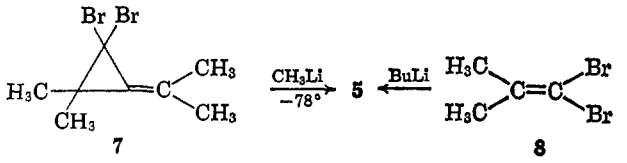


indicate that cyclopropylidene **4** is being formed in the metal halide exchange reaction, we have found isomeric, as yet unidentified, products when the reaction is run at 0°. Cumulene **5** is also formed from **8**.[12] The interesting features of further carbene addition to both the terminal[10,12] and central[12] double bonds of **5** have been noted and the present synthesis of the cumulene should facilitate further studies.[13]

(12) G. Kobrich and H. Heinemann, *Angew. Chem.*, 77, 590 (1965).

(13) NOTE ADDED IN PROOF. After submission of this article, G. Maier, *Tetrahedron Letters*, 3599 (1965), reported the synthesis of **5** from solvent-free decomposition of **1**. We experienced no difficulty in solvent decompositions when the product was removed as formed. We also favor the above stepwise decomposition over the intermediacy of a highly unstable dicarbene.

F. Thomas Bond, Diane E. Bradway
Department of Chemistry, Oregon State University
Corvallis, Oregon 97331
Received July 23, 1965

Studies on the Hydrolysis of Cyclophosphamide. I. Identification of N-(2-Hydroxyethyl)-N′-(3-hydroxypropyl)ethylenediamine as the Main Product[1]

Sir:

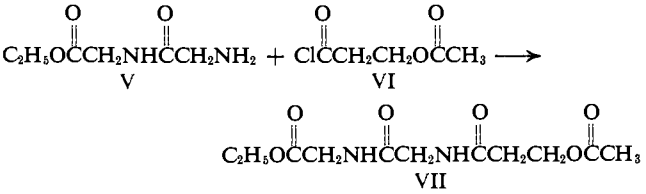
Cyclophosphamide (2H-1,3,2-oxaphosphorine, 2-[bis-(2-chloroethyl)amino]tetrahydro-2-oxide) (I), which was designed as a "transport" form of di(2-chloroethyl)-amine (norHN2),[2] is a known potent inhibitor of experimental tumors in animals that is now used clinically in certain types of human cancer. The compound is biologically inactive *per se* and becomes biologically active only upon appropriate activation *in vivo*. The nature of the activation process, which was presumed to involve release of norHN2, is still not clearly understood although a number of studies have been made.[3-7]

We report here the isolation and characterization of N-(2-hydroxyethyl)-N′-(3-hydroxypropyl)ethylenediamine (III) as the main hydrolytic product from cyclophosphamide[8] (I) when an aqueous solution (2%) of I was heated under reflux for 72 hr. The dioldiamine III was isolated directly as a crystalline dihydrochloride in 40% yield, m.p. 106–108°. *Anal.* Calcd. for $C_7H_{18}N_2O_2 \cdot 2HCl$: C, 35.73; H, 8.57; N, 11.91; Cl, 30.13. Found: C, 35.61; H, 8.60; N, 11.78; Cl, 30.02; Cl (ionic), 29.97. III was also obtained in 70% yield when isolated from the reaction mixture as a dipicrate, m.p. 209–211° (aqueous methanol). *Anal.* Calcd. for $C_7H_{18}N_2O_2 \cdot 2C_6H_3N_3O_7$: C, 36.77; H, 3.92; N, 18.10. Found: C, 36.53; H, 4.09; N, 18.08.

The identity of this hydrochloride and picrate of III was established on the basis of comparison of melting point, mixture melting point, R_f value (for the dihydrochloride, 0.16 in 1-propanol:water, 8:1), and infrared absorption spectra with authentic samples obtained through independent synthesis.

The mother liquor yielded a very small amount of another hygroscopic product, m.p. 147–151° (presoft), with R_f value 0.10 (1-propanol:water, 8:1), identical with that of an authentic sample of 1-(3-hydroxypropyl)-piperazine (IV). *Anal.* Calcd. for $C_7H_{16}N_2O \cdot H_3PO_4 \cdot HCl$: C, 30.2; H, 7.3; N, 10.1; P, 11.1. Found: C, 30.6; H, 7.4; N, 10.2; P, 11.1. The picrate of this product was found to be identical with the picrate derived from a synthetic sample of IV in respect to melting point, mixture melting point, and infrared absorption spectrum.

The dioldiamine III was synthesized by treating β-acetoxypropionyl chloride[9] (VI) with glycylglycine ethyl ester hydrochloride (V) (Schotten-Baumann) to give the acetoxydiamido ester VII in about 60% yield, m.p. 138–140° (ethyl acetate). *Anal.* Calcd. for $C_{11}H_{18}N_2O_6$: C, 48.16; H, 6.61; N, 10.21. Found: C, 48.4; H, 6.4; N, 10.3. The desired base III was obtained from VII by $LiAlH_4$ reduction, yield 56%, oil, b.p. 150–155° (0.1 mm.). *Anal.* Calcd. for $C_7H_{18}N_2O_2$: C, 51.82; H, 11.18; N, 17.27. Found: C, 52.0; H, 11.0; N, 17.2. The dihydrochloride had m.p. 108–110° (2-propanol). The dipicrate, m.p. 210–212° (aqueous methanol).



