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
Received August 14, 1967

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).

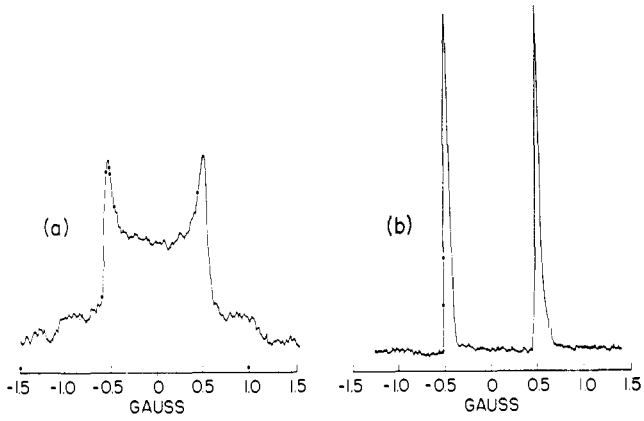

Figure 1. Deuterium spectra of a system containing 50% D_2O, 36% SDS, 7% DeOH, and 7% Na_2SO_4: (a) immediately after being placed in the magnet; (b) after being in the magnet for about 45 min.

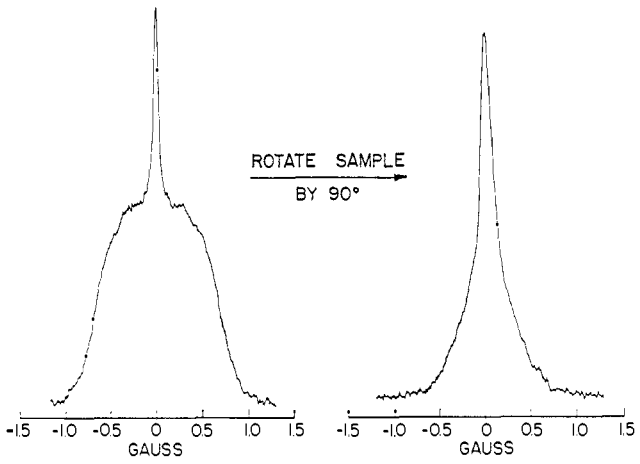

Figure 2. Proton spectra of the system of Figure 1. The gain is not the same for the two spectra.

the phase is present brackets the probe temperature range in most nmr spectrometers. Thus spectra can be obtained with good temperature homogeneity and stability in the same fashion as reported for eutectic mixtures of thermotropic nematic phases.[3,4]

The phase can dissolve both hydrophilic and lipophilic molecules because it possesses both aqueous and hydrophobic regions. The orientation of the phase in the magnetic field allows the nmr sample tube to be spun for improving field homogeneity. Typical line widths at half-height for solute molecules in spun samples are in the range of 3–9 Hz. Line widths for nonspinning samples are in the range of 10–30 Hz. Finally the phase seems to orient slowly in the magnetic field allowing rotation experiments to be performed on the host molecules. The amount of orientation may be monitored by detecting the H^2 spectra of D_2O (which can be substituted in place of water in making this phase). Figure 1a shows the deuterium spectrum immediately after the sample is placed in the spectrometer. Figure 1b shows the spectrum recorded about 45 min later. The first spectrum is typical of randomly oriented spin-1 nuclei in which the asymmetry

(3) H. Spiesicke and J. Bellion-Jourdan, *Angew. Chem. Intern. Ed. Engl.*, **6**, 450 (1967).
(4) D. Demus, *Z. Naturforsch.*, **22a**, 285 (1967).

