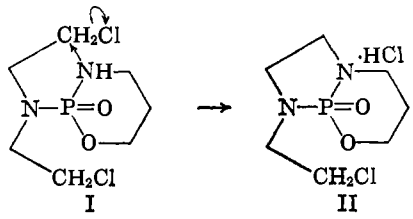
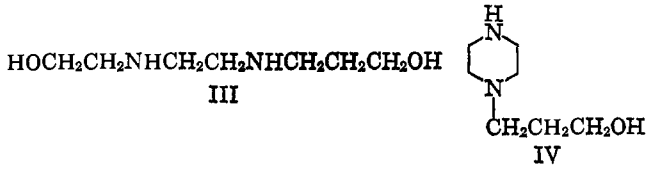
under equivalent conditions gave no trace of the dioldiamine III. This intramolecular alkylation mechanism is also consistent with the observation that cyclophosphamide gives essentially no reaction when tested as an alkylating agent with 4-(γ-nitrobenzyl)pyridine[11] even on prolonged heating. Work is in progress on the identification of the possible hydrolytic intermediates.



I II



III IV

The implications of these results do not support the various hydrolytic mechanisms that have been suggested for cyclophosphamide involving liberation of norHN2[12] or its hydrolysis products[4] as discrete entities. The possible relationship of the present results to the biological mechanism of action of cyclophosphamide is under investigation.

(11) E. Boger and O. M. Friedman, *Anal. Chem.*, 33, 906 (1961).
(12) H. Arnold and F. Bourseaux, *Angew. Chem.*, 70, 539 (1958).

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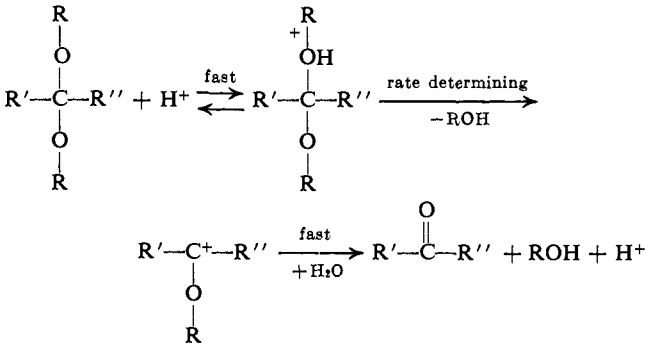
Neighboring Group Participation in Acetal Hydrolysis[1]

Sir:

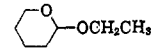
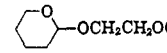
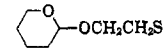
Previous work has indicated that hydrolysis of acetals and ketals, although probably proceeding *via* the carbonium ion mechanism



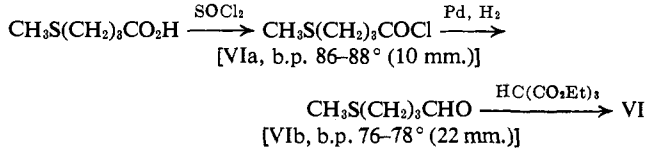
is not subject to rate enhancement by neighboring nucleophiles.[2] For example, Kreevoy and Taft[3] observed no significant departure in hydrolysis rates for phenylacetaldehyde diethyl acetal, benzyl methyl ketone diethyl ketal, and bromoacetone diethyl ketal from those rates anticipated on the basis of the combined inductive effects of the substituents.

We wish now to report an instance of apparent assistance by the methylthio group in hydrolysis of methylthioacetaldehyde diethyl acetal (IV); also recorded here are effects of methylthio and methoxy groups in hydrolysis of higher acetals and ketals. As shown in Table I, the hydrolysis rate determined for IV, although