1-(3-Hydroxypropyl)piperazine (IV), prepared by alkylation of the known N-benzylpiperazine with 3-bromo-1-propanol and subsequent catalytic (10% Pd–C) hydrogenolysis, was isolated as the dihydrochloride, m.p. 233–236° (95% ethanol). *Anal.* Calcd. for $C_7H_{16}N_2O \cdot 2HCl$: Cl, 32.72; N, 12.90. Found: Cl, 32.7; N, 12.9. The compound gave a dipicrate, m.p. 249–251° (aqueous methanol), corresponding closely to the reported value,[10] m.p. 252–253° dec. (water).

From the formation of these compounds it appears that an initial intramolecular alkylation of the type I → II takes place followed by subsequent hydrolysis of the amide (N–P) and ester (O–P) bonds and in the case of the minor product IV a second N-alkylation, at some stage in the process. This suggestion is supported from paper and vapor phase chromatography of the samples taken from the reaction mixture intermittently, where the presence of breakdown products bis(2-hydroxyethyl)amine, 3-hydroxypropylamine, or bis(2-chloroethyl)amine was not indicated, and from the fact that an equimolar mixture of 3-hydroxypropylamine and bis(2-chloroethyl)amine heated to reflux

(1) Supported by a research grant (CA-02130) from National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) H. Arnold, F. Bourseaux, and N. Brock, *Naturwiss.*, **45**, 64 (1958).

(3) G. E. Foley, O. M. Friedman, and B. P. Drolet, *Cancer Res.*, **21**, 57 (1961).

(4) H. Arnold and H. Klose, *Arzneimittel-Forsch.*, **11**, 159 (1961).

(5) H. Arnold and F. Bourseaux, *ibid.*, **13**, 927 (1963).

(6) H. M. Rauen, A. Reisch, and H. Schriewer, *ibid.*, [3] **14**, 176 (1964).

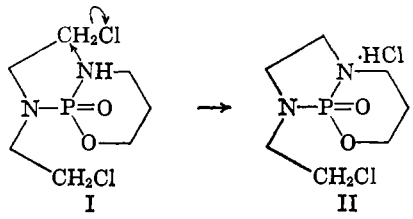
(7) H. Wilmanns, H. Graul, H. Hundeshagen, and H. Arnold, *Intern. Cancer Congr., 8th, Moscow, USSR*, 20 [1–2], 276 (1962).

(8) Kindly supplied by Dr. Harry B. Wood, Jr., Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

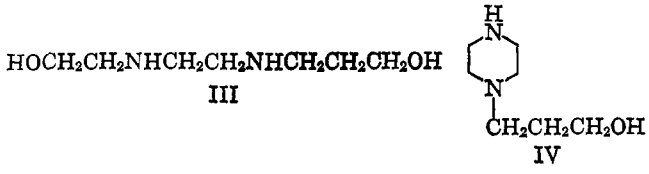
(9) T. L. Gresham, J. E. Jansen, and F. W. Shaver, *J. Am. Chem. Soc.*, 72, 93 (1950).

(10) S. M. McElvain and L. W. Bannister, *ibid.*, 76, 1126 (1954).

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.

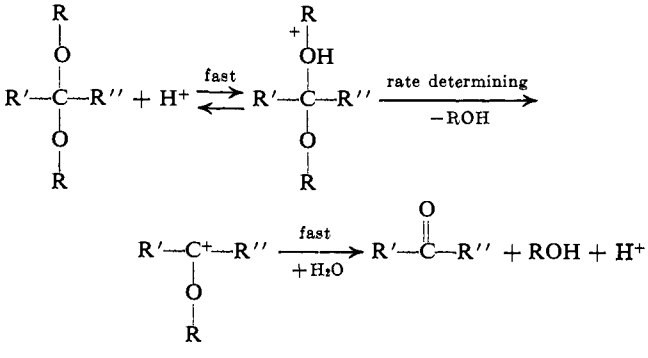
(11) E. Boger and O. M. Friedman, *Anal. Chem.*, 33, 906 (1961).
(12) H. Arnold and F. Bourseaux, *Angew. Chem.*, 70, 539 (1958).

Orrie M. Friedman, Shlomo Bien, Jiban K. Chakrabarti
Department of Chemistry, Brandeis University
Waltham, Massachusetts
Received September 13, 1965

Neighboring Group Participation in Acetal Hydrolysis[1]

Sir:

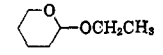
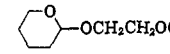
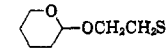
Previous work has indicated that hydrolysis of acetals and ketals, although probably proceeding *via* the carbonium ion mechanism



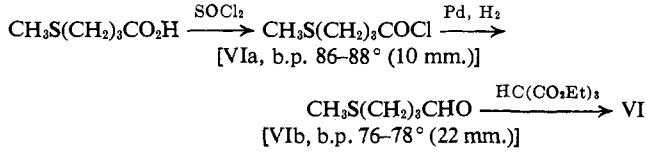
is not subject to rate enhancement by neighboring nucleophiles.[2] For example, Kreevoy and Taft[3] observed no significant departure in hydrolysis rates for phenylacetaldehyde diethyl acetal, benzyl methyl ketone diethyl ketal, and bromoacetone diethyl ketal from those rates anticipated on the basis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