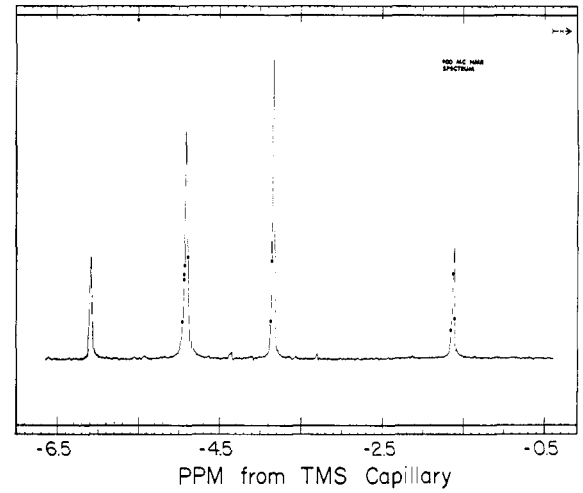

Figure 3. A 100-MHz proton spectrum of CH_3OH dissolved to a concentration of 1.66 *M* in the system of Figure 1. This spectrum is obtained from a spinning sample. The peak near −5 ppm comes from the HDO and the OH.

parameter is zero.[5] This type spectrum is normally found from D_2O in smectic, lyotropic mesophases.[6] The second spectrum is the type expected from a sample of spin-1 nuclei in which the electric field gradients have a preferred orientation with respect to the magnetic field axis.[7]

The separation of the doublet follows the expected $A(3\cos^2\theta - 1)$ dependence, with $A = 0.343$ kHz ($e^2qQ/h = 0.547$ kHz) for an oriented system containing 50% D_2O, 42% sodium decyl sulfate (SDS), 4% decyl alcohol (DeOH), and 4% Na_2SO_4. The proton spectrum demonstrates similar changes on rotation. Figure 2 shows the H^1 spectra taken with angles of 0 and 90° with respect to the equilibrium orientation in the magnetic field. The sharp peak in the center of the broader component comes from the residual HDO and the OH of the alcohol.

This kind of phase can be easily made from readily available chemicals. They have been prepared from the C_{10} and C_8 alkyl sulfates and alcohols; the C_{12} homologs produce a crystalline phase at room temperature. The phases can be prepared either by hydrolysis of the alkyl sulfate with acid (D_2SO_4) or by mixing together the various components and heating. The concentration ranges over which the phase forms have not yet been fully defined and, in fact, vary somewhat depending on the solute molecule used. The phase can be readily recognized using the polarizing microscope since it shows the "threaded" or "schlieren" texture normally found in nematic mesophases.[8] An example of the kind of spectra displayed from a solute molecule is given in Figure 3. It is seen that the degree of orientation of methanol is somewhat higher than that previously reported.[9] Additional experiments are in progress to determine the structure of the phase,

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(6) T. J. Flautt and K. D. Lawson, XIVth Colloque AMPERE, Ljubljana, Yugoslavia, Sept 6–11, 1966.
(7) M. H. Cohen and F. Reif, "Solid State Physics," Vol. 5, F. Seitz and D. Turnbull, Ed., Academic Press Inc., New York, N. Y., 1957.
(8) G. W. Gray, "Molecular Structure and the Properties of Liquid Crystals," Academic Press Inc., New York, N. Y., 1962.
(9) G. Englert and A. Saupe, *Mol. Crystals*, **1**, 503 (1966).

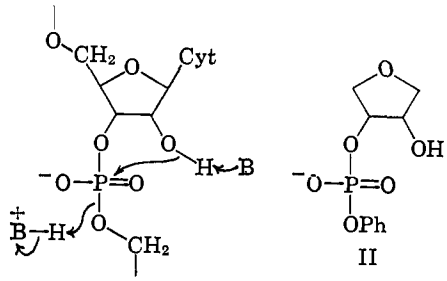
the detailed phase diagram, and the kinds of materials which can be dissolved.

K. D. Lawson, T. J. Flautt
The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45239
Received August 4, 1967

Models of Ribonuclease Action. I. General Species Catalysis in the Hydrolysis of a Nucleotide Diester Analog[1]

Sir:

The first step in the action of the enzyme ribonuclease-A on a ribonucleic acid or dinucleoside phosphate substrate is believed to be the formation of the nucleoside 2′,3′-cyclic phosphate,[2] and a considerable amount of evidence implicates the imidazole groups of histidine-12 and histidine-119 in the catalytic process.[3] In one of the currently discussed mechanisms for this reaction,[3,4] one imidazole acts as a general base toward the 2′-hydroxyl group while the other acts as a general acid, protonating the leaving group (I). However,



there has been to date no demonstration of the involvement of imidazole or of any other nitrogenous base in the nonenzymic hydrolysis of a nucleic acid or nucleic acid analog.[5] We now report that hydrolysis of the phenyl ester of *cis*-tetrahydrofuran-3,4-diol monophosphate (II), a model of a dinucleoside phosphate, shows general species catalysis by imidazole and by morpholine and involves initial formation of the cyclic phosphate III.

