Anal. Calcd for $C_{12}H_{21}NO_4S$: C, 52.36; H, 7.64; N, 5.09; S, 11.64. Found: C, 52.11; H, 7.53; N, 4.95; S, 11.22.

Hydrolysis of Thioacetate Oxazolidine XIa to Thioacetate XIIa.—The compound (57 mg) was treated with 10 ml of a 1:1 mixture of acetic acid and water for 15–30 min at room temperature. The aqueous solution was extracted with three portions of methylene chloride and the combined extracts were dried and evaporated first on a rotatory evaporator (water aspirator) and then on a high vacuum line, to remove the last traces of acetic acid. In this way, 41 mg (91% yield) of analytically pure XIIa was obtained: nmr ($CDCl_3$) δ 1.45, 1.5 (double singlet, 6), 2.45 (s, 3, SAc), 3.3 (s, 2, CH_2SAc), 4.1 (AB q, 2, J = 10 Hz, CH_2OC), 10 ppm (s, 1, CHO).

Anal. Calcd for $C_9H_{14}O_4S$: C, 49.54; H, 6.42; S, 14.68. Found: C, 49.41; H, 6.34; S, 14.48.

Registry No.—I, 5736-03-8; II, 38615-71-3; IIa, 38615-72-4; IIb, 38615-73-5; IIIa, 38615-74-6; IIIb, 38615-75-7; IVb, 38615-76-8; V, 38615-77-9; Va, 38615-78-0; VI, 38615-79-1; VII, 38615-80-4; VIIa, 38615-81-5; VIIb, 38615-82-6; VIIc, 38615-83-7; X, 38615-84-8; Xa, 38615-85-9; Xb, 38615-86-0; XIa, 38615-87-1; XIIa, 38615-88-2; 1,1-dimethylhydrazine, 57-14-7; trifluoromethanesulfonic anhydride 358-23-6; *N*-methylethanolamine, 109-83-1.

Relative Reactivities of Nucleophilic Centers in Some Monopeptides

CHARLES C. PRICE,* HIROSHI AKIMOTO,[1] AND RICHARD HO[1]

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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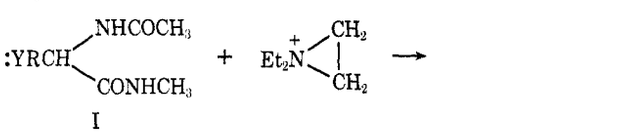
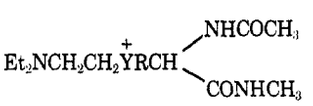
The "monopeptide" derivatives of a number of amino acids with nucleophilic centers have been prepared and their reactivity in water to diethylaziridinium ion, iodoacetic acid, and iodoacetamide determined. The reactivity of the imidazole ring of histidine, the mercaptan group of cysteine, the phenolate ion in tyrosine, and the sulfide group in methionine are all very nearly the same as that of these groups in model compounds. The sulfide group shows unexpectedly high reactivity when measured by iodoacetic acid or iodoacetamide.

As a sequel to studies of the effect of structure and conformation of nucleic acids on the reactivity of nucleophilic centers to alkylation,[2] we have now undertaken a similar investigation for proteins. As a prelude to study of the reactivity of nucleophilic centers in polypeptides, we have examined the nucleophilicity of some of the more significant nucleophilic centers in model "monopeptides," the *N*-acetylmethylamide derivatives of several amino acids. The alkylating agent used most generally has been the one utilized most extensively in the earlier nucleic acid studies, *N*,*N*-diethylaziridinium ion.



I

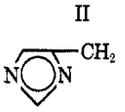


II

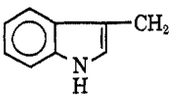
a, :YR = $HOC_6H_4CH_2$

b, :YR = $HSCH_2$

c, :YR = $CH_3SCH_2CH_2$



d, :YR =



e, :YR =

f, :YR = $HSCH_2CH_2$

Experimental Section

Materials.—*N*,*N*-Diethyl-2-chloroethylamine hydrochloride (Aldrich) was recrystallized from acetonitrile.

The monopeptides Ia, Ic, Id, and Ie were prepared from the corresponding amino acids essentially as described in the literature.[3a-c] Ib was prepared from the corresponding cystine derivative by zinc and acid reduction.[3d]

L-Homocysteine monopeptide (If) was prepared in much the same way as Ib. After addition of 1.56 g of thionyl chloride dropwise with stirring to a chilled suspension of 3.20 g of L-homocystine in 30 ml of absolute methanol, the reaction mixture was heated under reflux for 2 hr. After cooling, the solvent was removed *in vacuo* to give 4.4 g (theoretical yield) of colorless solid, L-homocystine dimethyl ester hydrochloride, which was used directly in the next step. This material and 5.35 g of triethylamine in 50 ml of $CHCl_3$ was chilled and 2.12 g of acetyl chloride was added dropwise with stirring. The reaction mixture was allowed to stand for 1 hr at room temperature and then washed and dried. After removal of the solvent, the crude product was recrystallized from ethyl acetate to give 4.3 g (94%) of *N*,*N*′-diacetyl-L-homocystine dimethyl ester as colorless needles, mp 105–106°, ir (Nujol) ν_{NH} 3800, ν_{CO} 1770, 1670 cm^{-1}.

