Aldrichimica acta

Volume 5, Number 1, 1972

Bifunctional Catalysis

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ABOUT THE COVER

When our chemist-collector bought this landscape (oil on panel, 20 x 29½ inches) with Elijah being fed by the ravens, he was told that it had been painted by a 16th century Flemish artist working in Italy, Lodewyk Toeput, called Pozzoserrato. Our chemist does not know the work of this artist, who died in 1604, but as this painting appears to be strongly influenced by Adam Elsheimer, the great German artist working in Italy early in the 17th century, it appears to be later, around 1615. Our collector suspects that it is by an artist whose works have been misunderstood as have almost no other artist's in the 17th century: David Teniers the Elder. Hundreds or even thousands of the early works of his son, David Teniers the Younger, and of the son's imitators, have been called works of the father, and so art-historians and collectors have connected the father's name with dim and often boring tavern interiors with peasants drinking and carousing. Actually, there is not a single documented work like this by the father, who painted beautifully balanced landscapes with biblical and mythological figures.

Perhaps we shouldn't question our chemist about paintings: whenever we do, we get an art-historical lecture, and when the painting is biblical, instruction in the Bible to boot. We asked whether he believed in the miracle of Elijah being fed by birds, and he said 'No, with all of life being a miracle, God does not resort to specific miracles,' and that the text may have been misunderstood: perhaps Elijah was fed by inhabitants of the town of Oreb, which means Raven, near the Brook of Cherith, whither he had fled—an opinion expressed in the Babylonian Talmud (Hullin 5a). All of which does not detract from the great charm of this painting: here at least Elijah is being fed by ravens, one bringing a steak, the other a twisted loaf of bread.

ALDRICHIMICA ACTA

Volume 5, Number 1
1972

Published by
ALDRICH CHEMICAL COMPANY, INC.
Milwaukee, Wisconsin

Editor, Kathleen D. Ryan

Bifunctional Catalysis

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A polyfunctional catalyst may be defined as a catalyst with two or more active groupings (functions) in the same molecule which act *simultaneously* and *cooperatively* upon the substrate to effect a single chemical transformation. Enzymes are generally considered to be multifunctional catalysts. The commonly encountered bifunctional catalyst contains two functional groups, one nucleophilic and one electrophilic, or one base and one acid. A catalytic process by such bifunctional catalysts is sometimes referred to as a "push-pull" mechanism.

One type of bifunctional catalyst distinguishes itself by repeatedly oscillating between two tautomeric forms in the course of catalyzing a chemical reaction. Such a process is known as *tautomeric catalysis*. In contrast to concerted general acid-general base catalysis which is rare, tautomeric catalysis is a definitely proven, general phenomenon in a variety of chemical reactions, especially in acyl transfer reactions.* While tautomeric catalysis in itself is not an explanation of enzyme action, it may be involved in the enzymic mechanism by shuttling protons between the side chains of different amino acid residues within the enzyme active site.

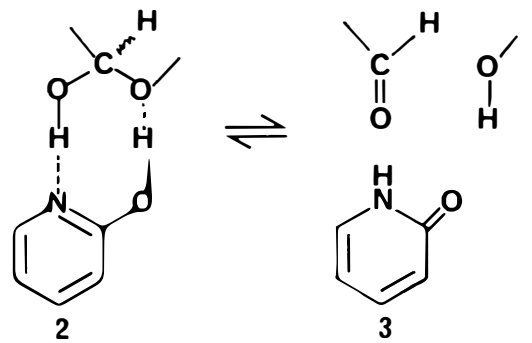
Pertaining to bifunctional catalysis, various model systems have been designed for mechanistic studies on the mode of action of enzymes. Other model systems have been constructed in hope of utilizing the enzymic mechanisms to achieve rapid and selective organic reactions. Examples are scattered in the literature. This article, while not attempting to be an exhaustive account of the title subject, presents some examples of bifunctional catalysis, tautomeric or nontautomeric, according to the types of chemical transformation.

Mutarotation of Tetramethylglucose

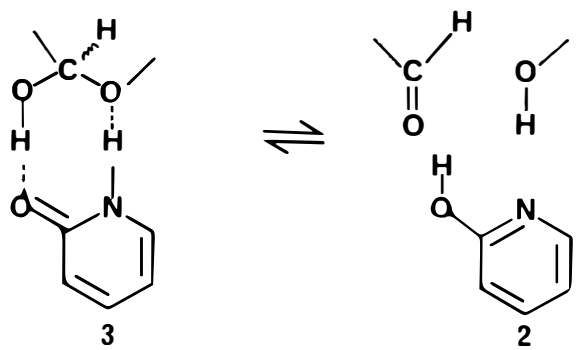
One of the classic examples of bifunctional catalysis was described by Swain and Brown.[1] Mutarotation of 2,3,4,6,-tetramethyl-α-D-glucose (**1**) in benzene, which involves a reversible hydrolysis of the hemiacetal linkage to the aldehyde, is a typical polar displacement reaction. Since a mixture of pyridine and phenol is a catalyst involved in a concerted mechanism for the mutarotation, Swain and Brown visualized 2-hydroxypyridine (**2**) as incorporating the functional features of pyridine and phenol, and suggested that it would act as a bifunctional catalyst. Indeed, 2-hydroxypyridine in benzene is more than 50 times as effective as an equimolar mixture of pyridine and phenol at the same concentration, and is 10 times as effective as hydronium ion in aqueous solution.

