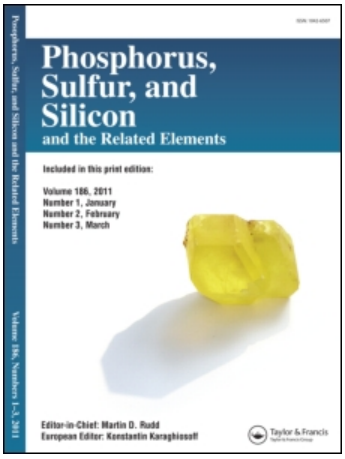

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ELECTROPHILIC CLEAVAGE OF SULFENATE ESTERS. I. POSSIBLE BIOLOGICAL IMPLICATIONS

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ELECTROPHILIC CLEAVAGE OF SULFENATE ESTERS. I. POSSIBLE BIOLOGICAL IMPLICATIONS†

by

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ABSTRACT

Base-assisted electrophilic cleavage of sulfenate esters was studied with reference to possible biological models. It is suggested that sulfenate esters (RSOR') may serve as intermediates in oxidations involving alcohol dehydrogenases. Models for the biological oxidation of alcohols *via* sulfenate ester intermediates are presented. The lipoic acid catalyzed dehydrogenation step in the actions of α-ketoacid oxidases (*e.g.*, pyruvic acid dehydrogenase and α-keto glutarate dehydrogenase) is also explained in terms of a possible sulfenate ester intermediate.

In the interaction of alcohols and amines, with membrane proteins, the possibility of reversible formation of sulfenate esters and of sulfenamide formation is suggested. Experimental support is given for the formation of carbonyl compounds, from alcohols--*via* sulfenate esters and subsequent electrophilic attack by *N*-iodosuccinimide on the esters. Such reactions of sulfenyl esters open virtually unexplored areas of chemistry and of the related biological implications. Methyl fluorosulfate ('magic methyl') in presence of base is also effective for the cleavage reaction.

INTRODUCTION

The unique role played by sulfur functions, especially by sulfhydryl and disulfide groups, in biochemical mechanisms is well known.[1,2] Sulfhydryl groups are known to be critically involved in the mechanism of action of most alcohol dehydrogenases which, among other requirements, also depend on Zn^{++} ions and essential histidyl residues for their activity.[3] Our interest in the biochemistry of prostaglandins led us to re-examine the general area of alcohol dehydrogenases because 15-hydroxy-prostaglandin dehydrogenase represents a key enzyme in governing the physiological activity of prostaglandins. Indeed, oxidation of the latter to the corresponding 15-keto compounds results in up to 90% loss of physiological activity.[4-6] Initial studies with 15-hydroxy-PGDH have indicated that it is similar to other alcohol dehydrogenases in structure and properties.[7,8]

The mechanism of action of alcohol dehydrogenases is still unclear. The theory of a direct stereospecific hydride transfer from alcohol to the coenzyme, NAD^+, and vice-versa, (*i.e.* from NADH to the carbonyl substrate), proposed by Theorell and others,[9,10] seems to be accepted by most investigators. The bulk of work currently in progress is directed towards resolving the exact role of zinc ions,[11-16] essential sulfhydryl groups,[17-19] and hystidyl residues[20] in the catalytic processes. Unfortunately, the precise roles of these variables in alcohol dehydrogenase function do not seem to be clearly understood and controversial views have been put forward. Thus, the role of Zn^{++} ions in binding the substrate and that of sulfhydryl groups in binding the coenzyme has been implicated,[9] but, not without contradiction.[15,19,21,22]

Scheme I shows the model of dehydrogenase action proposed by Theorell, which has also been supported by current crystallographic studies.[23]

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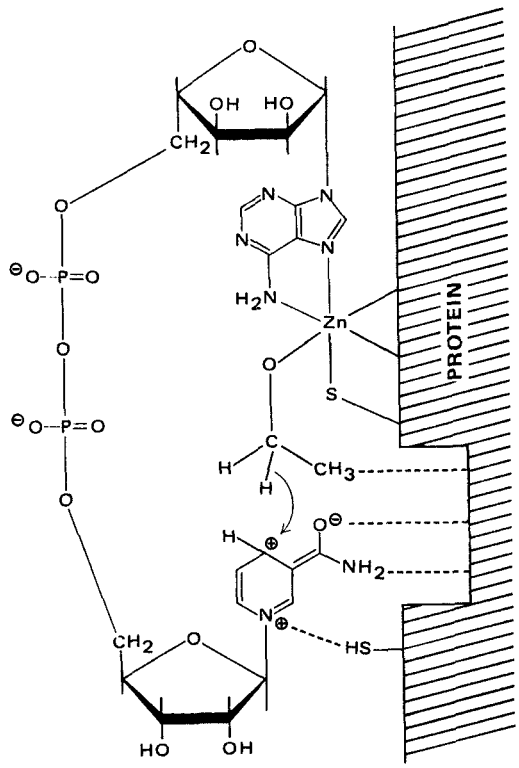