Anal. Calcd for $C_{14}H_{24}O_6N_2S_2$: C, 44.19; H, 6.36; N, 7.36; S, 16.85. Found: C, 44.07; H, 6.48; N, 7.39; S, 16.58.

After 3 days at room temperature, a solution of 3.8 g of this dimethyl ester in 40 ml of 40% aqueous methylamine was concentrated to dryness *in vacuo* to afford the desired compound in theoretical yield. The crude product was recrystallized from methanol to give 3.2 g (85%) of pure *N*,*N*′-diacetyl-L-homocystinmethylamide as colorless prisms, mp 188–189°, ir (Nujol) ν_{NH} 3350, ν_{CO} 1645, 1560, 1545 cm^{-1}.

Anal. Calcd for $C_{14}H_{26}O_4N_4S_2$: C, 44.42; H, 6.92; N, 14.80; S, 16.94. Found: C, 44.31; H, 6.86; N, 14.91; S, 16.76.

After 400 mg of zinc dust was added to 757 mg of this disulfide dissolved in 30 ml of 2 *N* aqueous acetic acid, 300 mg of concentrated sulfuric acid was dropped slowly into the stirred mixture over 15 min under nitrogen. The exothermic reaction raised the temperature to 35–40°. After this reaction mixture was warmed at 45–50° for 2 hr, it was concentrated to dryness *in vacuo*. The residue was extracted with three 25-ml portions of warm isopropyl ether, and the combined extract was evaporated *in vacuo* to give 670 mg (88%) of crude product. It was recrystallized from isopropyl ether under nitrogen atmosphere to yield 625 mg of *N*-acetyl-L-homocysteinmethylamide (If) as colorless prisms, mp 192–195°, ir (Nujol) ν_{NH} 3350, ν_{SH} 2600, ν_{CO} 1650 (sh), 1645, 1565, 1545 cm^{-1}.

Anal. Calcd for $C_7H_{14}O_2N_2S$: C, 44.19; H, 7.42; N, 14.72; S, 16.85. Found: C, 44.33; H, 7.71; N, 14.64; S, 16.81.

(1) Supported in part by NIH Grant No. NIGMS 19593.

(2) (a) C. C. Price, G. M. Gaucher, P. Koneru, R. Shibakawa, J. R. Sowa, and M. Yamaguchi, *Biochim. Biophys. Acta*, **166**, 327 (1968); (b) C. C. Price and M. T. L. Yip, in press; (c) Mei-Tak Lam Yip, "The Nitrogen Mustard Alkylation of Polynucleotides and Ribonucleic Acids," Ph.D. Dissertation, University of Pennsylvania, 1972.

(3) (a) H. Zahn, *Z. Phys. Chem.*, **344**, 75 (1966); (b) T. H. Applewhite and C. Niemann, *J. Amer. Chem. Soc.*, **81**, 2208 (1959); (c) H. T. Huang and C. Niemann, *ibid.*, **73**, 3223 (1951) (d) T. A. Martin, D. H. Causey, A. L. Sheffner, A. G. Wheeler, and J. R. Corrigan, *J. Med. Chem.*, **10**, 1172 (1967).

Alkylated *N*-Acetyl-L-homocysteinmethylamide (IIf).—A solution of 475 mg of If in 25 ml of distilled water was added to the cyclic imonium ion solution prepared from 645 mg of *N,N*-diethyl-2-chloroethylamine hydrochloride in 25 ml of distilled water as described below in the method of alkylation. The reaction mixture was maintained at pH 7.0 and 37° for 24 hr under nitrogen and concentrated to dryness on a warm bath under reduced pressure. The residue was extracted with three 30-ml portions of methanol and, after evaporation of the solvent, the resulting residue was chromatographed on both silica gel and alumina successively, using chloroform–methanol as an eluent. The white solid obtained was recrystallized from ether–*n*-hexane to give 452 mg (63%) of cotton-like, colorless needles: mp 91–92°; ir (Nujol) ν_{NH} 3410, ν_{CO} 1650, 1575, 1550 cm^{-1}; nmr ($CDCl_3$) τ 2.70 (broad, 1 H), 2.82 (broad, 1 H), 5.34 (q, 1 H), 7.35 (s, 3 H), 7.00–7.60 (m, 10 H), 7.90 (t, 2 H), 8.00 (s, 3 H), 8.98 (t, 6 H).

Anal. Calcd for $C_{13}H_{29}O_2N_3S$: C, 53.95; H, 9.40; N, 14.52; S, 11.08. Found: C, 53.77; H, 9.55; N, 14.32; S, 10.83.

