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Mixed Alkylation (Methylation and Ethylation) of Adenosine by Diazoethane in Aqueous 1,2-Dimethoxyethane[1]

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Synthesis of 2′-*O*-ethyladenosine by treatment of adenosine with diazoethane in aqueous 1,2-dimethoxyethane produced several unexpected alkylation products. Characterization of the products by several methods, including mass spectrometry of their trimethylsilyl ethers, indicated that methylation was occurring to approximately the same extent as ethylation. Analysis of the reaction conditions employing palmitic acid as an alkyl acceptor implicated the solvent (1,2-dimethoxyethane) as a potential source of the extraneous methyl groups since only ethylation was observed when diethyl ether was employed as an alternate solvent.

The reaction of adenosine and diazomethane in aqueous 1,2-dimethoxyethane has been used extensively to prepare 2′-Am[2] since the 2′-hydroxyl group is preferentially methylated under these conditions.[3-5] When adenosine was treated with diazoethane under similar reaction conditions, several unexpected products were observed. In this paper these products are identified and found to indicate the occurrence of mixed alkylation. Evidence is presented which is consistent with involvement of the solvent (1,2-dimethoxyethane) in the alkylation reaction under these conditions.

Adenosine and diazoethane were combined in a solvent of aqueous 1,2-dimethoxyethane, the reaction was permitted to reach completion, and the products were resolved by ion exchange chromatography.[6] Six prominent uv-absorbing fractions were observed as shown in Table I, whereas only four fractions were observed following alkylation of adenosine with diazomethane.[5]

Some preliminary conclusions concerning the nature of the six fractions may be reached from their relative yields and column retentions. By analogy to the reaction of adenosine and diazomethane, one of the major products would be 2′-Ae while lesser amounts of the 3′-ethyl ether and dialkylated products would be obtained.[3] Furthermore, the degree of retention of nucleosides on the ion-exchange column may be correlated with increasing ionization potential of available ribose hydroxyl groups.[7] Therefore, the expected order of elution is: dialkylation products, 2′-*O*-alkylation products, and finally 3′-*O*-alkylation products. Compounds with both 2′- and 3′-hydroxyl groups available are not eluted under these conditions. On the basis of yield and elution pattern, fractions 3 and 4 may contain 2′-alkyl ethers, one of which should be 2′-Ae, while fractions 5 and 6 may contain 3′-alkyl ethers.

Components of all six fractions were characterized by descending paper chromatography in the four solvent systems described in Table II. Fractions 3, 4, 5, and 6 each gave a single uv-absorbing spot in all four solvent systems and were estimated to be greater than 97% pure. Although the reaction of diazoethane with adenosine was expected to yield only ethylated products, surprisingly, the compound in fraction 4 migrated with 2′-Am in all four systems and the compound in fraction 6 migrated with 3′-Am in all four systems (Table II). The compounds in fractions 3 and 5 migrated faster than 2′-Am or 3′-Am, respectively, and were well resolved from each other by solvent D. These results were consistent with the tentative identification of fraction 3 as 2′-Ae and fraction 4 as 3′-Ae since the 3′-ethyl ether should have been retained longer than the 2′-ethyl ether on the ion exchange column as discussed above.

Both fractions 1 and 2 were resolved into several components by paper chromatography with the four solvents. Analytical studies on the first two column fractions in the comparable methylated adenosine series indicated they

Table I
Fractionation of Alkylated Nucleosides on Bio-Rad AG 1 Column[a]

Fraction no.	Identity	Registry no.	Tubes pooled in each fraction	Recovery, % of adenosine applied
1			12–24	3.8
2			38–44	1.7
3	2′-*O*-Ethyladenosine	52842-98-5	45–58	11.9
4	2′-*O*-Methyladenosine	2140-79-6	68–81	9.8
5	3′-*O*-Ethyladenosine	52928-62-8	105–120	3.8
6	3′-*O*-Methyladenosine	10300-22-8	179–210	2.8

[a] The column consisted of Bio-Rad AG 1-X2 (OH^-), 200–400 mesh, 4 × 40 cm, equilibrated with 40% ethanol prior to use. The crude reaction mixture (95,100 A_{260} units) was applied in 40% ethanol and eluted with 40% ethanol at a flow rate of 2 ml/min; the tube volume was 20 ml.

Table II
Paper Chromatography of Alkylated Adenosine Derivatives[a]

Compound	R_f Solvent A	Solvent B	Solvent C	Solvent D
Adenosine	0.37	0.19	0.23	0.63
Fraction 3	0.70	0.53	0.57	0.82
Fraction 4	0.61	0.38	0.47	0.76
2′-*O*-Methyladenosine	0.63	0.37	0.47	0.77
Fraction 5	0.73	0.51	0.55	0.68
Fraction 6	0.60	0.35	0.40	0.64
3′-*O*-Methyladenosine	0.59	0.37	0.40	0.64

[a] Solvents were: (A) 2-propanol–NH_4OH–0.1 *M* boric acid (7:1:2); (B) ethyl acetate–1-propanol–H_2O (4:1:2, upper phase); (C) 1-butanol–NH_4OH–H_2O (86:5:14); and (D) ethanol–1 *M* ammonium acetate (pH 7.5) (7:3); all solvent proportions by volume.

were 2′,3′-di-*O*-methyladenosine and 2′-*O*,N^6-dimethyladenosine, respectively.[5] Mixed alkylation occurring within a single molecule when adenosine was treated with diazoethane in aqueous 1,2-dimethoxyethane would account for the mixture of components noted above for fractions 1 and 2.