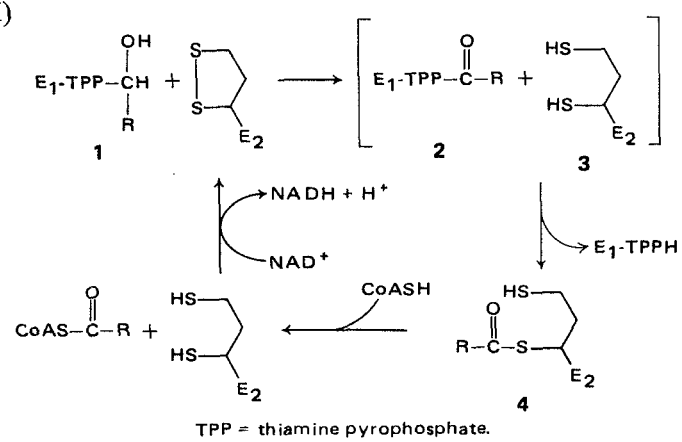
SCHEME I

Sulfur Functions in Biological Oxidations

Sulfur functions, especially the sulfhydryl and disulfide groups, have been frequently implicated in biological redox reactions.[24] Low redox potentials of most sulfhydryl disulfide couples[25] (in the range of −0.2 to −0.3 volts compared to −0.32 volts for a typical NADH/NAD$^+$ couple) indicate their easily reversible involvement in redox processes.

Except for thiol disulfide exchange reactions (RSH + R′SSR → RSSR′ + R′SH) relatively few cases are known where a definitive role has been assigned to sulfhydryl or disulfide groups in biochemical redox reactions.

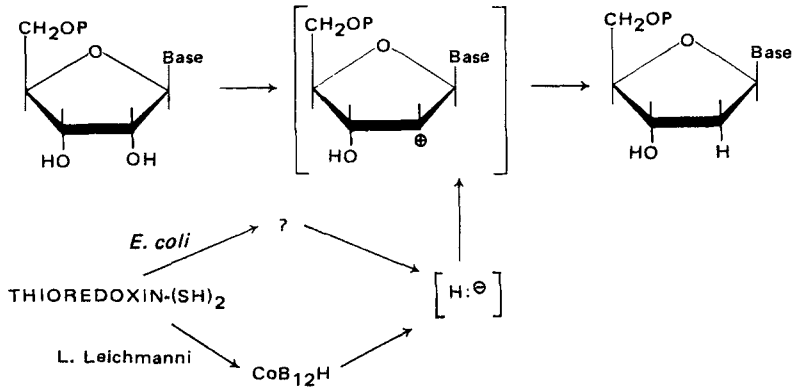
In the mechanism of α-keto oxidases (e.g., pyruvate dehydrogenase and α-ketoglutarate dehydrogenase) dihydrolipoic acid is believed to be reversibly involved in the dehydrogenation of the enzyme-bound active aldehyde residue from the keto acid[26] (Scheme II)



TPP = thiamine pyrophosphate.

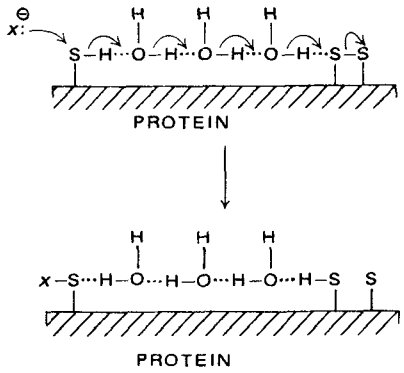
SCHEME II

Thioredoxin, a small low molecular weight protein,[27] with an active disulfide group, is reversibly involved in the reduction of ribonucleotides to deoxyribonucleotides, and in the NADPH-dependent reduction. The existing evidence indicates the possibility of a hydride ion transfer from the −SH group of thioredoxin to a carbonium ion derived from the ribonucleotide.[28] (Scheme III)