Alkylated L-homocysteine was obtained from 289 mg of IIf by dissolving in 5 ml of 6 *N* aqueous hydrochloric acid and heating on an oil bath (110–120°) for 15 hr. After evaporation of solvent *in vacuo*, the resulting residue was redissolved in a minimum amount of distilled water and chromatographed on an ion-exchange resin (25 ml of Dowex 50W-*X*8), using distilled water and 2.5% aqueous ammonium hydroxide as eluents, successively, to eliminate chloride ion. The ninhydrin-positive fractions, eluted by 2.5% aqueous ammonium hydroxide, were collected and evaporated to dryness *in vacuo* to yield a pale yellow solid, which was triturated with ether to give 165 mg (71%) of the desired amino acid: mp 210–215° dec; ir (Nujol) $\nu_{NH_3^+}$ 3000, ~2100, $\nu_{CO_2^-}$ 1640 (sh), 1590, 1550 cm^{-1}; nmr (D_2O) τ 6.50 (broad t, 1 H), 6.95 (q, 4 H), 6.75–7.40 (m, 8 H), 8.60 (t, 6 H).

Anal. Calcd for $C_{10}H_{22}O_2N_2S$: C, 51.25; H, 9.46; N, 11.95; S, 13.68. Found: C, 51.35; H, 9.24; N, 11.57; S, 13.39.

Alkylated *N*-Acetyl-L-histidinmethylamide (IId).—A solution of 1.056 g of *N*-acetyl-L-histidinmethylamide (Id) in 100 ml of distilled water was added to the aziridinium solution prepared from 0.860 g of *N,N*-diethyl-2-chloroethylamine hydrochloride in 20 ml of distilled water as described below. The reaction mixture was maintained at pH 7.0 and at 37° for 48 hr, concentrated to dryness on a warm bath under reduced pressure, and extracted with three 50-ml portions of methanol. After evaporation *in vacuo* to dryness, the resulting residue was chromatographed on silical gel, using 15% methanol in chloroform as an eluent, to give 708 mg (46%) of colorless, oily semisolid, which was recrystallized from benzene–*n*-hexane–ether to yield colorless needles having mp 128–129°. Our data do not indicate unequivocally whether the product is 1- or 3-alkylated *N*-acetyl-L-histidinmethylamide (IId): ir (Nujol) ν_{NH} 3400, ν_{CO} 1645, 1650 (sh), 1575, 1555 cm^{-1} (sh); nmr ($CDCl_3$) τ 2.60 (s, 1 H), 3.22 (s, 1 H), 5.42 (m, 1 H), 5.99 (d, 2 H), 6.12 (t, 2 H), 7.02 (d, 2 H), 7.29 (d, 3 H), 7.50 (q, 4 H), 7.50 (t, 2 H), 8.01 (s or d, 3 H), 9.40 (t, 6 H).

Anal. Calcd for $C_{15}H_{27}O_2N_5$: C, 58.23; H, 8.80; N, 22.63. Found: C, 57.77; H, 8.50; N, 22.59.

Alkylated *N*-Acetyl-L-tyrosinmethylamide (IIa).—A solution of 2.36 g of *N*-acetyl-L-tyrosinmethylamide (Ia) in 236 ml of distilled water was added to the aziridinium solution prepared from 8.61 g of *N,N*-diethyl-β-chloroethylamine hydrochloride in 150 ml of distilled water as described previously. After incubation at pH 7.0 and at 37° for 48 hr, the reaction mixture was concentrated to one-third volume on a warm bath under reduced pressure, acidified with concentrated hydrochloric acid, and then extracted with five 50-ml portions of ethyl acetate. The extracts were washed, dried, and evaporated *in vacuo* to dryness to give 1.05 g (45%) of the starting material. The aqueous layer was made alkaline with concentrated aqueous NaOH and extracted with five 50-ml portions of ethyl acetate. The combined extract was washed with 5% aqueous NaOH and saturated NaCl, dried over magnesium sulfate, and then evaporated *in vacuo* to dryness to afford 1.45 g of oily product, which rapidly solidified on trituration with *n*-hexane. The solid (630 mg) so obtained was collected and washed with *n*-hexane, then chromatographed on silica gel, using 20% methanol in chloroform as an eluent, to give the desired alkylated tyrosine monopeptide in a yield of 9.3% (320 mg), accompanied by a small amount of starting material and *N,N*-diethylethanolamine. The crude product was recrystallized from benzene–*n*-hexane–ether to give colorless needles: mp 140–141°; ir (Nujol) $\nu_{NH,OH}$ 3400, ν_{CO} 1655, 1550, 1525 cm^{-1}; nmr ($CDCl_3$) τ 2.98 (d, 2 H), 3.20 (d, 2 H), 5.34 (m, 1 H), 6.00 (t, 2 H), 7.02 (d, 2 H), 7.14 (t, 2 H), 7.32 (s, 3 H), 7.34 (q, 4 H), 8.04 (s, 3 H), 8.90 (t, 6 H).

