of −139 kcal/mol, or about 1.5 kcal/g of ester. This value is comparable to that for a number of explosives and is consistent with the observed properties. The exothermicity also exceeds singlet and triplet energies of formaldehyde and other carbonyl compounds.[10] We will report our observations of solution chemiluminescence from hyponitrites later.

## Experimental Section

Dry silver hyponitrite (2 g) was added at 0° to stirred methyl bromide (10 ml) that had been purified by bubbling through concentrated sulfuric acid and condensing at −78°. After 5 hr, the mixture was filtered. The excess methyl bromide was allowed to distil off at 25°; the remaining liquid was further concentrated on a vacuum line by warming repeatedly from −196° and removing vapor portionwise at low temperature. In later runs, mineral oil (1 ml/g $Ag_2N_2O_2$) was added before concentration. The fractionation was followed by means of a capillary bleed leading to a quadrupole mass spectrometer (Finnigan Model 4000). Methyl bromide displayed intense signals at *m/e* 79 and 81. Pure methyl hyponitrite froze to a white solid below 0°: $^1$H NMR ($C_6D_6$) $\delta$ 3.52 ($J_{^{13}C-H}$ = 145.3 Hz); $^{13}$C NMR ($C_6D_6$) $\delta$ 60.1 ppm downfield from internal TMS; mass spectrum (70 eV) principal *m/e* 90, 59, 31, 30, 29, 28, 15. In pure benzene-$d_6$ the $^1$H NMR signal of the hyponitrite decreased 50% after 1 week at 25°. For the product study in 1,4-cyclohexadiene, the hyponitrite at 2 Torr pressure was diluted with diene to a total pressure of 6 Torr, and the mixture was condensed out at −196° and removed from the vacuum line. The magnetic resonance experiments were determined with a Varian XL-100 NMR spectrometer.

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**Registry No.**—Silver hyponitrite, 7784-04-5; methyl bromide, 74-83-9; methyl hyponitrite, 29128-41-4.

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# Testing Proposed Reaction Mechanisms with Compounds Bound to Solid Supports

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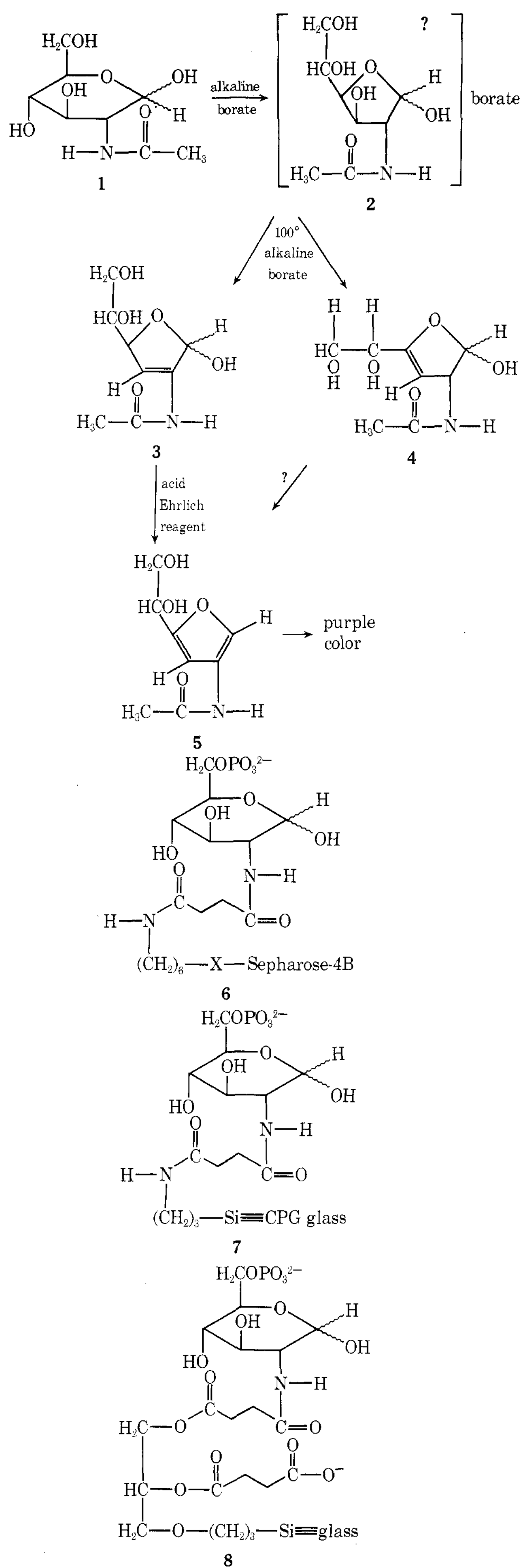
During a sequence of reactions, the fate of a functional group or side chain may be difficult to determine, particularly if one of the intermediates or the product is unstable. Determining the fate of various parts of a molecule may be facilitated if the compound can be bound to a solid support through the part of interest. This paper illustrates the value of this approach by following the fate of an *N*-acyl group through a sequence of reactions.

