The product was purified further by trituration three times with boiling acetone. The acetone-soluble portion was filtered through Celite and ether was added to the filtrate. Compound **10** separated on slow cooling; mp 105–106.5°, 72% recovery.

Anal. Calcd for $C_{10}H_{24}Cl_2NO_{10}S$: C, 28.75; H, 5.79; N, 3.35. Found: C, 29.01; H, 5.95; N, 3.45.

Registry No.—**1**, 21876-83-5; **2**, 21864-92-6; **3**, 21864-93-7; **4**, 21864-94-8; **5**, 21864-95-9; **6**, 21864-96-0; **7**, 21864-97-1; **8**, 21864-98-2; **9**, 21864-99-3; **10**, 21865-00-9; **11**, 21865-01-0; **12**, 21865-02-1; **13**, 21865-03-2; **14**, [21865-04-3; **15**, 21865-05-4; **16**, 21865-06-5; **17**, 21865-15-6; **18**, 21865-16-7; 3-(trimethylammonium)propane sulfobetaine, 21865-17-8; 3-(triethylammonium)propane sulfobetaine, 1887-93-0; 3-(pyridinium)propane sulfobetaine, 15471-17-7; 3-(trimethylammonium)-1,1,3-trimethylpropane sulfobetaine, 21865-20-3; 3-(pyridinium)-1,1,3-trimethylpropane sulfobetaine, 21865-21-4; 4-(triethylammonium)butane sulfobetaine, 21876-42-6; 4-(pyridinium)butane sulfobetaine, 21876-43-7; 4-ethyl-4-(trimethylammonium)butane sulfobetaine, 21876-44-8; 4-ethyl-4-(pyridinium)butane sulfobetaine, 21876-45-9; 6-(trimethylammonium)hexane sulfobetaine, 21876-46-0; 1-methyl-3-pyridinium sulfobetaine, 21876-47-1; trimethyl taurine, 7465-57-8; 2-methoxy-1-methylpyridinium perchlorate, 21876-49-3; 1-methyl-2-pyridinium sulfobetaine, 4329-93-5; 4-methoxy-1-methylpyridinium perchlorate, 21876-51-7; N,N,N′,N′-tetramethylbutylenediamine, 111-51-3; 4-(trimethylammonium-iodide)-1-dimethylaminobutane, 21876-53-9; 4-aza-4,4-dimethyl-8-trimethylammonium iodide octane sulfobetaine, 21876-54-0; 3,3′-bis(1,4-tetramethylammoniumbutane)propane sulfobetaine, 21876-55-1.

Relative Nucleophilicity. Methylation of Anions in Aqueous Media[1]

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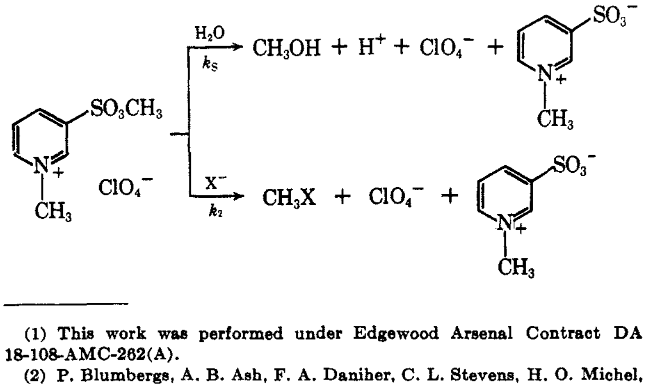
Alkylation rate data were obtained for 16 anion nucleophiles in aqueous media at 25° (and 37°) and constant salt concentration using 1-methyl-3-(methylsulfonate)pyridinium perchlorate as the alkylating agent substrate. The data at 25° are presented in terms of log k_2/k_w and the Swain and Scott equation is employed to obtain the substrate constant, *s* (0.715). Nucleophilic constants, *n*, are calculated and compared with published values for 12 anions, and new constants were determined for four phosphonate ion species.

The synthesis of a series of water-soluble alkylating agents has been described.[2] A pyridine analog, 1-methyl-3-(methylsulfonate)pyridinium perchlorate,[2] representing the most reactive agent of the series, was used as substrate, in the work reported herein, to measure nucleophilic constants by the Swain and Scott[3] method.