Anal. Calcd for $C_{18}H_{29}O_3N_3$: C, 64.45; H, 8.71; N, 12.53. Found: C, 64.17; H, 8.92; N, 12.52.

Alkylated *N*-Acetyl-L-cysteinmethylamide (IIb).—A solution of 352 mg of *N*-acetyl-L-cysteinmethylamide (Ib) in 20 ml of distilled water was added to the aziridinium solution prepared from 516 mg of *N,N*-diethyl-2-chloroethylamine hydrochloride in 20 ml of distilled water as described previously. The reaction mixture was maintained at pH 7.0 and 37° for 24 hr under nitrogen, and concentrated to dryness on a warm bath under reduced pressure. The resulting residue was extracted with three 25-ml portions of methanol, and, after evaporation of the combined extracts, the residue was chromatographed on both silica gel and alumina, successively, using chloroform–methanol (9:1) as an eluent, to afford 486 mg (88%) of white solid, which was recrystallized from a mixture of benzene–*n*-hexane–ether to give colorless needles: mp 124–126°; ir (Nujol) ν_{NH} 3360, ν_{CO} 1645, 1570, 1545 cm^{-1}; nmr ($CDCl_3$) τ 2.50 (broad d, 1 H), 3.02 (broad, 1 H), 5.50 (m, 1 H), 7.00–7.50 (m, 6 H), 7.34 (s, 3 H), 7.43 (q, 4 H), 8.00 (s, 3 H), 8.99 (t, 6 H).

Anal. Calcd for $C_{12}H_{23}O_2N_3S$: C, 52.32; H, 9.17; N, 15.26; S, 11.64. Found: C, 52.30; H, 9.15; N, 15.13; S, 11.81.

Alkylation Rates.—A 0.1 *M* solution of *N,N*-diethyl-2-chloroethylamine hydrochloride in distilled water was adjusted to pH 10.0, allowed to stand at 0° for 30 min, and then returned to pH 7.0. A 10.0-ml aliquot of this approximately 0.1 *M* aziridinium solution was added to 20.0 ml of 0.01 *M* monopeptide in distilled water. The reaction mixture was maintained at pH 7.0 and 37° usually for 48 hr. The extent of reaction was measured by selective colorimetry appropriate for each monopeptide, as described below.

Estimation of the cyclic imonium ion was carried out on 10.0 ml of *ca.* 0.1 *M* aziridinium solution added to 20.0 ml of distilled water and maintained at pH 7.0 and 37° for 48 hr. Then 10.0 ml of 0.1 *M* sodium thiosulfate was added to a 6.0-ml aliquot of this solution. After standing at room temperature for 3 hr, the excess thiosulfate was titrated by 0.1 *M* iodine solution using a starch indicator; the amount of the remaining aziridinium ion was 70.2% based on *N,N*-diethyl-2-chloroethylamine hydrochloride used, while at zero time, 88.8% of the original hydrochloride was found to be converted to aziridinium ion. From these data, the second-order rate constant for hydrolysis (k_w) is estimated as $k_w^{37} = 2.45 \times 10^{-8}$ l. mol^{-1} sec^{-1}. This is in good agreement with the value of $k_w^{45} = 3.0 \times 10^{-8}$ l. mol^{-1} sec^{-1} reported earlier.[1a]

Competition Factor for Histidine Monopeptide (Id).—Aliquots (1.0 ml) of the reaction mixture, prepared from 0.1 *M* cyclic imonium ion solution and 0.01 *M* histidine monopeptide solution, were taken at specified intervals and measured up to 100 ml by addition of distilled water. To 20.0 ml of this diluted reaction mixture was added 10.0 ml of 10% aqueous sodium carbonate and 5.0 ml of freshly prepared diazotized reagent, which was made from the same volumes of 0.5% sulfanilic acid in 3.5% aqueous hydrochloric acid and 5.0% aqueous sodium nitrite solution.[4] After 10 min at room temperature, the absorbancies of the solution and the control were measured at 490 mμ against a reagent blank. The results are summarized in Table I. From these

TABLE I
REACTION OF 0.0067 *M* HISTIDINE MONOPEPTIDE WITH 0.0295 *M* DIETHYLAZIRIDINIUM ION AT 37°, pH 7

t, min	240	550	1000	1350	1900	2880
% Alkylation	8.8	17.0	27.5	34.5	42.0	52.3

data, the second-order rate constant, k_a, for alkylation is estimated to be 2.35×10^{-4} l. mol^{-1} sec^{-1} and the competition factor k_a/k_w to be 9.6×10^3.