**Scheme I**



The Morgan–Elson[1] assay for 2-acylamido-2-deoxy-D-hexose sugars **1** and their 6-phosphate esters involves two main steps[2] (see Scheme I). In step A, heating the sugar with borate buffer at alkaline pH may produce a furanose–borate complex **2.** This is followed by dehydration to form a 2,3-anhydro sugar derivative[2] **3** and perhaps a 4,5-anhydro sugar derivative[3] **4.** In step B, treatment with Ehrlich reagent (*p-N,N*-dimethylaminobenzaldehyde in HCl–glacial acetic acid) dehydrates the monoanhydro sugars above to furan derivatives[2,3] **5** and complexes them with Ehrlich reagent, resulting in a purple color with absorption maxima at 550 and 590 nm.

It has been proposed that in the course of the Morgan–Elson reaction the *N*-acyl group of 2-acylamido-2-deoxy-D-hexose sugars may be eliminated during some stage of color development[2,3] because of differential behavior of *N*-acyl and *N*-alkyl hexosamines.

"These results show that, under the conditions of the Morgan–Elson procedure, a wide variety of *N*-acyl groups facilitates chromogen formation (possibly by binding the lone electron pair on the nitrogen atom during the $\beta$-elimination process) but that they do not affect the color formed by subsequent reaction with [*p-N,N*-dimethylaminobenzaldehyde]. *N*-Alkyl substituents, on the other hand, which do little to promote chromogen formation, have a marked effect upon the final color. This suggests that acyl, but not alkyl, groups are eliminated during the color development."[2]

The chromogens produced during the Morgan–Elson reaction are the monoanhydro sugars and furan derivatives mentioned above and seen in Scheme I.[2,3] These compounds do retain the acetamido group of the starting *N*-acetylhexosamine, so that if the *N*-acyl group is eliminated during color development, it probably occurs after the dehydration steps.

The hypothesis that the *N*-acyl group is eliminated has been tested using an *N*-acylhexosamine bound to a solid support via the other end of the *N*-acyl side chain. "*N*-Succinylglucosamine 6-phosphate Sepharose-4B" (**6**) and "*N*-succinylglucosamine 6-phosphate CPG-Glass" (**7**) were synthesized from 2-amino-2-deoxy-D-glucose 6-phosphate and succinylated solid supports using the water-soluble carbodiimide procedure.[4,5]

The Sepharose-4B **6** and the CPG-Glass **7** derivatives were subjected to a modified Morgan–Elson procedure,[6] and the typical purple color appeared in both the soluble and nonsoluble phases. Beads were placed in small columns and rinsed with borate buffer–Ehrlich reagent mix equivalent to the normal blank used in the quantitative assay. The soluble color was quickly removed, and the other color in the beads remained attached.

In order to determine the step during which chromogen is released from the Sepharose-4B solid support and the relative amounts of color remaining attached and released, the following experiment was performed. A sample of 0.4 ml of Sepharose derivative **6** was boiled in borate buffer (step A) and cooled. The sample containing the beads was placed on a small column and washed with the appropriate borate buffer while 0.7-ml fractions of the effluent were collected. The Sepharose beads were recovered from the column, and both the beads and fractions were treated with Ehrlich reagent (step B). The absorbance of the various fractions was measured at 585 nm and compared to standards of *N*-acetylglucosamine.

The above experiment could have several possible results. If color appeared in the washings of the Sepharose derivative subjected to the borate boiling above (step A), this would suggest that some of the "*N*-succinylglucosamine-6-P" and/or derivatives with longer side chains had been solubilized during the alkali heating step. If color did not appear after treatment of these fractions with Ehrlich reagent, this would suggest release of the chromogen during a later stage.