This alkylating agent, in common with other members of the series,[2] solvolyzes in water to form methanol, hydronium ion, perchlorate ion, and a stable, unreactive, water-soluble sulfobetaine. The alkylation of an anion results in the formation of the methylated anion, perchlorate ion, and the sulfobetaine. The equation for these (simultaneous) reactions is as follows, where k_s is the solvolysis rate constant and k_2 is the second-order anion alkylation rate constant.



The ratio of k_2/k_s and k_s is determined conveniently in separate experiments in a pH Stat. Hydronium ion is not generated in the anion alkylation reaction, whereas it is a product of the competing hydrolysis reaction. Accordingly, the reduction in the quantity of hydronium ion liberated at time *t*, relative to solvolysis in the absence of anions, is a measure of the extent of alkylation at time *t*. Mathematical treatment leads to the following general expression.

$$k_2/k_s = \frac{2.3 \log [S_0]/[S_t]}{[H^+_t]}$$

In this equation, $[S_0]$ is initial concentration of anion and $[S_t]$ is the concentration at time *t*, usually taken at infinity. The term $[H^+_t]$ is the molar hydronium ion formed by hydrolysis at time *t*; it is equal to the initial molar concentration of agent multiplied by the mole fraction of agent hydrolyzed.

The alkylation of 16 anion nucleophiles was studied kinetically in water at 25° (and 37°) and pH 7.0 with certain exceptions. The system was adjusted to 0.1 *M* in total salt; this is the sum of the agent and anion concentrations with sodium perchlorate added if required. The ratio of k_2/k_s is salt concentration dependent, decreasing with increasing salt concentration. For three sluggish nucleophiles, data were taken more conveniently at 0.5 *M* salt and extrapolated to 0.1 *M* salt.

The observed ratios k_2/k_s are multiplied by 55.4, the molar concentration of water, to give k_2/k_w. A conventional Swain and Scott[3] treatment is based on the equation log $k_2/k_s = sn$, where *n* is the anion nucleo-

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(1) This work was performed under Edgewood Arsenal Contract DA 18-108-AMC-262(A).

(2) P. Blumbergs, A. B. Ash, F. A. Daniher, C. L. Stevens, H. O. Michel, B. E. Hackley, Jr., and J. Epstein, *J. Org. Chem.*, **34**, 4065 (1969).

(3) C. G. Swain and C. B. Scott, *J. Amer. Chem. Soc.*, **75**, 141 (1953).

TABLE I

RELATIVE NUCLEOPHILICITIES. SWAIN AND SCOTT METHOD[3]

[substrate: 1- methyl-3-(methylsulfonate)pyridinium perchloroate (25°, 0.1 *M* total salt, pH 7.0[a])]

Anion	k_2/k_s, M^{-1}	k_2/k_w	log k_2/k_w	Nucleophilic Constant *n* Obsd[b]	Nucleophilic Constant *n* Lit.[c]
Nitrate	0.20	11	1.0	1.4	1.03
p-Nitrophenyl Methylphosphonate	0.27	15.0	1.18	1.7	. . .
Isopropyl Methylphosphonate	0.57	31.6	1.50	2.1	. . .
Fluoride	0.70	38.8	1.59	2.2	2.0
Sulfate	1.44	79.8	1.9	2.6	2.5
Chloride	2.61	145	2.16	3.02	3.04
Acetate	4.52	250	2.4	3.36	2.72
Chloromethylphosphonate (Dianion)	7.28	403	2.53	3.5	. . .
Bromide	10.9	604	2.78	3.89[d]	3.89
Bicarbonate	23	1300	3.1	4.3	3.8
Azide	27	1500	3.2	4.5	4.00
Thiocyanate	57.3	3170	3.5	4.89	4.77
Iodide	74.0	4100	3.61	5.05	5.04
Ethyl Methylthiophosphonate	150	8200	3.9	5.5	. . .
Hydroxide	480	26600	4.4	6.2	4.2
Thiosulfate	3500	190000	5.3	7.4	6.4