Competition Factor for Tyrosine Monopeptide (Ia).—After 48 hr, 5.0-ml aliquots of the reaction mixture, prepared from 0.1 *M* cyclic imonium ion solution and 0.01 *M* tyrosine monopeptide, were diluted to 100 ml by addition of 0.05 *N* aqueous sodium hydroxide solution. The optical densities of this solution and the control solution were measured at 295 mμ to be 0.678 and 0.710, respectively. From these values, the percentage of the alkylated tyrosine monopeptide was calculated to be 4.4%, giving

(4) H. T. MacPherson, *Biochem. J.*, **40**, 470 (1946).

a value for k_a of 1.0×10^{-5} l. mol^{-1} sec^{-1} and for the competition factor k_a/k_w of 400.

Competition Factor for Ionized Tyrosine Monopeptide.—The alkylations of the tyrosine monopeptide at pH 8.0 and 9.0 were carried out in the same way as described above. The rate constants for aziridinium ion hydrolysis (k_w) at both pH 8.0 and 9.0 were determined by the sodium thiosulfate–iodine method as described previously. Furthermore, using the value of the dissociation constant for tyrosine itself ($K = 4.0 \times 10^{-11}$),[5] the second-order rate constant for the phenolate ion was calculated from the data at pH 9 to be $k_a = 2.2 \times 10^{-3}$ l. mol^{-1} sec^{-1}, giving the competition factor $k_a/k_w = 7 \times 10^4$ (Table II).

TABLE II

REACTION OF 0.0067 *M* TYROSINE MONOPEPTIDE WITH 0.0295 *M* DIETHYLAZIRIDINIUM ION (37°, 48 HR)

	pH 7.0	pH 8.0	pH 9.0
% Alkylation	4.4	5.5	57.7
Rate constant k_w ($\times 10^8$)	2.45	2.76	3.15
Apparent k_a ($\times 10^5$)	1.0	1.15	9.5
Apparent competition factor	400	420	3200

Competition Factor for Cysteine Monopeptide (Ib).—A 1.0-ml aliquot of the reaction mixture prepared from aziridinium ion and cysteine monopeptide as described previously was taken periodically and diluted with distilled water up to 100 ml. A 1.0-ml aliquot of this diluted reaction mixture, and 0.02 ml of 5,5′-dithiobis(2-nitrobenzoic acid) solution, which was prepared from 19.8 mg of 5,5′-dithiobis(2-nitrobenzoic acid) and 5.0 ml of pH 7.0, 0.1 *M* phosphate buffer, were added to 2.0 ml of pH 8.0, 0.05 *M* phosphate buffer solution in a photometer cell.[6] After rapid color development, the adsorbancy at 412 mμ was measured against a reagent blank at each time. The data are summarized in Table III.

TABLE III

REACTION OF 0.0067 *M* CYSTEINE MONOPEPTIDE WITH 0.0295 *M* DIETHYLAZIRIDINIUM ION AT 37°, pH 7.0

t, min	50	150	330	645	1230	1890	2880
% Alkylation	8.0	19.9	30.0	38.5	56.9	70.2	81.5

From these data, the rate constant is estimated as $k_a = 1.0 \times 10^{-3}$ l. mol^{-1} sec^{-1} and the competition factor as $k_a/k_w = 4.2 \times 10^4$.

Competition Factor for Methionine Monopeptide (Ic).—A 5.0-ml aliquot of the reaction mixture, prepared from aziridinium solution and methionine monopeptide solution as described previously, was added after 24 hr to a mixture of 10.0 ml of distilled water and 20.0 ml of 0.2% aqueous pentacyanoaminoferrate ammonium disodium salt.[7] After 1 min at room temperature, 2.0 ml of glacial acetic acid and then 1.0 ml of 10% aqueous sodium nitrite were added to the above mixture. The optical densities of the reaction mixture and the control, read at 515 mμ against a reagent blank within 30 min, were 0.305 and 0.395, respectively. From these values, the percentage of the alkylated methionine monopeptide was calculated to be 23%, the second-order rate constant k_a to be 5.9×10^{-5} l. mol^{-1} sec^{-1}, and the competition factor to be $k_a/k_w = 2400$.

Analysis of Methionine Monopeptide Alkylation by Amino Acid Analyzer.—As the direct isolation of the alkylated methionine monopeptide was very difficult, the hydrolyzed products of the alkylated methionine monopeptide were measured and identified by amino acid analyzer, as proposed by Gundlach.[8a] A 1.0-ml aliquot of the above reaction mixture was oxidized with performic acid and, after lyophilization, hydrolyzed with 5.0 ml of 6 *N* aqueous hydrochloric acid by heating on an oil bath at 115–120° for 24 hr. After evaporation of hydrochloric acid *in vacuo*, and suitable dilution for amino acid analyzer with distilled water, the sample solution was analyzed and determined to contain 9.8% of methionine sulfoxide, 78.1% of methionine sulfone, 7.6% of homoserine, 1.2% of homoserine lactone, 2.3% of methionine, and 1.2% of the alkylated homocysteine (IIc).[8b] The peaks were identified by comparison with authentic samples. The formation of methione sulfoxide must arise from the alkylated methione monopeptide, since only methionine sulfone and *no* sulfoxide was observed when methione monopeptide was treated in the same way.

