Carcinogenicity of Epoxides, Lactones, and Peroxy Compounds. III. Biological Activity and Chemical Reactivity

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The uncatalyzed rates of reaction of a number of epoxides of known biological activity with thiosulfate, azide, and iodide ion were measured. The neutral hydrolysis rates and thiosulfate cleavage of several β- and γ-lactones were also determined. Carcinogenic epoxides and lactones have, with some exceptions, a higher reaction rate with the nucleophiles examined. The noncarcinogenic γ-lactones do not react with thiosulfate and are not hydrolyzed under the conditions used. A correlation of water solubility with carcinogenicity suggests that solubility plays a role in facilitating the expression of biological activity by these materials. The relationship between carcinogenesis and cross linking of deoxyribonucleic acid (DNA) by bifunctional alkylating agents is discussed in the light of possible reaction sites in DNA and interatomic distances between active functional groups. It is concluded that cross linking of the type under discussion is impossible for several of these carcinogenic materials.

In parts I and II of this series the carcinogenicity of a series of epoxides, lactones, and peroxy compounds was described.[1,2] As a part of this program dealing with the carcinogenicity and mode of action of these compounds it was important to examine their chemical reactivity, physical properties, and stereochemistry and to relate these factors to carcinogenic activity.

The present report gives the results obtained in the neutral hydrolysis, thiosulfate cleavage of lactones, and the uncatalyzed thiosulfate, azide, and iodide opening of epoxides. The compounds selected for these experiments included carcinogenic as well as noncarcinogenic materials. In addition, the water solubility and stereochemical aspects of these compounds were examined.

Experimental Section

Materials.—The epoxides and lactones were prepared and purified as described in parts I and II of this series.[1,2] Two of the compounds used in the present work were not described in our earlier studies and are described here.

β-Propiolactone.—Commercial quality β-propiolactone (Celanese Corp.) was purified by distillation, b.p. 114° (190 mm.), n^{22}D 1.4074, lit.[3] b.p. 45.8° (8 mm.).

Epichlorohydrin.—Commercial grade material (Aldrich Chemical Co.) was distilled, b.p. 114–115° (760 mm.), n^{25}D 1.4310; lit. b.p. 115–117°,[4] n^{20}D 1.438.[5]

Rate of Reaction of Epoxides with Thiosulfate, Iodide, and Azide Anions.—Standard solutions, 0.1 N, of potassium iodide, sodium thiosulfate, and sodium azide were buffered with 0.001 N Tris buffer. For kinetic runs, a known volume of a standardized solution of the nucleophile (0.025–0.10 M) was placed in the titration vessel of an automatic recording constant pH titrator (Radiometer Co., Copenhagen). The titration vessel was placed in a constant-temperature bath kept at 37.0° (±0.1°). A known amount of the epoxide (0.010–0.17 M) was then introduced; the base liberated when the epoxide reacts with the nucleophile was measured by titration with standard perchloric acid. For slow reactions this acid was 1 N, for faster reactions the acid was 3 N or 6 N. The instrument was set to maintain pH 7.0, and the perchloric acid was added automatically through a capillary-tip buret. The amount of acid added in a known time interval was recorded. In all cases, at least three kinetic runs were carried out for the determination of a rate constant using different concentrations of epoxide and/or nucleophile. The uncatalyzed hydrolysis of epoxides is slow at 37°[6]; the rate constants were therefore not corrected for this hydrolysis rate.

Neutral Hydrolysis Rates of Lactones.—A known quantity of the lactone (0.001–0.05 M) was placed in a volumetric flask and unbuffered CO_2-free water was added. The flask was placed in a constant-temperature bath at 37° and aliquots were withdrawn at varying times and then added to a standardized solution of thiosulfate. After heating the solution, the thiosulfate consumed (and hence lactone present) were determined by iodimetric titration.[3]

Rate of Reaction of Lactones with Thiosulfate.—The same iodimetric titration procedures as described above were used to determine these reaction rates.