[a] Except acetate, 7.1; bicarbonate, 8.3; azide and chloromethylphosphonate, 9.0 (equivalence point). [b] Calculated from *s* = 0.715 (see footnote *d*). [c] All values from Swain and Scott,[3] except nitrate.[4] [d] Bromide ion taken as standard; *n* = 3.89.[3]

philic constant. A plot of log k_2/k_s *vs.* *n* is employed normally to determine the slope, *s*, the substrate constant.[3] In the present study, a plot of log k_2/k_s *vs.* the published nucleophilic constants[3] for chloride, bromide, and iodide ions was linear within 0.02 log units. Accordingly, bromide ion (*n* = 3.89)[3] was selected as standard to establish the substrate constant *s* as 0.715, and the nucleophilic constants listed in Table I were calculated by dividing log k_2/k_w by 0.715. The value of *s* of 0.715 for 1-methyl-3-(methylsulfonate)pyridinium perchlorate is comparable in magnitude with another sulfonate ester, ethyl *p*-toluenesulfonate (0.66).[3]

The alkylation of isopropyl methylphosphonate anion was confirmed by nmr and product isolation studies.

At 37°, with bromide ion as standard, the slope was 0.702, reflecting the decrease in k_2/k_s to 9.6 M^{-1} (from 10.9 M^{-1} at 25°). Nucleophilic constants for nine anions at 37° were in agreement with those observed at 25° within 0.1 log unit or less.

Experimental Section

A recording Sargent pH-Stat with thermoelectric temperature control (0.1°) was used. Solution volumes were 10–15 ml, 10^{-2}–10^{-3} *M* in agent, using 0.02–0.06 *N* sodium hydroxide as titrant with a nitrogen sweep. Sodium perchlorate was the added electrolyte to adjust the total salt concentration to 0.1 *M*. The infinity concentration of hydronium ion was adjusted for 50% of the volume of titrant. Five or more runs were made for each nucleophile in most cases and the results are reported to the number of significant figures warranted by the precision of the data under study. Nucleophilic constants expressed to two significant figures have a probable error of 0.1 log unit. Solvolysis rate constants for 1-methyl-3-(methylsulfonate)pyridinium perchlorate were determined in water (0.1 *M* sodium perchlorate) at pH 7.0 at 25°. The k_s was 8.18 (±0.10) × 10^{-4} sec^{-1}; at 37°, k_s is 3.07 (±0.12) × 10^{-3} sec^{-1}. The rate decreases with increasing salt concentration (see below).

Phosphonate and Thiophosphonate.—The alkylation of isopropyl methyl phosphonate was studied at 25°, 0.1 *M* in anion and 5 × 10^{-3} *M* in substrate. The ratio of k_2/k_s was 0.56 M^{-1}· at 0.5 *M* salt, the ratio was 0.41 M^{-1}. For *p*-nitrophenyl methylphosphonate, 0.5 *M* in anion, the ratio of k_2/k_s was 0.19 M^{-1}. Isopropyl methylphosphonate was extensively studied also at 37° and the ratios varied with phosphonate concentration as follows: 0.7 *M*, 0.23; 0.5 *M*, 0.25; 0.17 *M*, 0.35. A comparative study of isopropyl methylphosphonate was made with five analogous agents at 37°, 0.70 *M* in phosphonate and 7 × 10^{-3} *M* in agent; the values of k_2/k_s ranged from 0.26 to 0.29, or constant within experimental error. To establish a nucleophilic constant for a phosphonate dianion, chloromethylphosphonic acid of high purity was used. The study was made at the equivalence point (pH 9.0), pK_{a_1} 2.14 and pK_{a_2} 6.41, as determined in water at 25°. In a system 0.5 *M* in dianion, the ratio of k_2/k_s at 25° was 5.20, corrected to 7.28 at 0.1 *M* salt concentration. The alkylation of ethyl methylthiophosphonate anion was studied at 25°, 1.72 × 10^{-3} *M* in substrate and 4.96 × 10^{-3} *M* in thiophosphonate adjusted to 0.1 *M* total salt with sodium perchlorate. The ratio k_2/k_s was 146 M^{-1}; at 37°, two runs gave the values 116 and 122 M^{-1}.