Alkylations of Cystine and Tryptophan Monopeptides.—Although the alkylations of both cystine monopeptide (IIa) and tryptophan monopeptide (IId) were undertaken in exactly the same procedure as described above, no detectable alkylation of either monopeptide was observed. The fluorescein–mercuric acetate method was used[9] for cystine monopeptide and the *p*-dimethylaminobenzaldehyde method[10] for tryptophan monopeptide. Furthermore, no alkylated monopeptides were isolated from the reaction mixtures, and only starting materials were recovered in almost theoretical yields (*ca.* 99%).

Alkylation of Histidine Monopeptide and Methionine Monopeptide with Iodoacetic Acid.—The alkylations of the monopeptides with iodoacetic acid were carried out in exactly the same way as for the aziridinium ion. The extent of reaction was estimated at each period by colorimetric methods, the sulfanilic acid method for the histidine monopeptide and the pentacyanoaminoferrate method for the methionine monopeptide. The data obtained are summarized in Table IV.

Alkylations of pyridine, imidazole, and diethyl sulfide (0.0067 *M*) by aziridinium ion (0.0295 *M*) were measured at pH 7.0 and 37° by determining unreacted aziridinium ion by reaction with excess 0.1 *M* thiosulfate, followed by titration of unreacted thiosulfate with 0.01 *M* iodine. After 48 hr, pyridine was 55% alkylated (C. F. = 1.2×10^4), imidazole 62% (C. F. = 1.3×10^4), and diethyl sulfide 25% (C. F. = 2.6×10^3). The results are included in Table V.

Alkylations of the above three nucleophiles under the same conditions with iodoacetic acid were monitored by measurement of iodide liberated. The iodide from 5.0-ml aliquots was converted to iodine, extracted by chloroform, and diluted to 10 ml and the optical density was read at 510 mμ. Imidazole was 3.0% alkylated in 1 hr, 29% in 17 hr; pyridine 9.0% in 1 hr, 28.8% in 3 hr; diethyl sulfide 36% in 1 hr, 50.5% in 2 hr. Values for k_a and k_a/k_w calculated from these data are included in Table V.

The solvolysis rate constant, k_w, for 0.0333 *M* iodoacetamide was measured at pH 7.0 and 37°. After 48 hr, iodide liberated indicated 2.69% reaction, corresponding to $k_w = 2.9 \times 10^{-9}$ l. mol^{-1} sec^{-1}, exactly the same as for iodoacetic acid.

Alkylation by iodoacetamide was carried out as for iodoacetic acid. Imidazole was 6.6% alkylated in 4 hr, 20.8% in 17 hr; pyridine 13.1% in 3 hr, 32.2% in 12 hr; diethyl sulfide 12.1% in 1 hr, 28% in 3 hr; histidine monopeptide 2.3% in 3 hr, 14% in 24 hr; methione monopeptide 7.9% in 1 hr, 24.8% in 3 hr.

The Competition Factors for Sodium Thiosulfate with Iodoacetic Acid and Iodoacetamide.—As the reaction of sodium thiosulfate with iodoacetic acid or iodoacetamide was too fast to be measurable by the usual iodine methods described previously, the reaction was quenched by an excess amount of iodine solution and the remaining iodine was then back-titrated with standard sodium thiosulfate. Solutions of 0.1 *M* alkylating agent and 0.01 *M* sodium thiosulfate were held at pH 7.0 and 37° for 30 min. After quick addition of 2.0 ml of 0.01 *M* sodium thiosulfate solution to 1.0 ml of the alkylating agent, the mixture was allowed to react for a specified time and then quenched by quick addition of 3.0 ml of iodine solution with stirring. The remaining iodine was back-titrated by sodium thiosulfate. The thiosulfate was 66% alkylated in 1 min with iodoacetic acid, 45% with iodoacetamide. Values for k_a and k_a/k_w are included in Table V.

(5) F. Yamazaki, K. Fujiki, and Y. Murata, *Bull. Chem. Soc. Jap.*, **38**, 8 (1965); E. Coates, P. G. Gordan, and B. Rigg, *Trans. Faraday Soc.*, **62**, 2577 (1966). The value for tyrosine monopeptide estimated from pH titration was $K_a = 3.7 \times 10^{-11}$, as determined by Dr. Arthur C. Breyer, Beaver College, Glenside, Pa.

