chlorine isotopes. Since the two isotopes have slightly different nuclear moments, slight differences in the hyperfine splitting will be expected. These small splittings are not resolvable and hence a composite splitting, due to the isotopic mixture, is observed.

The bis(trichloromethyl) nitroxide is not as stable as the fluorinated analog,[1,3] and the esr signal decays to one-half of its original intensity in 7 hr.

Bis(trichloromethyl) nitroxide is considered to be formed during the synthesis of trichloronitrosomethane by attack of a trichloromethyl radical on the nitroso compound.

$$CCl_3\cdot + CCl_3\cdot NO \longrightarrow (CCl_3)_2NO$$

A free-radical mechanism was suggested for the synthesis of trichloronitrosomethane, but free trichloromethyl radicals were not postulated.[6] In view of the present work it would appear that at least some trichloromethyl radicals must be formed during the synthesis of trichloronitrosomethane. A possible source of trichloromethyl radicals is the decomposition of the postulated sulfoxide intermediate

$$CCl_3SO_2\cdot \longrightarrow CCl_3\cdot + SO_2$$

although other sources could also be envisaged.

Further work on this, and related systems, is in progress.

H. Sutcliffe, H. W. Wardale
Department of Chemistry and Applied Chemistry
University of Salford, Salford 5, Lancashire, England
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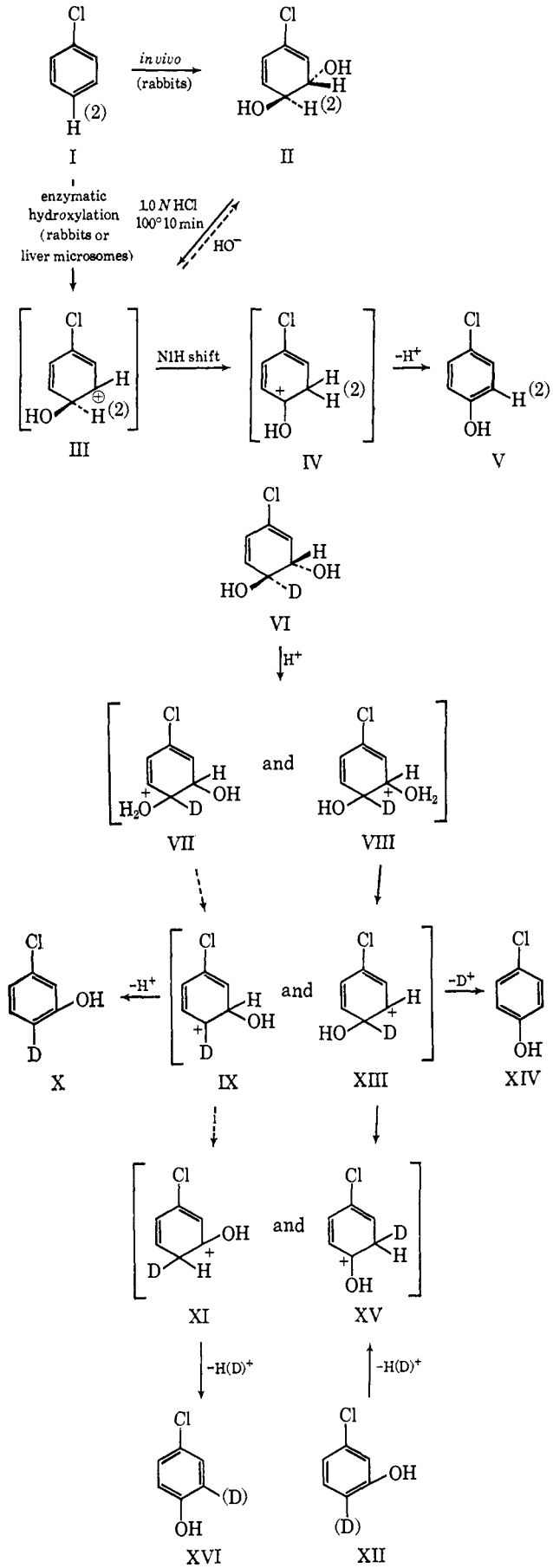
Deuterium Migration during the Acid-Catalyzed Dehydration of 6-Deuterio-5,6-dihydroxy-3-chloro-1,3-cyclohexadiene, a Nonenzymatic Model for the NIH Shift