SCHEME III

Based on studies of interactions of protein sulfhydryl and disulfide groups with simple disulfides, Klotz[29] has postulated that protein-mediated electron transport might be effected by movement of hydride ions across the water bridge from a sulfhydryl group to an S–S, or an equivalent oxidizable group acting as the acceptor of the hydride ion (Scheme IV)



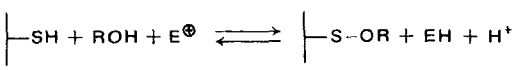
SCHEME IV

The importance of the above observation was pointed out, especially in regard to long-distance electron transport in a fixed matrix such as in mitochondria and in chloroplasts.[29]

Glyceraldehyde phosphate dehydrogenase is another example where a thiol group has been shown to play a specific catalytic role.[30] The enzyme can oxidize acetaldehyde to acetyl phosphate, and the reaction is believed to proceed *via* formation of a thio-acetal resulting from addition of the enzyme-thiol group to the carbonyl group of the substrate.

Sulfur Involvement in Alcohol Dehydrogenases

In view of these observations we are inclined to consider a more specific chemical role of thiol functions in the mechanism of alcohol dehydrogenases. Hypothetically, we envisage alcohols interacting with –SH or –S–S– groups, with possible formations of sulfenate ester (eqs. 1 and 2).

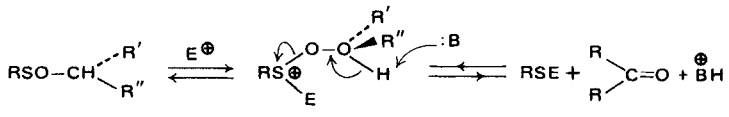


(1)

(2)

$E^{\oplus}$ may represent an electrophilic species or an electron-accepting co-factor of an enzyme. Sulfenates could also be formed by prior *in vivo* oxidation of an –SH group to SOH, followed by reaction with the alcohol.

Sulfenates are attractive as possible intermediates in the oxidations of alcohols because a base-catalyzed electrophilic cleavage of the esters could readily lead to corresponding carbonyl compounds. (eq. 3)



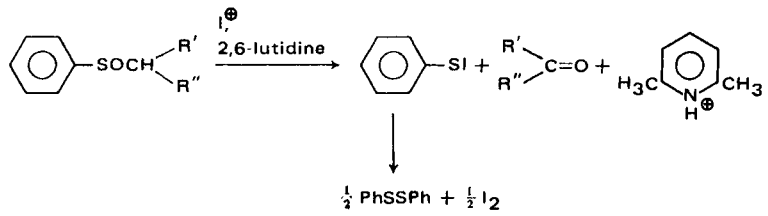
(3)

5

Intermediates of type 5. *e.g.* have been successfully used by Corey and coworkers in oxidations of alcohols in high yields, although sulfenates were not the starting material in those reactions.[31]

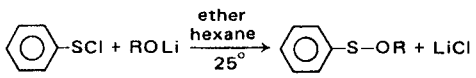
We have examined the feasibility of the reaction of equation 3 and found that starting with sulfenate esters, this type of cleavage can be effected under suitable conditions. Thus, sulfenate esters were synthesized from cyclohexyl, benzyl and *n*-octyl alcohols by titrating a dilute solution of benzenesulfenyl chloride with the appropriate lithium alcoholates (Table I). Subsequently, when each of the sulfenates was refluxed with 1 equivalent of *N*-iodosuccinimide and 8 equiv. of 2.6 lutidine in 1:1 CCl_4-$C_2H_4Cl_2$ solution, the corresponding carbonyl compounds were formed, albeit in yields of only 10–20% (Table II).

A major fraction of the sulfenate was cleaved back to the alcohol in each case; elemental iodine and diphenyl disulfide were the other major identifiable products. The reaction to form the carbonyl product is considered to occur as in eq. 4.