Based on the above chromatographic characterizations, fractions 4 and 6 were expected to contain a methyl ether on the ribose moiety while fractions 3 and 5 were expected to contain an ethyl ether on the ribose moiety. Nucleoside alkyl ethers treated with perchloric acid release the alkyl group as free alcohol,[8] which can be identified by gc of the hydrolysate.[9] Alkyl groups occurring on the base do not interfere with the analysis since the hydrolysis specifically released ether groups from the ribose moiety.[9] As expected, fractions 4 and 6 released methanol when characterized in this manner, while fractions 3 and 5 released ethanol. These results confirm the presence of ribose methyl ethers in fractions 4 and 6 and ribose ethyl ethers in fractions 3 and 5.

With these preliminary characterizations in mind, a mass spectral analysis of the nucleosides as their trimethylsilyl derivatives was performed to confirm structures. Fractions 4 and 6 were shown to be 2′-Am and 3′-Am, respectively, by comparison with authentic samples. Fractions 3 and 5 were identified as 2′-Ae and 3′-Ae, respectively, by comparison of the mass spectra of their trimethylsilyl derivatives with those of their 2′- and 3′-*O*-methyl isomers. (See paragraph at the end of paper regarding supplementary material.)

The occurrence of extensive methylation during the reaction of adenosine and diazoethane raised the question as to the origin of the methyl groups. One possibility was the presence of a potential methyl donor in the alkylating reagent, *e.g.*, the inadvertant generation of diazomethane during the preparation of diazoethane. An alternate possibility was the spontaneous formation of a methyl donor under the reaction conditions. These possibilities could be distinguished by employing diethyl ether as an alternate solvent in a diazoethane reaction. Palmitic acid was chosen as an appropriate acceptor molecule since it is soluble in both diethyl ether and 1,2-dimethoxyethane. Palmitate esters were formed under the two reaction conditions described in Table III. The products were dissolved in hexane and characterized by their gc retention times relative to standards and by combined gas chromatography–mass spectrometry.

Significant levels of methyl palmitate were produced during the reaction in the presence of aqueous 1,2-dimethoxyethane, although ethyl palmitate was the major product (Table III). However, only ethyl palmitate was formed when the reaction occurred in diethyl ether. The presence of as little as 0.01% of methyl ester relative to ethyl ester could have been detected under the conditions employed. The results indicate that the diazoethane preparation was not contaminated with a potential methyl donor such as diazomethane, but suggest the spontaneous formation of a methyl donor in the presence of aqueous 1,2-dimethoxyethane.

Table III
Gas Chromatography of the Alkyl Esters of Palmitic Acid

	% of total ester product	Rel retention[a]
Ester standards		
Methyl palmitate		0.78
Ethyl palmitate		1.00
Diazoethane in aqueous 1,2-dimethoxyethane[b]		
Methyl palmitate	6.8	0.78
Ethyl palmitate	93.2	1.00
Diazoethane in diethyl ether[c]		
Methyl palmitate	<0.01	
Ethyl palmitate	100.0	1.00

[a] Gc conditions: Hewlett-Packard F&M 402; column of U-shaped glass tubing (6 ft × ⅛ in. i.d.) packed with 3% SE-30 on silanized Supelcoport (100–200 mesh); N_2 carrier gas at 50 ml/min; oven temperature, 155°. [b] Palmitic acid (23 mg) was placed in 1 ml of water and heated to 80°, and 2 ml of 1,2-dimethoxyethane containing diazoethane at 18° was added. The reaction proceeded for 1 hr at room temperature. The aqueous phase was extracted two times with 4 ml of ether, the ether layers were combined and washed with 4 ml of water, and the ether phase was evaporated in a stream of N_2. [c] Palmitic acid (23 mg) was dissolved in 1 ml of diethyl ether and 2 ml of ethereal diazoethane was added at room temperature. The reaction proceeded for 1 hr, at which time the sample was evaporated under a stream of N_2.

The source of the methyl groups occurring in the products of these alkylation reactions has not been characterized, but a potential source is the methyl ether groups of the solvent, 1,2-dimethoxyethane. The exchange of the ethyl donor with these methyl groups could involve formation of a trialkyloxonium ion intermediate. Both triethyloxonium ion[10] and trimethyloxonium ion[11] have been prepared as their fluoroborate salts. This postulated intermediate could then dissociate to form a methyl donor or serve as a direct alkylating agent.