Table I. Hydrolysis of Acetals and Ketals in 50% Dioxane-Water (v./v.) at 25.0°

No.	Compound[a]	k_2, l. mole^{-1} sec.$^{-1}$ [c]
I	$CH_3CH(OCH_2CH_3)_2$	0.254
II	$CH_3OCH_2CH(OCH_2CH_3)_2$[b]	2.0×10^{-4}
III	$CH_3CH_2OCH_2CH(OCH_2CH_3)_2$[b]	2.0×10^{-4}
IV	$CH_3SCH_2CH(OCH_2CH_3)_2$	2.33×10^{-2}
V	$CH_3(CH_2)_3CH(OCH_2CH_3)_2$	0.177
VI	$CH_3S(CH_2)_3CH(OCH_2CH_3)_2$	0.125
VII	$CH_3(CH_2)_3C(OCH_2CH_3)_2CH_3$	8.59×10^{2}
VIII	$CH_3O(CH_2)_3C(OCH_2CH_3)_2CH_3$	3.79×10^{2}
IX	$CH_3S(CH_2)_3C(OCH_2CH_3)_2CH_3$[b]	2.5×10^{2}
X		1.59×10^{-2}
XI		2.05×10^{-2}
XII		1.53×10^{-2}

[a] All substrates in these experiments were carefully purified by fractional distillation, usually immediately before carrying out kinetic runs. Distillation from sodium borohydride facilitated removal of peroxides and free aldehyde or ketone from these substances in certain instances. VI, b.p. 102–103° (10 mm.), VIII, b.p. 93° (14 mm.), IX, b.p. 112.5–113° (12 mm.), XI, b.p. 80–82° (15 mm.), and XII, b.p. 114–116.5° (15 mm.), are new compounds. VI was prepared as follows.



[VIa, b.p. 86–88° (10 mm.)]

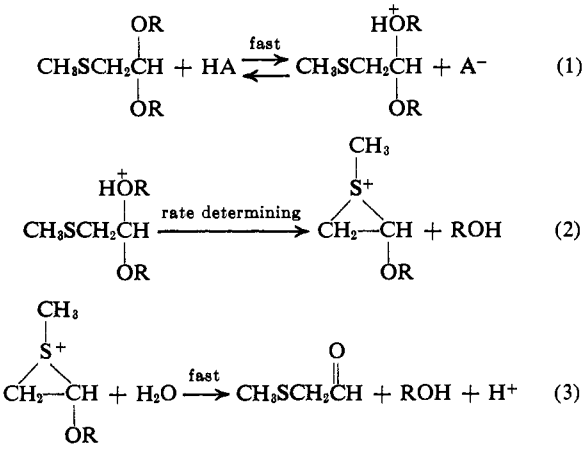
[VIb, b.p. 76–78° (22 mm.)]

VIII and IX were synthesized by reaction of $NaOCH_3$ and $NaSCH_3$, respectively, with $Cl(CH_2)_3C(OCH_2CH_3)_2CH_3$ [VIIIa, b.p. 92–93° (13 mm.)] which was prepared from 5-chloro-2-pentanone by reaction with triethyl orthoformate. XI and XII were synthesized by reaction of the appropriate alcohol with dihydropyran. Elemental analyses of VI, VIa, VIb, VIII, VIIIa, IX, XI, and XII by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by Micro-Tech Laboratories, Inc., Skokie, Ill., agreed closely with theoretical values. [b] Hydrolysis of these substances was subject to a primary salt effect, and the k_2 values given here are those obtained on extrapolation to zero ional strength. Apparently, dependence of the second-order constant for hydrolysis of III on ionic strength has not been recognized before this; Kreevoy and Taft[3] give 8.62×10^{-4} as the k_2 for this compound. [c] All rate determinations were based on increments in absorbance at the wave length corresponding to the $n \rightarrow \pi^*$ transition for the carbonyl compound formed on hydrolysis. Equilibria between hydrated and unhydrated forms of these aldehyde or ketone products appeared to be established sufficiently rapidly so as not to complicate these measurements; the same was apparently true of the equilibrium between $HO(CH_2)_4CHO$ and its cyclic hemiacetal, products from hydrolysis of X, XI, and XII. The values for k_2 are in each instance the average of several runs.

(1) Supported by a grant (GM 05524-07) from the National Institutes of Health.
(2) Alkaline cleavage of the alkali-sensitive glycosides, of the type represented by phenyl β-D-glucoside, is in a sense an exception, for in this reaction oxygen at C-2 undoubtedly participates by nucleophilic attack at C-1 with release of the phenoxide ion. This kind of glycoside scission proceeds, however, without apparent protonation of the glycosidic oxygen, and the final products are phenoxide ion and the 1,6-anhydro sugar. For a discussion of alkali-sensitive glycosides see C. E. Ballou, *Advan. Carbohydrate Chem.*, 9, 59 (1954).