(4)

TABLE I



R	Yield	B.P.	IR
Benzyl	94%	102/0.2 mm	949 cm^{-1}, 908 cm^{-1}
Cyclohexyl	96%	103/0.3 mm	943 cm^{-1}, 913 cm^{-1}
n-Octyl	92%	141/1.3 mm	960 cm^{-1}, 895 cm^{-1}

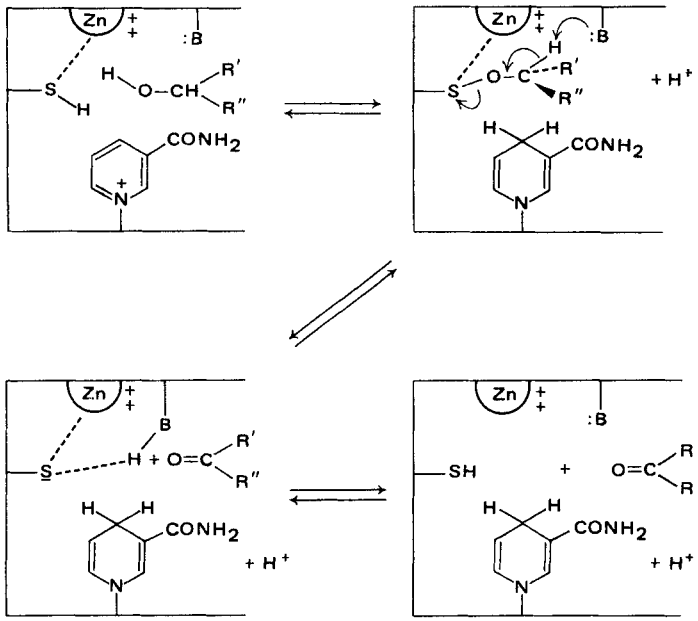
TABLE II

Sulfenate Cleavage by *N*-Iodosuccinimide in 2,6-Lutidine, 1:1 CCl_4-$C_2H_4Cl_2$

Sulfenate	Carbonyl Product	Yield*
PhSO- 	 =O	10–22%
PhSO–CH_2Ph	PhCHO	8–20%
PhSO-C_8H_{17}-*n*	*n*-C_7H_{15}CHO	7–15%

* In more recent work, CH_3^+, from CH_3OSO_2F ('magic methyl'), or from $CH_3OSO_2F_3$, was used as the electrophile, giving improved yields of the carbonyl products and accompanying formation of methyl phenyl sulfide.

In alcohol dehydrogenases, the possibility of sulfenate formation seems possible because the specific sulfur electrophiles such as Zn^{++} ions, –SH groups and basic histidyl residues are intimately involved in alcohol dehydrogenase action. If the sulfenates are formed as intermediates in the process, with the essential thiol group of the enzyme involved, a mechanism as in Scheme V may be postulated, involving hydride transfer mediated by the sulfhydryl group of the enzymes at a hydrophobic site.



SCHEME V

Thus, the electrons from the α-carbon of the alcohol molecules are transferred in a cyclic process *via* a basic group (possibly histidyl residue) and a sulfhydryl group.

In a hydrophobic environment, a stereospecific hydride transfer from alcohol to NAD^+ could be feasible, with such a mechanism, and hydrogen atoms from the α-carbon of the alcohol will be transferred to the C-4 of NAD^+.

The following observations may now be made in support of the above postulates.

1) Such an oxidation pathway should be energetically favorable. The direct hydride transfer reaction has a highly unfavorable free energy change ($\Delta G^\circ = -0.14$ volts; $K_{eq} = 2.1 \times 10^{-5}$).

2) Current work by Shore *et al.*[32] with liver alcohol dehydrogenases indicates that during formation of a ternary complex between the enzyme, NAD^+ and the alcohol, release of one equivalent of protons occurs prior to and uncoupled from the catalytic hydrogen-transferring step. It is postulated that protons are released from the enzyme during turnover, as a result of perturbation of the pK_a of a functional group of the enzyme from 9.6 to 7.6 and direct binding of the alcohol OH group to the basic form of the perturbed functional group. In the mechanism postulated in Scheme V, proton release could account for the initial activation of the alcoholic –OH group as a result of deprotonation under the influence of the said perturbed functional group of the enzyme. Such an activation of alcoholic –OH group would be desirable in view of the need for a nucleophilic attack by alcoholic –OH group at the sulfhydryl sulfur atom.