Other Anions.—Bromide ion, the standard, was studied at 37° over a range of total salt concentration. The observed values of k_2/k_s (M^{-1}), a function of total salt concentration (substrate plus bromide ion), are as follows: 0.026 *M*, 12.0; 0.088 *M*, 9.9; 0.100 *M*, 9.6; 0.26 *M*, 9.0; 0.30 *M*, 7.6; 0.50 *M*, 6.8; 0.71 *M*, 6.7. The corresponding values for k_s (sec^{-1}) × 10^3 are as follows: 0.026 *M*, 3.4; 0.088 *M*, 3.2; 0.100 *M*, 3.1; 0.126 *M*, 3.0; 0.30 *M*, 2.8; 0.71 *M*, 2.6. Alkylation of thiosulfate ion exceeded 90% even at a 1:1 mole ratio of substrate to the anion; the results were reproducible within 0.1 log unit although the absolute error may be greater. Bicarbonate ion was studied at pH 8.30 over a wide range of mole ratios of bicarbonate to substrate; k_2/k_s values ranged from 21 to 26 M^{-1}, increasing (in this case) with increasing salt concentration. Azide ion was determined at pH 9.0 where the system appeared to be more stable than at pH 7.0. Thiocyanate ion was studied at pH 7.0 with good reproducibility. Chloride ion was checked independently using a sister agent, methyl 3-(trimethylammonium perchlorate)-sulfonate, both in the pH-Stat and by a sample-withdrawal titration technique.

The less reactive nucleophiles (the first five anions of Table I) were studied in water at 25°, 0.5 *M* in anion and 10^{-3} *M* in substrate. The results, k_2/k_s, were as follows (M^{-1}): nitrate, 0.17; *p*-nitrophenyl methylphosphonate, 0.19; isopropyl methylphosphonate, 0.41; fluoride, 0.51; sulfate, 1.02. Isopropyl methylphosphonate and sulfate ions were studied at a concentration of 0.1 *M* in anion plus agent to give k_2/k_s ratios of 0.57 and 1.44 M^{-1}. This corresponds, in both cases, to a factor of 1.4 in

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(4) W. L. Petty and P. L. Nichols, Jr., *J. Amer. Chem. Soc.*, **76**, 4385 (1954).

k_2/k_s between 0.5–0.1 *M*. This factor was used to estimate k_2/k_s at 0.1 *M* total salt for the other three less reactive anions (see bromide ion data above).

The ratios of k_2/k_s (M^{-1}), listed in Table I, were determined at 25°. At 37°, corresponding data (0.1 *M* total salt) for nine anions are as follows: isopropyl methylphosphonate, 2.0; fluoride, 2.1; chloride, 3.09; acetate, 3.42; bromide, 3.89 (standard); azide, 4.7; thiocyanate, 4.86; ethyl methylthiophosphonate, 5.5; iodide, 5.01.

Nmr Studies.—Nmr studies were made of the solvolysis of a sister agent, methyl 3-(trimethylammonium perchlorate)propane sulfonate,[2] in deuterium oxide, and the alkylation of sodium isopropyl methylphosphonate was studied in chloroform and dueterium oxide. All studies were carried out in an nmr tube using a Varian DP-60 operating at 60 Mcps. Hydrolysis of a saturated solution (7%) of the agent in deuterium oxide was followed by the disappearance of the signal due to protons on the $SOCH_3$ group at τ 6.1 and the appearance of the signal due to methanol at τ 6.6. Alkylation of isopropyl methylphosphonate anion was studied with the same substrate, but the substrate anion was isopropyl methylphosphonate instead of perchlorate. The chloroform solution initially showed the presence of $SOCH_3$, but, after several hours, a $POCH_3$ doublet appeared and the $SOCH_3$ peak decreased in intensity. A solid precipitated, identified as 3-(trimethylammonium)propane sulfobetaine. Methyl isopropyl methylphosphonate was isolated; the infrared and nmr spectra of the compound in carbon tetrachloride were identical with those of an authentic sample. The nmr spectrum contained a POCH multiplet centered at τ 5.35 (one proton), and a $POCH_3$ doublet at τ 6.36 (J = 11 cps). A PCH_3 doublet occurred at τ 8.66 (J = 18 cps), and a CCH_3 doublet (two methyls, six protons) appeared at τ 8.71 (J = 6 cps). This experiment was repeated in deuterium oxide at a concentration of substrate of *ca.* 20%. Although solvolysis predominated, the $POCH_3$ peak was observed; methyl isopropyl methylphosphonate was isolated and confirmed by an nmr spectrum (CCl_4). A control study showed that isopropyl methylphosphonic acid was not esterified by methanol.

Registry No.—1-Methyl-3-(methylsulfonate)pyridinium perchlorate, 21876-83-5.