A clear precedent for solvent participation in diazoalkylation reactions is not known, but it is interesting to note that methyl ether formation of glucopyranosyl derivatives with diazomethane was catalyzed by the presence of methanol.[12] In addition, Williams and Sweeley[13] have observed that the use of methanol as a component of the solvent in esterification of *p*-hydroxybenzoic acid by diazomethane resulted in complete formation of the *O*-methyl ether in addition to the ester; in the absence of methanol, only about 1–5% of the *O*-methyl ether was formed.

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Registry No.—Adenosine, 58-61-7; diazoethane, 1117-96-0; 1,2-dimethoxyethane, 110-71-4.

Supplementary Material Available. The mass spectral characterization of column fractions 3–6 and authentic 2′-Am and 3′-Am as their trimethylsilyl derivatives will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JOC-74-3674.

References and Notes

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(2) The abbreviations used in this paper are as follows: gc, gas chromatography; 2′-Am, 2′-*O*-methyladenosine; 3′-Am, 3′-*O*-methyladenosine; 2′-Ae, 2′-*O*-ethyladenosine; 3′-Ae, 3′-*O*-ethyladenosine.
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Kinetics of the Formation of *N*-Arylsydnones from *N*-Nitroso-*N*-Arylglycines

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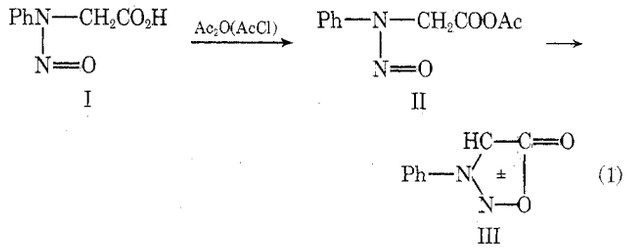
The formation of *N*-arylsydnones by the reaction of *N*-nitroso-*N*-arylglycines with acid anhydrides (mainly dichloroacetic anhydride) has been kinetically studied by means of uv spectrophotometry of the produced sydnones in dioxane or other solvents. The rate is expressed as $v = k_2[R^1C_6H_4N(NO)CHR^2COOH][(RCO)_2O]$, and virtually no effect was observed on addition of RCO_2H or pyridine. An electron-withdrawing group on *N*-phenyl (R^1) retards the reaction, while the same group on the α carbon (R^2) accelerates it. An electron-withdrawing group on acid anhydride accelerates the reaction in general. These findings suggest a mechanism involving rate-determining cyclization of the hydrogen-bonded acid anhydride of the substrate by a nucleophilic attack of the nitroso oxygen on the carbonyl carbon.

Since the discovery of sydnone,[1] which was prepared by the condensation of *N*-nitroso-*N*-phenylglycine with acetic anhydride, some analogous mesoionic compounds have been prepared, but no kinetic study on the formation has appeared.[2,3]

A mechanism involving acetyl glycinoyl anhydride (II) (eq 1) has been postulated on the basis of the formation of II by the reaction of I with acetyl chloride, the formation of sydnone (III) from II on heating, and some other facts.[3b]



However, since no kinetic study was done, the mechanism is still obscure. That is, is the main path truly that *via* anhydride II? Which step is rate determining? Does an attack of nitroso oxygen on carbonyl carbon or an attack of carbonyl oxygen on nitroso nitrogen occur? To clarify these uncertainties, we carried out kinetic studies of the reaction by means of uv spectrophotometry of the product with some *N*-nitroso-*N*-arylglycines and with some substituted acetic anhydrides. The following is a summary of our results which suggest a probable and more accurate mechanism for the reaction.

Table I
Second-Order Rate Constant for the Reaction of *N*-Nitroso-*N*-phenylglycine with Dichloroacetic Anhydride in Dioxane at 23°

Initial concn (10^{-4} *M*)		
Substrate	Acid anhydride	k_2, M^{-1} sec^{-1}
2.74	6.00	9.5
2.74	9.01	11.5
2.74	12.01	8.6
2.74	30.03	11.7
3.28	6.00	9.4
3.83	6.00	11.7
4.38	6.00	10.1
4.93	6.00	11.6
5.47	6.00	10.0

Results

Kinetics. The rates of the reactions of all the glycines and acid anhydrides studied can be expressed as $v = k[ArN(NO)CH_2CO_2H][(RCO)_2O]$. A typical kinetic run for the reaction of *N*-nitroso-*N*-phenylglycine with dichloroacetic anhydride in dioxane is shown in Table I, where the second-order rate constants hold a satisfactory constancy. No reaction occurs with *N*-nitrosophenylglycine ester in the presence of dichloroacetic anhydride, in agreement with the observation in acetic anhydride by Baker, *et al.*[3b]

Effect of Acid and Base. The reaction gives rise to acetic acid, but, as shown in Table II, virtually no effect of ace-