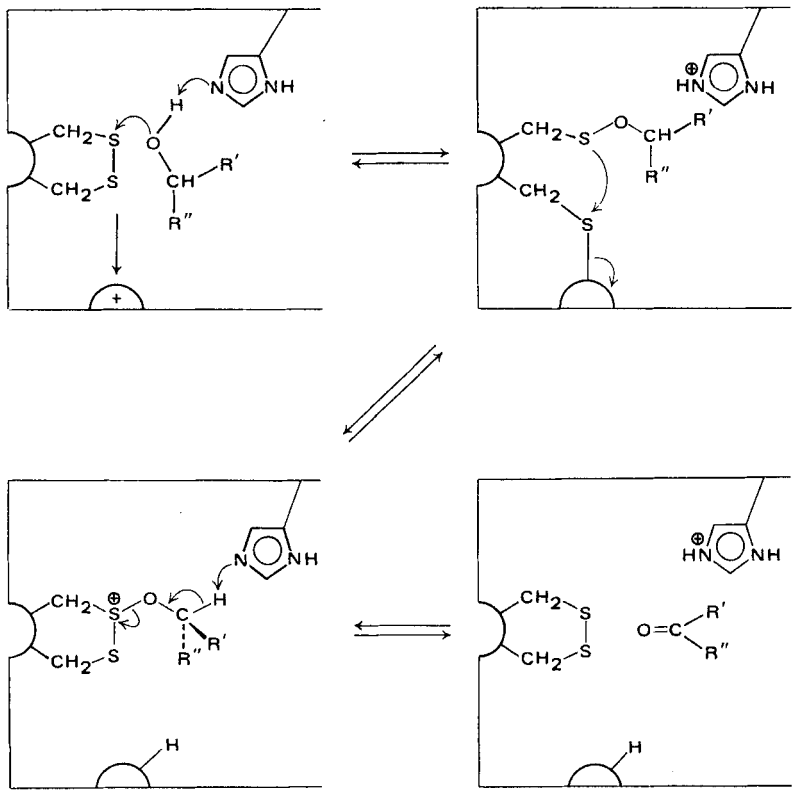
3) Schellenberg[33,34] in his experiments with yeast alcohol dehydrogenase using tritium labelled ethanol found that tritium was incorporated into the methylene group of a tryptophan residue of the enzyme protein. This led him to postulate that a trytophan residue participated as intermediate in the hydrogen transfer mechanism, a claim which has been subsequently refuted.[35,36]

Schellenberg also found that tritium incorporation into the enzyme protein could be decreased by silver ions without completely inhibiting the enzyme.[34] This led to the suggestion that an essential sulfhydryl group was involved in the labelling reaction as well as in the catalytic process. This seems to be a significant observation with regard to our postulate, because even if the labelling of protein occurred subsequently to and not prior to NADH formation, as has been claimed,[35] the possibility that hydride transfer can occur from NADH *via* a sulfhydryl group and reverse is thus clearly indicated.

4) Jacobs *et al.*[37] recently observed that although *p*-chlorobenzaldehyde is reduced about 100 times faster than *p*-methoxybenzaldehyde, with sodium borohydride, the rate ratio was approximately 2 for the reduction with NADH over liver alcohol dehydrogenase. The difference was explained in terms of the activating influence of the Zn^{++} ion in polarizing the carbonyl group of the aldehyde. The reversal of the mechanism in Scheme V for the reduction of the carbonyl group requires nucleophilic addition at the oxygen end of the carbonyl group by a thiolate ion (which seems chemically unfavorable). The electronic effects would be reversed under these conditions. It should be pointed out, however, that reduction of carbonyl compounds by alcohol dehydrogenases may not necessarily be the alcohol oxidation mechanism in reverse.

Sulfenate formation *via* electrophilic cleavage of a disulfide bond (Eq. 2) represents, in our opinion, a possibly plausible way in which alcohols may interact with reactive protein disulfide functions. Preliminary work in our laboratory indicates that in principle such reactions can occur. Thus one equivalent of cyclohexanol refluxed with one half equiv. of diphenyl disulfide, 1.5 equiv. of *N*-iodosuccinimide and 2 equiv. of pyridine in 1:1 dichloroethane–carbon tetrachloride, gave among the other products a substantial amount of cyclohexyl benzenesulfenate, as shown in the ir spectrum of the crude product. With unsymmetrical disulfides, such a reaction would be more feasible because of the greater reactivity of the S–S bond in these cases.

Scheme VI represents a hypothetical model of how a disulfide could be involved, reversibly, in the oxidation of an alcohol. Obviously, such reactions could have important biological implications.