Next, if Ehrlich reagent treatment of the recovered Sepharose beads produced only insoluble color, this would suggest that the *N*-succinyl bridge of **6** remains attached to glucosamine-6-P during the entire Morgan–Elson reaction. On the other hand, if this treatment produced either both soluble and insoluble color, or just soluble color, the result would be ambiguous because any of several bonds could be cleaved.

The results of the experiment were straightforward. The early washings from the beads produced color upon treatment with Ehrlich reagent, and accounted for about 137 (16%) of the original 880 nmol of N-succinylglucosamine-6-P attached to the starting material. Compounds covalently bound to Sepharose and glass beads are released in appreciable amounts at pH 8–10 at 4° during an 18-hr period;[7] therefore it is reasonable to expect that 9 min at 100° will also cause release. Consistent with this, treatment of the recovered Sepharose beads with Ehrlich reagent produced only insoluble color, suggesting that the *N*-succinyl group is not eliminated during color development with Ehrlich reagent. However, these experiments do not eliminate the possibility of an internal rearrangement during the Morgan–Elson reaction.

Possible release of soluble factors important to the reaction was tested by running part of the reaction under flow conditions on a column. The CPG-Glass derivative **7** was heated in borate buffer (step A) and then placed in a small column and rinsed with borate buffer. During step B, color developed in the beads on column under flow conditions using borate buffer–Ehrlich reagent mix. This suggests that no soluble factors necessary to the reaction are produced during heating in borate buffer (step A) or during treatment with Ehrlich reagent. The colored pigment is probably not physically trapped because it remains attached to the glass after grinding with a mortar and pestle.

The necessity of the side arm remaining intact for the production of insoluble color by the action of the Morgan–Elson reaction has been tested by including a linkage which is labile to one of the conditions of the sequence. Fully acetylated glucosamine (1,3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy-D-glucose, $\alpha$ and $\beta$ anomers) is Morgan–Elson positive[3] owing to *O*-deacetylation during step A. An ester linkage included in the side chain of a derivative similar to **7** should be labile to alkaline hydrolysis and cause all of the color to be soluble after subjection to the Morgan–Elson procedure. A compound with an ester linkage was synthesized[8] with structure **8**, and upon assaying it by the Morgan–Elson procedure, all of the color appeared in the soluble phase. Thus, cleavage of the ester linkage in the side arm brings about release of the purple pigment.

Finally, it was possible that the purple color bound to the solid support, especially Sepharose, might be linked through some unspecified covalent bonds formed during the harsh conditions of the Morgan–Elson reaction. To examine this possibility, we subjected 0.4 ml of aminohexyl-Sepharose-4B and 1 $\mu$mol of 2-succinamido-2-deoxy-D-glucose-6-phosphoric acid[9] to the Morgan–Elson procedure. The resulting purple color was quickly removed when the beads were placed in a small column and washed with appropriate borate buffer–Ehrlich reagent mix, eliminating the possibility of an unspecified covalent linkage formed after solubilization of *N*-succinylglucosamine-6-P.

These results suggest that the *N*-acyl group is not elimi-

nated during the Morgan–Elson reaction, and any mechanism proposed for this reaction should take this into account. Because the colored products of the Morgan–Elson reaction are unstable and disappear in a few hours, I have not attempted to identify the colored compounds bound to solid supports. These experiments represent one example of an approach to elucidating reaction mechanisms. In cases where the product may be removed from the solid support and its structure identified, this method will lead to useful models of reaction mechanisms.

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**Registry No.**—7 (minus support group), 54814-95-8; 8 (minus support group), 54814-96-9.