Sir:

In most enzymatic hydroxylations of aromatic substrates the substituent (^2H, ^3H, or halogen) present at the position of the entering oxygen migrates to either one of the adjacent ring positions. This migration has been called the NIH shift.[1] In a variety of chemical hydroxylating systems only one reagent, trifluoroperacetic acid, produced these hydroxylation-induced migrations.[2] The electrophilic nature of trifluoroperacetic acid[3] suggests cationoid intermediates. A general mechanism involving such cationoid intermediates would conveniently rationalize the migration observed both in nonenzymatic and enzymatic hydroxylation reactions. This communication provides further evidence that enzymatic hydroxylations with concomitant migration and retention of isotopic label may proceed *via* cationoid intermediates. This has been accomplished by starting with a selectively deuterated aromatic substrate and by arriving at the same phenolic metabolite V by enzymatic (I → III → IV → V) and nonenzymatic (II → III → IV → V) pathways which intersect at the cationic intermediate III.



Following the procedure of Smith, *et al.*,[4] a dihydro-

(1) G. Guroff, J. Daly, D. Jerina, J. Renson, B. Witkop, and S. Udenfriend, *Science*, in press.
(2) D. Jerina, J. Daly, W. Landis, B. Witkop, and S. Udenfriend, *J. Am. Chem. Soc.*, **89**, 3347 (1967).
(3) C. A. Buehler and H. Hart, *ibid.*, **85**, 2177 (1963).
(4) J. N. Smith, B. Spencer, and R. T. Williams, *Biochem. J.*, **47**, 284 (1950).

benzenediol has been isolated from the urine of rabbits to which 4-deuteriochlorobenzene[5] had been administered. Eleven New Zealand white female rabbits (~1 kg) were injected intraperitoneally with 2.0 g each of 4-deuteriochlorobenzene applied in a 1:1 emulsion with water containing 0.25% each of oleic acid and triethanolamine. Urine was collected for 48 hr and adjusted to pH 8. The ether extract provided about 4 mg (0.014%) of 6-deuterio-5,6-dihydroxy-3-chloro-1,3-cyclohexadiene (VI) as a crystalline solid (mp 129–130°, lit.[4] 129–130° for the hydrogen compound) *containing exactly 1.0 deuterium atom.* The stability of this glycol to acid[4,6] and triacetylosmate[4] suggests the *trans* configuration VI (only one antipode is pictured arbitrarily).

About 1 mg of the glycol VI was dehydrated with 1.0 *N* HCl at 100° for 10 min. The phenolic products were methylated with dimethyl sulfate in oxygen-free 1.0 *N* NaOH at 80° for 2–3 min, conditions under which no appreciable exchange occurs. The resulting chloroanisoles were examined by combined glpc–mass spectrometry with Carbowax columns operating at 115–130°. The mixture consisted of 99% 4- and 1% 3-chloroanisole. The 4-chloroanisole contained 0.23, and the 3-chloroanisole approximately 0.84 deuterium atom per molecule. When the dehydration was conducted at room temperature in 1.0 *N* HCl for 4 days, the isolated *p*-chloroanisole contained 0.19 deuterium atom per molecule. The retention of 19–23% deuterium in the 4-chlorophenol (XVI) by acid-catalyzed dehydration of the glycol VI compares with 54% retention *in vivo* where the phenol XVI is probably formed directly (I → III → IV → V) rather than *via* the glycol II or VI.