Rate of Reaction of Diketene.—Diketene hydrolyzes very rapidly, and the rate of hydrolysis was therefore determined using the automatic recording titrator described above. The instrument maintained a constant pH of 7.0 by automatic addition of standardized base *via* a capillary buret.

Results and Discussion

The second-order rate constants of nine epoxides with three nucleophiles, thiosulfate, azide, and iodide are given in Table I. Carcinogenicities of these compounds as determined in our earlier work[1,2] are also included in this and subsequent tables. Some of the reaction rate constants showed slight drifting until over 90% reaction, *e.g.*, epoxycyclohexane, whereas others showed pronounced drifting of rate constants after *ca.* 10% reaction, *e.g.*, ethyleneoxy-3,4-epoxycyclohexane. This drifting of rate constant may be due to substrate hydrolysis. To minimize this error, reaction rate constants were determined as early in the reaction as possible. The neutral hydrolysis rates and rates of reactions of eight lactones with thiosulfate ion were determined and are recorded in Table II.

Chemical Reactivity.—Although chemical structure–biological activity relationships among aromatic and heterocyclic carcinogens have received a great deal of attention,[7] comparatively little is known about this aspect of the simple oxygenated carcinogens under discussion. On the basis of the known chemical reactivity of epoxides and β-lactones, it has been suggested that these materials function as alkylating agents and that they react with any of a number of nucleophilic (elec-

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(6) J. N. Brönsted, M. Kilpatrick, and M. Kilpatrick, *J. Am. Chem. Soc.*, **51**, 428 (1929).

(7) D. B. Clayson, "Chemical Carcinogenesis," Little, Brown and Co., Boston, Mass., 1962, p. 135.

Table I

Comparison of Chemical Reactivity with Carcinogenicity of Epoxides

Compd.	Carcinogenicity	Rate constants, $k \times 10^2$, l. mole^{-1} sec.$^{-1}$ at pH 7.0 and 37°: $S_2O_3^{2-}$	N_3^-	I^-
dl-Diepoxybutane	++	2.50	0.60	...
meso-Diepoxybutane	++	2.25	0.68	0.15 (0.43[a])
Glycidaldehyde	++	1.78	0.023	...
1-Ethyleneoxy-3,4-epoxycyclohexane	++	0.57	0.040	...
1,2,5,6-Diepoxyhexane	+[b]	1.1	...	...
1,2-Epoxybutene-3	−	1.5	0.78	0.18 (0.65[a])
Epichlorohydrin	−	0.02	0.12	0.35 (0.098[a])[c]
Epoxycyclohexane	−	0.40	...	...
Ethyleneoxycyclohexane	−	0.33	...	...

[a] K_2 at 20°. [b] Gave benign tumors in carcinogenicity testing. Lit.[c] 0.10×10^{-2}.

Table II

Rate Constants for Lactones

Compd.	Carcinogenicity	Hydrolysis rate constant k, min.$^{-1} \times 10^3$ (37°)	Second-order rate constant for reaction with thiosulfate ion k, l. mole^{-1} sec^{-1} $\times 10^2$ (24°)
β-Propiolactone	++	11.3 (11.9, 37.3[a])	13 (19, 25[a,b])
β-Butyrolactone	+	2.98 (2.48, 35[a,c])	0.19
4,5-Epoxy-3-hydroxyvalerie acid β-lactone	+[d]	0.017	3.4
Diketene	−	120 (25°)	
2,2,4-Trimethyl-3-hydroxy-3-pentenoic acid β-lactone	−	*Ca.* 2×10^{-3} (30% acetone-water)	No reaction (60% ethanol-water)
α-Angelicalactone	−	Slow	No reaction
β-Angelicalactone	−	No reaction	No reaction
γ-Butyrolactone	−	No reaction	No reaction

[a] Ref. 12. [b] Ref. 3. [c] A. R. Olson and P. V. Youle, *J. Am. Chem. Soc.*, **73**, 2468 (1951). [d] Gave benign tumors only in carcinogenicity testing.

tron-rich) centers in tissue. The various reactions which epoxides can undergo with tissue constituents were recently reviewed.[8] The reactivity of these materials with nucleophiles is therefore of significance in relation to their biological activity.

