was varied in the range from 0.2 to 2.0 N at 25 °C, with ionic strength adjusted to 2.0 by appropriate addition of KCl. The second component of the product mixture was analyzed by first removing 6-mercaptopurine ribonucleoside by adsorption on acid-washed charcoal in 1 N HCl and then subjecting the product to hydrolysis in a sealed tube for 20 h at 110 °C. Thin layer chromatography on cellulose, using phenol–water (3:1) for development and ninhydrin for visualization, showed that the cysteine residue of glutathione had been converted quantitatively to serine. The same procedure was used to determine the final product of reaction with *N*-acetylcysteine and showed that it had been completely converted to *N*-acetylserine. When the reactant was ethanethiol, quantitative analysis of the products with alcohol dehydrogenase[7] showed that it had been completely converted to ethanol; ethanethiol itself was found to be without activity as a substrate for alcohol dehydrogenase.

These results suggested that the overall reaction involved hydrolytic displacement of 6-mercaptopurine ribonucleoside, since β-elimination would have generated ethylene from ethanethiol. The possibility remained, in the cases of glutathione and *N*-acetylcysteine, that the alkaline reaction might involve β-elimination, followed by hydration of the resulting dehydroalanine derivative to yield a serine derivative as the final product. This possibility was examined by allowing the reactions of glutathione and *N*-acetylcysteine to proceed to completion in the presence of 99% D_2O and examining the products for possible incorporation of deuterium at the α position of serine derived from the original cysteine. No such incorporation was found to have occurred when the products were examined by proton magnetic resonance spectroscopy, indicating that these reactions had also proceeded by hydrolytic displacement.

The initial alkylation was rapid for all thiols examined, but the course of the hydrolytic reaction was found to be strongly dependent on the structure of the thiol. Exposed to 1 N KOH at 25 °C, products of alkylation decomposed with an apparent rate constant of 1.3×10^{-1} min^{-1} for glutathione, 1.6×10^{-3} min^{-1} for *N*-acetylcysteine, and 2.3×10^{-5} min^{-1} for ethanethiol. The hydrolytic reaction was also very slow for 2-mercaptopropionic acid and for coenzyme A. 2-Mercaptoethanol and unsubstituted cysteine, equipped with a second nucleophilic group near the thiol group, gave quite different results. Following alkylation of the thiol group, alkaline decomposition in these cases yielded materials with ultraviolet spectra resembling those of 6-methoxypurine ribonucleoside and 6-methylaminopurine ribonucleoside,[3] respectively, indicating that intramolecular displacement of sulfur (by the hydroxyl group of 2-mercaptoethanol or by the amino group of cysteine) had occurred.

Aside from their synthetic uses, the present reactions involving intermolecular sulfur transfer are of interest as possible models for the formation of thiolated bases in transfer RNA in biological systems. These biosynthetic modifications of transfer RNA have been shown to require cysteine and ATP,[8,9] and it seems reasonable to speculate that they may proceed through hydrolysis of a thioether intermediate, formed by an ATP-dependent condensation between cysteine and the sulfur acceptor.

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References and Notes

(1) M. Sokolovsky and A. Patchornik, *J. Am. Chem. Soc.*, **86**, 1859–1860 (1964).
(2) M. Sokolovsky, T. Sadeh, and A. Patchornik, *J. Am. Chem. Soc.*, **86**, 1212–1216 (1964).
(3) B. T. Walsh and R. Wolfenden, *J. Am. Chem. Soc.*, **89**, 6221–6225 (1967).

0002-7863/78/1500-5943$01.00/0

(4) L. Polgar and M. Bender, *J. Am. Chem. Soc.*, **88**, 3153-3154 (1966).
(5) K. E. Neet and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U.S.A.*, **56**, 1606-1611 (1966).
(6) P. I. Clark and G. Lowe, *J. Chem. Soc., Chem. Commun.* 923-924 (1977).
(7) N. O. Kaplan and M. M. Clotti, "Methods in Enzymology", Vol. 3, Academic Press, New York, N.Y., 1957, pp 253-255.
(8) R. S. Hayward and S. B. Weiss, *Proc. Natl. Acad. Sci. U.S.A.*, **55**, 1161-1168 (1966).
(9) M. N. Lipsett and A. Peterkofsky, *Proc. Natl. Acad. Sci. U.S.A.*, **55**, 1169-1174 (1966).

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A Kinetic Study of the Friedel-Crafts Benzylation Reaction in Excess Aromatic and in Nitromethane

Sir:

Recently reported benzylation results in excess aromatic hydrocarbon and in nitromethane indicate the reaction to be moderate in rate and free of undesirable isomerization and disproportionation side reactions.[1] Benzylation therefore is not subject to the sort of criticism raised concerning the validity of data for fast competitive reactions (nitration[2] and halogenation[3]), where mixing rates may be slow compared with the rate of reaction. Thus, the relatively slow benzylation reaction would seem to be an ideal system to test the Brown selectivity relationship in the ongoing debate regarding mechanisms of electrophilic aromatic substitution.[4]

Olah has reported noncompetitive as well as competitive kinetic data for this benzylation reaction.[1] However, the consistency between these results is open to question in that his noncompetitive k_T and k_B values were calculated from first-order plots which are curved (Figures 6 and 7 of ref 1) at early reaction times. Recently, we decided to reexamine the reaction at 30 °C between benzyl chloride and benzene or toluene in excess aromatic and in nitromethane using the mild catalyst $TiCl_4$ to minimize reaction speed as well as side reactions.