The relatively slow aromatization of the *trans*-glycol VI suggests a cationic transition state XIII rather than a concerted (*cis*) elimination. This migration, concomitant with dehydration, is reminiscent of pinacol rearrangements in which the migratory group is hydrogen.[7] Migrations of hydrogen are known to occur enzymatically in the dehydration of aliphatic pinacols where they are then subject to stringent steric and mechanistic requirements.[8]

These results allow two conclusions: (i) retention of deuterium in the dehydration of the glycol VI involving cationic intermediates, such as XIII, is significant; (ii) the nonenzymatic formation of a 3-chlorophenol (isolated as 3-chloroanisole), from the glycol VI with 16% of the deuterium lost, suggested initially that hydrogen underwent migration by pathway IX → XI → XII. However, in control experiments 3-chlorophenol, but not 2- and 4-chlorophenols, exchanged labile nuclear hydrogen under the acidic conditions used for dehydration to the extent of 18%. Migration and loss of hydrogen by an enzymatic pathway IX → XI → XII leads to 3-chlorophenol with retention of 84% ^2H. The 16% loss of deuterium in this instance provides *the first evidence for the NIH shift of hydrogen itself.*

Six additional metabolites of chlorobenzene were isolated from the urine after ether extraction. Following treatment with β-glucuronidase the urine was adjusted to pH 4.7 and again extracted with ether. The major metabolites in this ether extract were 3-chlorophenol,[9] 4-chlorophenol, and 4-chlorocatechol. Two minor metabolites were 2-chlorophenol[9] and an O-methyl ether of 4-chlorocatechol.[9] Strong acidification of the remaining urine and further ether extraction led to the isolation of 4-chlorophenylmercapturic acid (S-4-chlorophenyl-N-acetylcysteine). The deuterium retentions observed in all the metabolites isolated are presented in Table I. The same three monophenols were isolated after *in vitro* hydroxylation of chlorobenzene with microsomal preparations as well as with liver slices. The retention of deuterium (tritium) in metabolites of selectively labeled aromatic substrates will permit a more detailed understanding of the multiple pathways and interrelationships in the breakdown of physiological and pharmacological agents *in vitro* and *in vivo*.

Table I. Retention of Deuterium in the Phenolic Metabolites from 4-Deuteriochlorobenzene after *in vivo* Hydroxylation in Rabbits

Metabolite[a]	Retention[c]
6-Deuterio-5,6-dihydroxy-3-chloro-1,3-cyclohexadiene (VI)	1.0
4-Chlorophenol	0.54
3-Chlorophenol	0.84
2-Chlorophenol	0.94
4-Chlorocatechol	0.01
4-Chlorocatechol O-methyl ether[b]	0.0
4-Chlorophenylmercapturic acid	0.02

[a] The phenols and catechols were examined after methylation while the remaining compounds were isolated as crystalline solids. [b] The position of the O-methyl group has not yet been established in this compound. [c] Atoms of deuterium per molecule.

Acknowledgment. The authors wish to express their thanks to Drs. Sidney Udenfriend and Gordon Gurofl of the National Heart Institute for their interest and advice.

(5) 4-Deuteriochlorobenzene was prepared by the neutralization of 4-chlorophenylmagnesium bromide with D_2O. The mass spectrum showed the presence of 1.0 deuterium atom.

(6) *Cf.* M. Nakajima, I. Tomida, and S. Takei, *Chem. Ber.*, **92**, 163 (1959).

(7) *Cf.* J. Ley and C. A. Vernon, *J. Chem. Soc.*, 2987, 3256 (1957).

(8) *Cf.* mechanism of dehydration of 1,2-propanediol to propionaldehyde: J. Retey, A. Umani-Ronchi, J. Seibl, and D. Arigoni, *Experientia*, **22**, 502 (1966).

(9) This material has not been previously reported as a metabolite of chlorobenzene in rabbits. Unlabeled chlorobenzene also produced this metabolite.

Donald M. Jerina, John W. Daly, Bernhard Witkop
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland 20014
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Magnetically Oriented Lyotropic Liquid Crystalline Phases

Sir:

Nematic liquid crystal phases have been demonstrated to be useful as matrices for the spectroscopic study of dissolved molecules, especially for nmr studies of relative bond distances, bond angles, anisotropy of chemical shifts, and the signs of indirect coupling constants.[1,2] This communication describes a new kind of nematic phase formed by a mixture of C_8 or C_{10} alkyl sulfates, the corresponding alcohol, sodium sulfate, and water in approximate proportions of 40, 5, 5, and 50, respectively. The temperature range (10–75°) over which

(1) A. Saupe and G. Englert, *Phys. Rev. Letters*, 11, 462 (1963).
(2) G. Englert and A. Saupe, *Z. Naturforsch.*, **19a**, 172 (1964).