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(5) The purpose of synthesizing these compounds is for affinity chromatography of glucosamine phosphate isomerase (E.C. 5.3.1.10) which is activated allosterically by 2-acetamido-2-deoxy-D-glucose 6-phosphate. The 2-succinamido-2-deoxy-D-glucose-6-P moiety mimics the physiological activator, binding the enzyme to the immobile Sepharose phase via the allosteric site. Subsequently, the enzyme can be eluted with a basic gradient: R. L. Benson, *Fed. Proc., Fdd. Am. Soc. Exp. Biol.*, **33**, 1477 (1974).
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## Asymmetric Decarboxylation of Ethylphenylmalonic Acid in a Cholesteric Liquid Crystal Solvent

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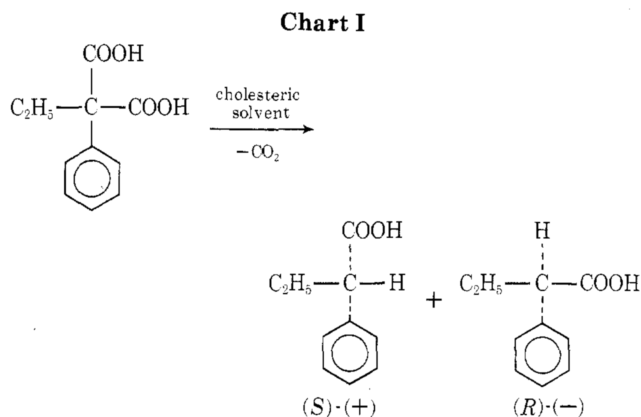
In connection with our interest in liquid crystals,[1] we have been investigating the use of cholesteric liquid crystals as chiral media for asymmetric reactions. Cholesteric or twisted-nematic phases occur in many derivatives of steroids, most commonly cholesterol, as well as in some nonsteroidal compounds. Common features of molecules which exhibit cholesteric mesomorphism are that they are relatively rigid, have a molecular length considerably greater than their breadth, and are, without exception, chiral. The model of the cholesteric phase is that of a layered nematic liquid twisted about an axis at right angles to the molecular layers. Along the direction of the twist axis a gradual change in molecular orientation within the layers occurs, imparting a helical macrostructure to the liquid. Thus, in contrast to the more usual optically active solvents, cholesteric liquid crystals appear particularly attractive as solvents for asymmetric reactions, since they possess not only molecular chirality but also an overall macrochirality owing to the helical arrangement of the mesophase.

Previous asymmetric reactions in isotropic chiral media have been reviewed by Morrison and Mosher.[2] Generally, stereoselectivities are in the range of 5–10%. The only report pertinent to the present work is a recent communication of the use of a cholesteric liquid crystal solvent for the Claisen rearrangement of methylallyl *p*-tolyl ether.[3] The methylallylphenol rearrangement product exhibited optical activity but the absolute configuration and optical purity of the phenol are unknown.

In this report we describe the results of the asymmetric decarboxylation of ethylphenylmalonic acid in the liquid crystal phase of cholesteryl benzoate (Chart I) and in the isotropic chiral solvent, bornyl acetate.

**Chart I**




Ethylphenylmalonic acid is an achiral molecule but contains two prochiral ligands. The carboxyl group at the top of the structure in Chart I is the pro-*R* ligand, since preferential loss of this group would yield the (*R*)-(−) enantiomer of 2-phenylbutanoic acid. The other carboxyl group is then the pro-*S* one.

A solution containing 10 mol % of the malonic acid in cholesteryl benzoate (2 g in 50 g) was smoothly decarboxylated by heating at 160° for 2 hr.[4] Vacuum distillation of the reaction mixture afforded 1.6 g of 2-phenylbutyric acid (80% yield), which was shown by a combination of TLC and VPC to be free of contaminants. Determination of the rotation utilizing a photoelectric polarimeter gave $[\alpha]^{27}D$ −14.2° (*c* 1.3, absolute EtOH). Based on the highest reported rotation for 2-phenylbutanoic acid of $[\alpha]^{25}D$ 78.5° (absolute EtOH),[5] the phenylbutanoic acid formed in this asymmetric decarboxylation has a minimum optical purity of 18%. This value is based on the assumption that the 20% of unrecovered acid has the same enantiomeric composition as the distilled material. If it does not, the stereoselectivity would be different from the observed 59:41 ratio. However, stability experiments involving the distillation of optically active and racemic 2-phenylbutanoic acid gave material having unchanged rotation in the former case and zero rotation in the latter case.

In contrast to the 18% enantiomeric excess found in the ordered cholesteric solvent, decarboxylation of ethylphenylmalonic acid in bornyl acetate, an isotropic chiral solvent, yielded 2-phenylbutanoic acid which was essentially racemic.

The stereoselectivity in the present asymmetric decarboxylation is relatively high compared to typical asymmet-