In excess aromatic these benzylation reactions proved to be exceedingly difficult to run in an homogeneous, reproducible fashion. Results depended *strongly* upon the amount of moisture present. Reaction mixtures prepared according to standard vacuum-line procedures with aromatic solvents dried to 0.0006 wt % water showed almost *immediate* precipitation in half the benzene runs and in all but one toluene run. Gas chromatographic data obtained from this toluene run gave a product isomer distribution of 39% ortho, 7% meta, and 54% para. At this point the obtained rate law, first order in benzyl chloride and second order in $TiCl_4$, yielded a k_T/k_B of ~6. The isomer percentages and rate constant ratio are in good agreement with values reported in the literature.[1]

However, with improved vacuum-line methods to minimize water transfer, reaction mixtures could be prepared that showed no precipitation. Average toluene product isomer percentages obtained are essentially unchanged at 41 ± 2% ortho, 6 ± 2% meta, and 53 ± 3% para.[5] The products must be formed under kinetically controlled, i.e., nonisomerizing, conditions because individual isomer percentages remain constant with time in any given experiment.

These isomer percentages are well within experimental error of those obtained from the reaction of benzyl chloride with toluene catalyzed by a wide variety of catalysts: $InCl_3$,[6] $FeSO_4$, $Fe_2(SO_4)_3$,[7] ZnO, TiO_2, ZrO_2, and TiO_2-ZrO_2.[8] This lack of sensitivity to the nature of the catalyst supports the possibility of the benzyl cation as a common electrophile in all these reactions.[2]

Even with improved techniques the rate law remained first order in benzyl chloride and second order in $TiCl_4$, although the individual rates of reaction for both benzene and toluene showed marked deceleration. With the initial methods, we obtained $k_B = 1.04 \times 10^{-2}$ L^2 mol^{-2} s^{-1}. With our improved methods, the average value dropped by a factor of five to $k_B = 2.0 \times 10^{-3}$ L^2 mol^{-2} s^{-1}. The k_T obtained = 3.9×10^{-2} L^2 mol^{-2} s^{-1}. Typical pseudo-first-order plots are given in Figure 1.

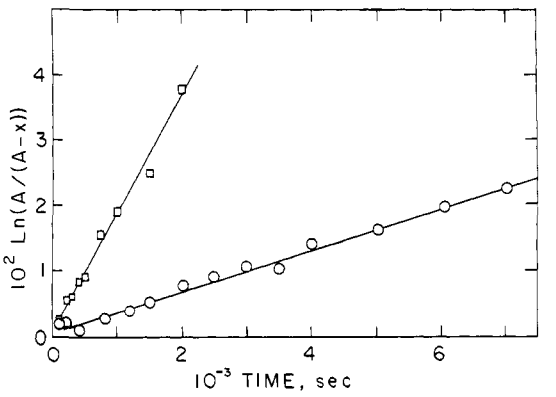

Figure 1. Typical first-order plots for the reaction of benzyl chloride with benzene or toluene in excess aromatic at 30 °C: A = initial concentration of benzyl chloride; □ refers to a toluene run with $[TiCl_4]_0 = 0.0174$ M; ○ refers to a benzene run with $[TiCl_4]_0 = 0.0404$ M.

The relative rate value, $k_T/k_B = 20 \pm 12$ exhibits a high standard deviation, reflecting our lack of complete success in controlling the moisture problem. Considering the experimental scatter in the data, our results fit the Brown selectivity relationship as well as could be expected. From the calculated partial rate factors $o_f = 25$, $m_f = 4$, $p_f = 64$ and the selectivity factor $S_f = 1.25$, the slope "b" of the linear free-energy equation $\log p_f = bS_f$ is calculated to be 1.4 in good agreement with Stock and Brown's[9] least-squares slope of 1.31 ± 0.10 (standard deviation).

This is most significant. Olah has already shown the benzylation reaction in excess aromatic to fit the selectivity relationship *when the aromatic was attacked by weak electrophiles*, e.g., benzyl chloride molecules containing electron-donating substituents.[1] We now find a similar fit to the selectivity relationship with a strong electrophile, the benzyl cation itself. Thus, although k_T/k_B values and product isomer percentages vary markedly through this series, thus implying different transition states, it now seems clear that for benzylation in excess aromatic *all* transition states resemble benzenium (σ complex) ions.

In nitromethane the benzylation reaction was readily followed at 30 °C. There were no difficulties with phase separation in this solvent. Solution homogeneity was maintained even after we purposefully injected small amounts of H_2O.[10] However, minor amounts of H_2O did affect the reaction rate. Only after lowering the H_2O content of the solvent to 0.005 wt % did we obtain consistent results.

From a noncompetitive initial rate study the rate law was found to be rate = k_4[benzyl chloride][$TiCl_4$]$_0^3$.

There is an internal check possible for this unusual rate law because the rate expression can be written as rate = k_{app}·[benzyl chloride] where $k_{app} = k_{n+1}[TiCl_4]_0^n$. Values of rate constants corresponding to "n" = 2, 3, 4 were calculated and the best agreement found was for "n" = 3. The pseudo-first-order plots were linear and passed through the origin. From four benzene runs, $k_B = 2.14 \pm 0.11$ M^{-3} s^{-1}; from three toluene runs, $k_T = 2.19 \pm 0.59$ M^{-3} s^{-1}.

We should like to point out the significance of the zero-order dependence of the rate upon the aromatic hydrocarbon concentration for this reaction. It indicates that the rate-determining step is the formation of the electrophile—not the sub-

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