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TABLE IV

REACTION OF 0.0067 *M* HISTIDINE AND METHIONINE MONOPEPTIDES WITH 0.0333 *M* IODOACETIC ACID AT 37°

t, min	60	120	240	360	1080	1650	2880
Histidine							
% Alkylation			4.9		19.0	28.2	44.5
C. F. ($\times 10^{-4}$)[a]			3.8		3.8	3.8	4.0
Methionine							
% Alkylation	22.2	38.8	54.2	69.0	82.4		
C. F. ($\times 10^{-5}$)[a]	7.8	8.0	6.7				

[a] The second-order rate constant for hydrolysis of iodoacetic acid was measured by iodometry to be $k_w = 2.9 \times 10^{-9}$ l. mol^{-1} sec^{-1}

TABLE V

SECOND-ORDER RATE CONSTANTS k_a (AND COMPETITION FACTORS k_a/k_w) FOR REACTION OF VARIOUS NUCLEOPHILES WITH DIETHYLAZIRIDINIUM ION, IODOACETIC ACID, AND IODOACETAMIDE AT pH 7 AND 37° IN WATER

Registry no.	Nucleophile	Az	IAcONa	Iodoacetamide
14383-50-7	$S_2O_3^{2-}$	0.13[a]	0.55	0.29
		(5.3×10^6)[b]	(1.9×10^8)	(1.0×10^8)
288-32-4	Imidazole	3.2×10^{-4}	2.5×10^{-4}	1.5×10^{-4}
		(1.3×10^4)	(8.5×10^4)	(5.0×10^4)
	Histidine monopeptide	2.4×10^{-4}	1.1×10^{-4}	5.5×10^{-5}
		(9.6×10^3)	(3.8×10^4)	(1.9×10^4)
110-86-1	Pyridine	3.0×10^{-4}	1.0×10^{-3}	3.8×10^{-4}
		(1.2×10^4)	(3.4×10^5)	(1.3×10^5)
	Methionine monopeptide	5.9×10^{-5}	2.3×10^{-3}	7.2×10^{-4}
		(2.4×10^3)	(8.0×10^5)	(2.5×10^5)
352-93-2	Diethyl sulfide	6.4×10^{-5}	4.3×10^{-3}	1.0×10^{-3}
		(2.6×10^3)	(1.5×10^6)	(3.5×10^5)
	Cysteine monopeptide	1.0×10^{-3}		
		(4.2×10^4)		
	Tyrosine monopeptide	1.0×10^{-5}		
		(400)		
38616-08-9	Anion (pH 9)	2.2×10^{-3}		
		(7.0×10^4)		
7732-18-5	Water (k_w)	2.45×10^{-8}	2.9×10^{-9}	2.9×10^{-9}

[a] Estimated graphically from the data in ref 1; k_a in l. mol^{-1} sec^{-1}. [b] The nucleophilic constant n used by others[11-13] is log (competition factor).

Discussion

There have been many efforts made to correlate relative reactivities in SN2 nucleophilic substitutions.[11-14] Most of these published data and correlations rate the nucleophilicities of various nucleophiles in the same relative order and are in general agreement with our values for diethylaziridinium ion as alkylating agent. The most extensive listing, especially with many nucleophiles analogous to those we have studied, is for methyl iodide in ethanol.[12] The authors indicate that their nucleophilic constants are 1.4 times those of Swain and Scott,[11] which are very close to those for aziridinium and sulfonium compound.[15] Our values for imidazole ($n = \log k_a/k_w = 4.1$), pyridine (4.1), diethyl sulfide (3.4), phenolate ion (4.8), and thiosulfate (6.7) are all reasonably close to the adjusted values of Pearson,[12] 3.55, 3.7, 3.8, 4.1, and 6.4, respectively.

It was thus a considerable surprise to find that the relative order of imidazole (a "hard" base[12]) and diethyl sulfide (a "soft" base[12]) were markedly altered on going to iodoacetic acid or iodoacetamide as alkylating agent. The sulfide group of methionine changes from about fivefold lower reactivity with aziridinium ion to about 20-fold greater reactivity with the iodoacetate alkylating agents. Thus, selection for alkylation of histidine units would be favored with aziridinium-type alkylating agents, while selection for methionine would be favored by iodoacetic acid or iodoacetamide. We are currently investigating a variety of alkylating agents to explore the structural features contributing to this reversal of order. Incidentally, none of the many general equations developed for relating nucleophilicities at sp^3 carbon accommodates such a reversal.

Examination of the results summarized in Table V clearly indicates the very remarkable reactivity of thiosulfate as a nucleophile in substitution at sp^3 carbon. This is in marked contrast to its very low reactivity as a nucleophile in reactions at sp^2 carbon, such as in attack on an ester carbonyl.[16] This dramatic reversal, and the failure to observe the "α effect" so prominent in attack at sp^2 carbon[16] in substitution at sp^3 carbon,[17] clearly support markedly different factors influencing nucleophilicity in attack at sp^3 and sp^2 carbon.