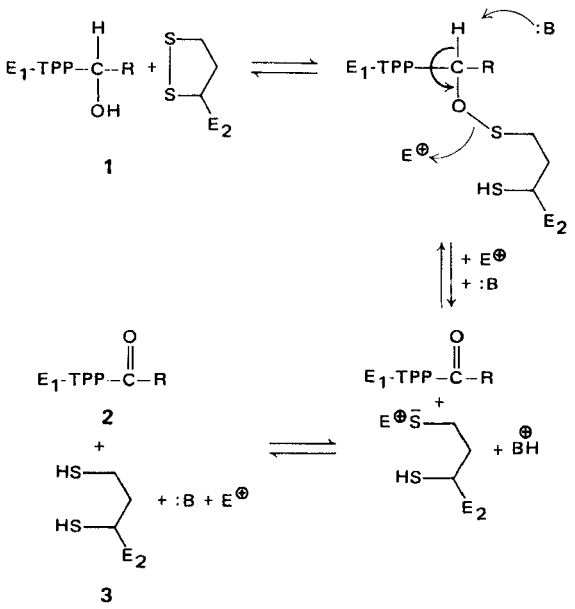


SCHEME VI

Although there is no evidence that a disulfide group occurs at or near the active site of an alcohol dehydrogenase, such a mechanism could be important in view of the fact that most alcohol dehydrogenases have one or more pairs of essential sulfhydryl groups and reversible oxidation of a pair of properly oriented –SH groups to a reactive disulfide bond may readily occur. In this connection it should be noted that recent kinetic studies by Dunn[38] report the non-equivalence of the two active sites of equine liver alcohol dehydrogenase, and that the so called "flip-flop" mechanism[39] has been suggested for alcohol dehydrogenase.

The mechanism of keto acid dehydrogenases (Scheme II) is instructive with regard to the possible involvement of a disulfide bond. Here, the enzyme-bound active aldehyde form is oxidized by lipoic acid to thiol ester **4**, with intermediate formation of acyl-enzyme derivative **2** and dihydrolipoic acid **3**. We suggest that this can be readily explained

in terms of the intermediate formation of a sulfenate ester followed by a base-catalyzed electrophilic cleavage. (Scheme VII)



SCHEME VII

It is thus clear that although no direct evidence exists at this time that sulfenate esters are formed as intermediates in the situations described above (no one has probably looked for these) their possible involvement should be considered. In the action of alcohol on membrane proteins, reversible addition of the –OH group to a reactive disulfide bond could be important. Current observations that disulfide groups may be involved at or near the prostaglandin receptor sites[40] may have some bearing on this. We suggest also that the similar reactions of amines with sulfenic acids or disulfides could produce sulfenamides, which could also undergo electrophilic attack by I^+ or CH_3^+. In the presence of *t*-amine, this would lead to imines, and by hydrolysis to carbonyl compounds (cf. ref. 42).

Experimental Section

A. Synthesis of Sulfenate Ester

1. *Benzenesulfenyl chloride*

The published procedure[41] for the synthesis of benzenesulfenyl chloride was modified in that pyridine was *not* added to the reaction mixture. Under absolutely anhydrous conditions and a nitrogen atmosphere, highly pure samples of benzenesulfenyl chloride can be prepared. The distilled product can be stored at 0–5° for several weeks without decomposition.

2. *Sulfenate esters*

The following synthesis of cyclohexyl benzenesulfenate illustrates the procedure for sulfenates. To a stirred solution of 10.0 g cyclohexanol (0.1 mol) in 150 ml anhyd. ether, under dry nitrogen, 42 ml of a 2.4 *M* solution of *n*-butyl lithium in hexane was added dropwise. A fine suspension of the lithium alcoholate so obtained was titrated by dropwise addition of 14.5 g (0.1 mol) of benzenesulfenyl chloride in 20 ml anhyd. ether. After this addition, a light yellow color persists in the reaction mixture. After 30 min. of additional stirring the insoluble precipitate of lithium chloride was filtered off, and the ether was evaporated from the filtrate. The residue was distilled to obtain 20 g (96% yield) of a light yellow liquid; bp 108°/0.5 mm.

B. Sulfenate Cleavage

The example below illustrates the conditions for electrophilic cleavage of sulfenates with *N*-iodosuccinimide. 431 mg *N*-iodosuccinimide and 399 mg of cyclohexyl benzenesulfenate in 10 ml 1:1 $C_2H_4Cl_2$–CCl_4 and 0.7 ml 2,6-lutidine were refluxed for 18 hrs. The solvent was evaporated under suction and the residue extracted with 15 ml of pentane. The light yellow pentane solution was washed with aq. thiosulfate, water, dil. HCl, water, and finally with saturated brine. After drying over anhyd. Na_2SO_4, the pentane was removed. Examination of the residue by ir indicated cyclohexanone, cyclohexanol, and diphenyl disulfide as the major identifiable products. In the case described the yield of cyclohexanone, as determined by gas chromatography over a 3% OV 17/Gaschrome Q column at 100°C, was 15%.

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