Ross[9,10] has studied the reaction rates of a number of epoxides with thiosulfate ion and has compared these rates with tumor-inhibitory activity but not with carcinogenic activity. The measurements of Ross were made in aqueous acetone in the presence of 0.2 *M* sodium thiosulfate and are not strictly comparable to those reported here.

The results indicate no large differences in the reactivities of the epoxides studied. However, three of the carcinogenic epoxides have a higher order of reactivity toward thiosulfate than the four noncarcinogens listed. Several exceptions are noted. 1-Ethyleneoxy-3,4-epoxycyclohexane has relatively lower reactivity than the other carcinogenic epoxides. The relatively high reactivity of the noncarcinogenic 1,2-epoxybutene-3 with thiosulfate and azide ion is probably due to the electron-withdrawing properties of the double bond.[11]

Glycidaldehyde appears to have a very low reactivity with azide ion. This may be due to a rapid reaction of azide ion at the aldehyde carbonyl; if the epoxide is not cleaved, base is not liberated, and since the disappearance of the nucleophile is measured by decrease in acidity, the apparent rate of reaction of glycidaldehyde with azide ion is relatively low.

β-Lactones undergo alkyl–oxygen cleavage in the pH range 1–7 and acyl–oxygen cleavage in basic and in strongly acid pH[12–14]; the *in vivo* reactions of β-lactones are therefore expected to occur by alkyl–oxygen cleavage, followed by alkylation of nucleophiles, as in the case of epoxides. For this reason the neutral hydrolysis rates and rate of reaction of these lactones with thiosulfate ion were determined. Three γ-lactones previously tested for carcinogenic activity are also included in this series although they were not expected to show significant reactivities. The order of agreement between carcinogenicity of the lactones and reactivity is better than for epoxides and shows, in fact, only one exception, *viz.*, diketene.

Diketene, which is the anhydride of acetoacetic acid, hydrolyzes rapidly in water[15]; it is highly reactive toward many nucleophilic and electrophilic reagents.[16] This high reactivity may preclude its reaction at biological sites important for carcinogenesis and hence account for its lack of carcinogenic activity. Its high hydrolysis rate made the determination of its thiosulfate reactivity impractical.

The noncarcinogenic γ-lactones, as expected, showed no reaction with thiosulfate at 37° and only α-angelicalactone showed any tendency to hydrolyze (very slowly) at this temperature. The γ-lactones do not undergo alkyl–oxygen cleavage but rather acyl–oxygen cleavage in base-catalyzed hydrolysis.

Dickens[17] has suggested the significance of α,β-unsaturation in γ-lactones for carcinogenicity. In the mouse skin carcinogenicity experiments[1,2] α- and β-angelicalactone gave negative results. In addition the presence of α,β-unsaturation does not appear of importance in facilitating alkyl–oxygen cleavage.

Water Solubility.—Reactivity toward nucleophiles in solution obviously does not account for carcinogenic potency and other factors must be involved. Among these is the solubility of these materials in water. The relationship between lipohydrophilic character of organic compounds and biological activity has been studied by several workers.[18] The water solubility of a number of epoxides was determined in the present work. Most carcinogens are in the highly water-soluble group, *e.g.*, *meso*- and *dl*-diepoxybutane, diepoxypentane, 1-ethyleneoxy-3,4-epoxycyclohexane, and glycidaldehyde. There were no carcinogens among the slightly soluble epoxides, *e.g.*, resorcinol diglycidyl ether, squalene hexaepoxide, and 1,2,5,6-diepoxycyclooctane. In the β-lactone series, β-propiolactone and β-butyrolactone are carcinogenic and water soluble, whereas diketene is inactive and water insoluble. This correlation suggests that solubility plays some role in

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(18) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964), and references cited therein.

facilitating the expression of biological activity by these materials.