Another feature of the data in Table V is that much of the difference in competition factor, and therefore the nucleophilic constant n, for the nucleophiles studied arises from the difference in the rate of reaction with

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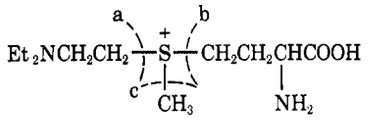
water. For example, the tenfold difference in competition factor for imidazole–aziridinium ion *vs.* imidazole–iodoacetate arises from the nearly tenfold difference in k_w for the alkylating agents. It is thus a serious question whether it is, in this case, more revealing to compare k_a's or competition factors. Since there is no compelling reason, other than convenience and custom, to pick water as the reference nucleophile, it is not the absolute magnitude of the nucleophilic constants which is significant but their relative order. Thus the significant change in relative rates for "hard" and "soft" nucleophiles with the iodoacetate alkylating agents, like the marked change in relative order for the thiosulfate ion in nucleophilic attack at sp^2 *vs.* sp^3 carbon, must signal major changes in the factors affecting the transition states involved.

The alkylation of methionine monopeptide provided a difficult problem in isolating the reaction product. The primary product is a sulfonium salt. This product can then undergo hydrolytic cleavage at each of the three C–S^+ bonds to regenerate methionine or to form homoserine (or its lactone) or alkylated homocysteine.[8,18] Our data indicate that the cleavage conditions we used favored removal of the diethylamino group (a, 12.1%) followed by the amino acid residue (b, 8.6%) and least of all the methyl group (c, 1.2%), or a:b:c 55:40:5, respectively. This ratio, which involves the assumption that the methionine sulfoxide measured by amino acid analyzer must have arisen from IIc, is supported by the fact that the sum of the



IIc

cleavage products observed (21.9%) is in good agreement with the extent of alkylation measured indirectly by colorimetry (23%).

Registry No.—Ia, 6367-14-2; Ib, 10061-65-1; Ic, 29744-03-4; Id, 6367-11-9; Ie, 6367-17-5; If, 38615-99-5; IIa, 38616-00-1; IIb, 38616-01-2; IId, 38616-02-3; IIf, 38616-03-4; L-homocystine, 626-72-2; L-homocystine dimethyl ester hydrochloride, 38616-04-5; *N,N'*-diacetyl-L-homocystine dimethyl ester, 38616-05-6; *N,N'*-diacetyl-L-homocystinmethylamide, 38616-06-7; alkylated L-homocysteine, 38616-07-8; diethylaziridinium ion, 18899-07-5; iodoacetic acid, 64-69-7; iodoacetamide, 144-48-9.

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Carbon-13 Magnetic Resonance Spectroscopy of Steroids. Estra-1,3,5(10)-trienes[1]

THOMAS A. WITTSTRUCK* AND KENNETH I. H. WILLIAMS

The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

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The natural abundance carbon-13 magnetic resonance spectra of estra-1,3,5(10)-triene and 17 derivatives are reported. Substituent effects upon the chemical shifts of each carbon atom are determined and discussed in terms of factors known to influence ^{13}C chemical shifts.

With the advent of instrumentation for the determination of high-resolution nuclear magnetic resonance spectra of ^{13}C in natural abundance, a number of papers have appeared describing its application to the structural elucidation of natural products. The techniques of ^{13}C magnetic resonance spectroscopy appear to promise to have as great an impact upon such studies as did the techniques of proton magnetic resonance in the last decade. Just as steroids provided model compounds from which much was learned with regard to the relationship between the observed nmr parameters (chemical shifts and spin–spin coupling constants) and molecular structure in proton spectroscopy,[2] so too this class of compounds, because of their well-defined structures, promises to aid in relating the ^{13}C magnetic resonance parameters to molecular structure.

In 1969, Reich, *et al.*, published the first extensive ^{13}C investigation of steroids, examining chiefly the spectra of cholestane derivatives.[3] These authors indicated that in general carbon resonances are far more informative than proton resonances for structural analysis of steroids.

Since we have available to us a large number of steroids of verified structure, incorporating a variety of the common functional groups, we have initiated a study of the carbon-13 magnetic resonance spectra of steroids. It is hoped that through these studies the nature of substituent effects upon ^{13}C chemical shifts may be better understood. In the present paper, the ^{13}C spectra of a number of derivatives of estra-1,3,5(10)-triene are reported, together with correlations of chemical shifts with carbon atoms of the steroids. Substituent effects upon the chemical shifts of the aromatic carbon of ring A are discussed, as well as a preliminary report of substituent effects upon the atoms of the nonaromatic portion of the molecule. A fuller discussion of these latter substituent effects will be presented in a later paper.

Experimental Section

The steroids used in this study, all known compounds, are listed in Table I. They were dissolved in dioxane, whose ^{13}C signal was used as the internal lock signal. Concentrations of steroids ranged from about 0.06 *M* to about 0.35 *M*. Three milliliters of solution was used in each case for the analysis, and was contained in a 12-mm o.d. sample tube. The tube was spun at 18 rps during the analysis, which was performed at room temperature.

Spectra were obtained at 25.1 MHz using a Varian Associates HA-100-15 spectrometer, together with a Varian Associates

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