Electron Spin Resonance of Trityl Alkyl Nitroxides. Spin-Labeled Amino Acids[1]

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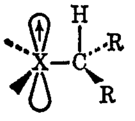
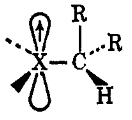
The electron spin resonance spectra of a variety of trityl alkyl nitroxides have been obtained by the reaction of *p*-nitroperbenzoic acid with the appropriate secondary amine in dioxane (or aqueous solution in some cases). Included are the nitroxides of certain trityl amino acids (or esters) and trityl dipeptides (or esters). The β-hydrogen hyperfine coupling constant is characteristic for the structure of the alkyl group. Evidence is obtained for restricted freedom of motion about the β-carbon atom. Different stable conformations are detected in the nitroxides of certain trityl dipeptides. Trityl alkyl (but no aryl) nitroxides with substituted β-carbon atoms dissociate to produce trityl radicals. Preliminary evidence indicates that this reaction may be reversible.

The angular dependence of β-hydrogen coupling in alkyl-substituted free radicals is well known to follow a $\cos^2 \theta$ relationship[2] where θ is the dihedral angle between the orbital containing the unpaired electron (normally a p orbital) and the carbon–hydrogen bond of the attached alkyl group. Maximum coupling is

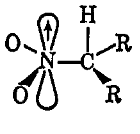


$\theta = 0°$

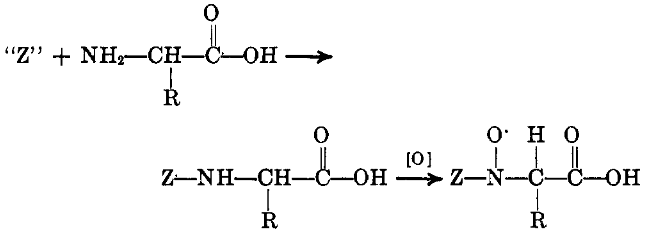


$\theta = 90°$

observed when θ = 0° and minimum coupling when θ = 90°. β-Hydrogen coupling thus depends on substitution since bulky groups restrict the conformations of the radical to those conformations with minimum steric crowding.[3] In most cases the β-hydrogen coupling decreases with increased substitution. For example in nitromethane, nitroethane, and 2-nitropropane radical anions, the β-hydrogen coupling is 11.4, 9.75, and 4.60 G for the methyl, methylene, and methine hydrogens, respectively.[4] Some variation in coupling is also observed in differently substituted radicals with methylene or methine hydrogens; *e.g.* for the methylene and methine hydrogens of 1-nitro- and 2-nitrobutane the coupling is 9.98 and 3.19 G, respectively.[4]



The object of this work is to investigate whether the β-hydrogen coupling in a suitably substituted radical is sensitive enough to a change in the bulk of the β-alkyl group to serve as a diagnostic tool for structure determination. Specifically, it is our aim to see if individual amino acids or peptides can be identified from the esr spectrum of a suitably substituted amino acid nitroxide produced by the oxidation of the N-substituted amino acid. The most convenient and only



$$\text{“Z”} + NH_2\text{—}\underset{R}{CH}\text{—}\overset{O}{\overset{\|}{C}}\text{—}OH \longrightarrow$$

$$Z\text{—}NH\text{—}\underset{R}{CH}\text{—}\overset{O}{\overset{\|}{C}}\text{—}OH \xrightarrow{[O]} Z\text{—}\overset{O\cdot}{N}\text{—}\underset{R}{\overset{H}{C}}\text{—}\overset{O}{\overset{\|}{C}}\text{—}OH$$

nitrogen substituent (Z) which gave the desired results was the triphenylmethyl (trityl) group (see Experimental Section for other attempts). This group is frequently used as a "blocking" group in peptide synthesis. Many of the N-trityl amino acid esters used in this work were kindly supplied to us by Professor

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(1) This work was supported by the Atomic Energy Commission, Contract AT-(40-L)-2851. Grateful acknowledgment is hereby made.

(2) C. Heller and H. M. McConnell, *J. Chem. Phys.*, **32**, 1535 (1960); see also M. C. R. Symons, *J. Chem. Soc.*, 277 (1959).

(3) For a review of conformational studies in electron spin resonance spectroscopy, see D. H. Geske, *Prog. Phys. Org. Chem.*, **4**, 125 (1967).

(4) L. H. Piette, P. Ludwig, and R. N. Adams, *J. Amer. Chem. Soc.*, **84**, 4212 (1962).