Cross Linking and Carcinogenicity.—The monofunctional epoxides are largely devoid of carcinogenic activity[1,2] but are potent mutagenic agents.[19] Bifunctional epoxides, on the other hand, are carcinogenic[1,2] and exhibit mutagenicity on the same order as that of monoepoxides.[19] These characteristics raise the question of the role of the often-mentioned, cross-linking ability of bifunctional alkylating agents in accounting for their biological activity.

It has been suggested, for example, that bifunctional alkylating agents cross link the strands of the double helix of DNA at two guanine moieties. This cross linking may then result in incomplete duplication of genetic material and these processes may be involved in carcinogenesis.[20]

Nevertheless, it must be remembered that the classical carcinogenic alkylating agent, β-propiolactone, has only one active alkylating center. This compound is one of the few alkylating agents which has noticeable carcinogenic as well as mutagenic activity.

In order to evaluate the role of cross linking of DNA by these alkylating agents more accurately, the interatomic distances between the reactive centers in several epoxides were measured by use of scale drawings and Dreiding atomic models. Because of free rotation in the open-chain epoxides, the distances of closest approach and of greatest separation between terminal reactive centers were measured and are listed in Table III. Several observations become apparent from these measurements. Thus, in glycidaldehyde, which has only one epoxide function, the distance between the terminal oxygen-bearing carbon atoms is only 2.6 Å. which would make it impossible to reach from a guanine moiety on one strand to the nearest base on the adjacent strand of the DNA helix. Similarly, for β-propiolactone, cross linking of this type between bases cannot occur. The relatively short distances between epoxide functions in the diepoxybutanes and diepoxypentane also render cross linking between bases unlikely. Even without exact measurements of interatomic distances between the N-7 positions on two adjacent base pairs it is immediately apparent that cross linking can occur only when there is a much longer distance between epoxide functions. However, resorcinol diglycidyl ether, in which there are eleven atoms between the terminal epoxy-bearing carbon atoms, is inactive as a carcinogen.[1] These considerations suggest a re-evaluation of the possible sites of action of alkylating agents concerned with carcinogenesis.

TABLE III
INTERATOMIC DISTANCES IN EPOXIDES

Compd.	Distance between reactive carbon atoms (Å.) Closest approach	Greatest separation
dl-Diepoxybutane	2.5	4.0
meso-Diepoxybutane	2.5	4.0
1,2,4,5-Diepoxypentane	1.5	5.2
1,2,5,6-Diepoxyhexane	0.0	6.6
1,2,6,7-Diepoxyheptane	Overlap	7.7
1-Ethyleneoxy-3,4-epoxycyclohexane	4.6	5.4
Glycidaldehyde	2.6 (fixed)	

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Synthesis and Study of β-Chloroethylamines, Sulfides, and Sulfones Structurally Related to Dibenzyline

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Several N-arylthioisopropyl-N-benzyl-2-chloroethylamines and their corresponding sulfones were obtained. The former do not have a sulfonium structure despite their rapid rate of hydrolysis, especially the disulfurated derivative. In general they lack adrenolytic activity as do the sulfones of low hydrolytic rate. From this it can be inferred that no relationship can be established between the rate of hydrolysis and the pharmacological activity of this group of substances. The hydrolysis mechanism takes place probably *via* the sulfonium ion.

The advent of dibenzyline, N-phenoxyisopropyl-N-benzyl-2-chloroethylamine,[1] and its promising activity as an adrenolytic agent induced us to attempt the synthesis of analogous sulfides, with the object of studying their properties and the possibility of obtaining new drugs in this series. In 1950[2] a series of adrenolytic 2-haloethylamines were obtained with the following formula. The cited patent included only one

$$\mathrm{ArO(CH_2)_nCHR}$$
$$\mathrm{ArCH_2NCH_2CH_2X}$$

R = alkyl, aryl, or aryloxy
X = Cl or Br

sulfide, N-phenylthioisopropyl-N-benzyl-2-chloroethylamine hydrochloride; its properties were not described.

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(2) Smith Kline and French International Co., British Patent 673,509 (June 4, 1952).