(3) M. M. Kreevoy and R. W. Taft, Jr., *J. Am. Chem. Soc.*, 77, 5590 (1955).

approximately 10-fold smaller than that for the parent compound I, is more than 100 times larger than the hydrolysis rates measured for the diethyl acetals of methoxy- and ethoxyacetaldehyde (II and III, respectively). Inasmuch as the σ^* values for the methylthio, methoxy, and ethoxy groups are nearly the same,[4] these data clearly indicate a rate-augmenting effect that does not quite compensate for the retardation expected from the inductive effect of the methylthio group. The relative hydrolysis rates for methylthioacetaldehyde and methoxy- and ethoxyacetaldehyde acetals may be complicated by protonation of the methoxy and ethoxy oxygen, in contrast to the improbability of protonation under these conditions of sulfur of the methylthio group,[5] but this possibility does not explain the abnormally fast hydrolysis of the methylthioacetaldehyde acetals.[6] It seems reasonable, then, to assume that the methylthio group assists in hydrolysis of IV by nucleophilic attack at the acetal carbon and that the following scheme approximates the course of these reactions.



If the cyclic sulfonium ion is a true intermediate in these reactions, then its formation is rate determining (as indicated in eq. 2), for during hydrolysis of IV 2 moles of ethanol appeared with each mole of aldehyde; *i.e.*, there was no evidence for rapid release of 1 molecule of ethanol followed by slower formation of the second molecule of ethanol and a molecule of the aldehyde as would have been observed if reaction 3 in this scheme were the slow step. Ethanol concentrations in these reaction mixtures were determined enzymatically, after quenching the reaction in 0.02 *M* sodium bicarbonate, by the horse liver alcohol dehydrogenase catalyzed reduction of nicotinamide–adenine dinucleotide coupled to a diaphorase (*Clostridium kluyveri*) mediated reduction of methylene blue.

Hydrolysis of 4-methylthiobutanal diethyl acetal (VI) is slower than that of the parent compound V; similarly, hydrolysis of IX is slower than that of VII. As before, stable sulfonium intermediates do not seem to be formed on hydrolysis of VI and IX, for enzymatic assay by the procedure described above revealed no initial burst of ethanol during hydrolysis of IX. Present knowledge of field effects makes it difficult to estimate the degree of assistance by the methylthio group in the hydrolysis of VI and IX, although it would appear from the relative hydrolysis rates for IX and its methoxy analog VIII that there is little, if any, rate enhancement by the methylthio group in these reactions. Among the 2-tetrahydropyranyl ethers, however, which are more sterically favorable for nucleophilic attack at the acetal carbon, the hydrolysis rate for the 2-methylthioethyl derivative XII is almost exactly equal to that of the ethyl analog X, indicating that, if a field effect by the methylthio group in XII retards hydrolysis, nucleophilic assistance compensates for this. When methoxy is substituted for the methylthio group of XII it appears that the resultant of opposing nucleophilic and field effects favors hydrolysis even more, for hydrolysis of XI is slightly faster than that of X.

These experiments are pertinent to glycosidase and transosylase catalytic mechanisms for they show that it is entirely reasonable for methionine sulfur to participate as a nucleophile with formation of S-glycosylsulfonium derivatives as reactive intermediates during reactions catalyzed by these enzymes.

Acknowledgment. We wish to thank Mr. Donald L. Schneider for redetermining the rate of acetaldehyde diethyl acetal hydrolysis.

(4) R. W. Taft, *J. Phys. Chem.*, **64**, 1805 (1960).
(5) *Cf.* E. M. Arnett, *Progr. Phys. Org. Chem.*, **1**, 308 (1963).
(6) Similar results have been observed for methylthioacetaldehyde dimethyl acetal hydrolysis in water and in 50% dioxane–water; they will be included in the complete report of these experiments.
(7) National Institutes of Health Predoctoral Fellow, 1965–1966.
(8) National Science Foundation Undergraduate Research Participant.

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