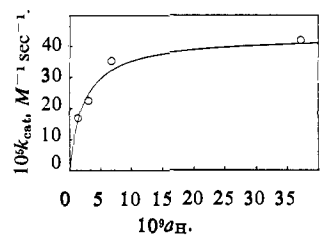


Figure 1. The dependence of k_{cat} on a_H (morpholine buffers; 50°; ionic strength 0.1). The rate and equilibrium constants given in the text give rise to the solid line; the experimental points are denoted by circles.

The phenyl ester II[6] was prepared by the reaction of phenyl phosphate with *cis*-tetrahydrofuran-3,4-diol using trichloroacetonitrile as the condensing agent.[7] The comparison ester IV was prepared by the reaction of diphenyl phosphorochloridate[8] with *cis*-4-methoxy-3-tetrahydrofuranol, followed by the removal of one phenyl group. Analyses for C, H, and P for all new compounds and for the cyclic phosphate III[9] were within 0.2% of theory. The rate of reaction of II was measured in a Cary Model 15 spectrophotometer by following the rate of formation of phenol. All of the experiments were carried out at 50° at an ionic strength of 0.1 (maintained with potassium chloride); the pH values of the buffer solutions were measured before and after each run, and the ultraviolet spectra of the hydrolysis products were the same as those of a mixture of phenol with the appropriate buffer.

The rate of production of phenol from II was found to be subject to general species catalysis,[10] but for morpholine the standard method[11] of dividing the catalytic effect between that due to general base and that due to general acid was inappropriate; our present data show that the catalysis changed from apparently general acid at high buffer ratio (base:base hydrochloride) to apparently general base at low buffer ratio. This is shown in Figure 1 and is reasonably well fit by an equation of the form

$$k_{cat} = \frac{(43.3 \times 10^{-5})a_H}{a_H + (2.26 \times 10^{-9})}$$

where the constants were obtained by a linear least-squares analysis of a plot of the experimental values of $1/k_{cat}$ *vs.* $1/a_H$.

The cyclic phosphate is the sole initial product, as was shown by paper chromatography and by isolation of the cyclic phosphate (infrared spectrum of the lithium salt) from the reaction mixture after ten half-lives (morpholine buffer, 1:1, 0.1 *M* free base).[12] It is unlikely that the reaction involves nucleophilic attack on phosphorus by the general base followed by ring closure: (1) nitrogen bases are not particularly efficient

(1) We thank the National Institutes of Health for support of this research under Grant No. GM 13335.
(2) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952).
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(5) Hydrolysis of the cyclic triester, methyl ethylene phosphate, is subject to general base catalysis (F. Covitz and F. H. Westheimer, *J. Am. Chem. Soc.*, **85**, 1773 (1963)) and could be considered to be a model for the second step of this reaction.
(6) The only aryl esters of a similar *cis*-1,2-glycol phosphate that have been previously reported are the 2′- and 3′-uridine α-naphthyl phosphates (M. Z. Kowalczewska, H. Sierakowska, and D. Shugar, *Acta. Biochim. Polon.*, **13**, 237 (1966)); these were not obtained in analytically pure form.
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(8) D. M. Brown, *Advan. Org. Chem.*, 75 (1963).
(9) P. Carré, *Ann. Chim. Phys.*, [8] **5**, 394 (1905).
(10) The production of phenol from II is of course also subject to specific acid and base catalysis. The latter may for simplicity be considered to be a concerted reaction in the example given here.
(11) R. P. Bell and E. C. Baughan, *J. Chem. Soc.*, 1947 (1937).
(12) The general species catalyzed process accounts for one-third of the rate in this buffer.