* See ref. 2 for a discussion on the potential scope and synthetic implications of tautomeric catalysis.

In the transition state, the nitrogen atom and the hydroxyl group of **2** act on the hemiacetal linkage at the same time to promote proton transfers accompanied by breaking of the ether bond. Subsequently, **2** emerges from the reaction as its tautomeric form, 2-pyridone (**3**). A similar transition state can be drawn for **3**. Therefore, 2-hydroxypyridine or 2-pyridone is a tautomeric catalyst. This is borne out by the fact that 2-methoxypyridine and N-methyl-2-pyridone, which are incapable of tautomerism, are monofunctional catalysts by kinetic order criterion.

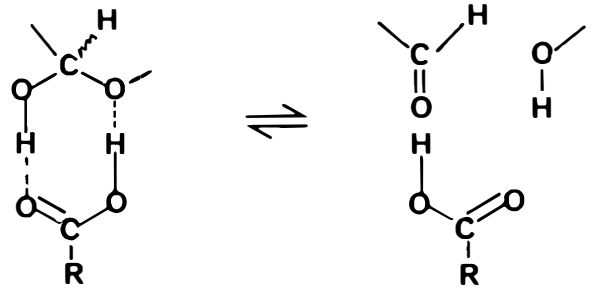


2 **3**



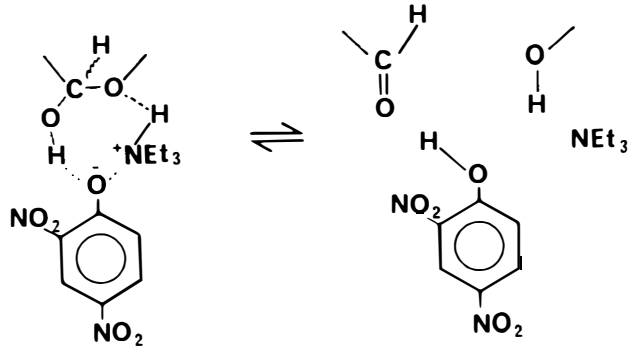
3 **2**

Other compounds demonstrated by Swain and Brown[1] to be bifunctional catalysts for mutarotation of **1** in benzene are 2-hydroxy-4-methylquinoline, 2-aminopyridine, picric acid (but not other phenols), benzoic acid, and trichloroacetic acid. The catalysis of mutarotation of **1** by benzoic acid, trichloroacetic acid, and other carboxylic acids was also described by Rony,[2] and by Blackall and Eastham.[3] The simultaneous transfer of two protons *via* bifunctional catalysis may be expressed as follows:



Eastham and co-workers[4] found that mutarotation of **1** and of 2,3,4,6-tetraacetyl-β-D-glucose in anhydrous pyridine was markedly accelerated by certain electrolytes. Perchlorate ion is one of the most powerful catalysts.

The observation by Swain and Brown that phenols (except picric acid) were not bifunctional catalysts was confirmed by Blackall and Eastham.[3] However, mutarotation of **1** in benzene was catalyzed by an ion pair derived from 2,4-dinitrophenol and triethylamine.[5] Kergomard and Renard[5] suggested a seven-membered cyclic transition state for the bifunctional catalysis, in analogy to the eight-membered cyclic transition state for 2-pyridone.



In connection with the catalytic ability of an ion pair, it is interesting that carbohydrate mutarotation can be catalyzed by ionic micelles. In fact, catalysis by ionic micelles in many organic reactions is well established. Fendler and co-workers[6] have reported that mutarotation of **1** in benzene is markedly enhanced by micellar dodecylammonium propionate (**4**) and dodecylammonium benzoate (**5**). Rate constants for the mutarotation of **1** in water at pH 5.43 (catalysis by hydronium ion) or at the same pH in the presence of 2-pyridone are 15-13 fold smaller than that catalyzed by **4** in benzene. Although the site of catalytic interaction is not certain, it seems that **1** is stabilized at the charged interior of the reversed micelles of **4** or **5** so as to achieve a favorable initial and transition state orientation toward the ammonium and carboxylate groups which provide sites for simultaneous two-proton transfer.

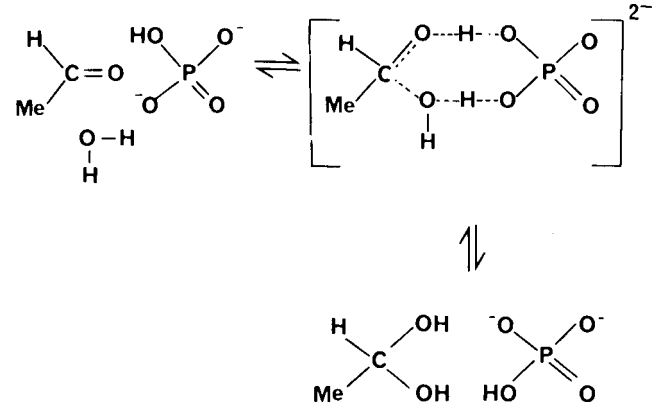
Hydration of Carbon Dioxide, Dehydration of Carbonic Acid, and Related Addition Reactions to Aldehydes

The reversible hydration of CO_2 and dehydration of carbonic acid have been studied as models of carbonic anhydrases by many groups of investigators. Roughton and Booth[7] reported that inorganic oxyacid ions, such as selenite, sulfite, tellurate, borate, chromate, cacodylate, and phosphate, were strong catalysts for the hydration of CO_2.

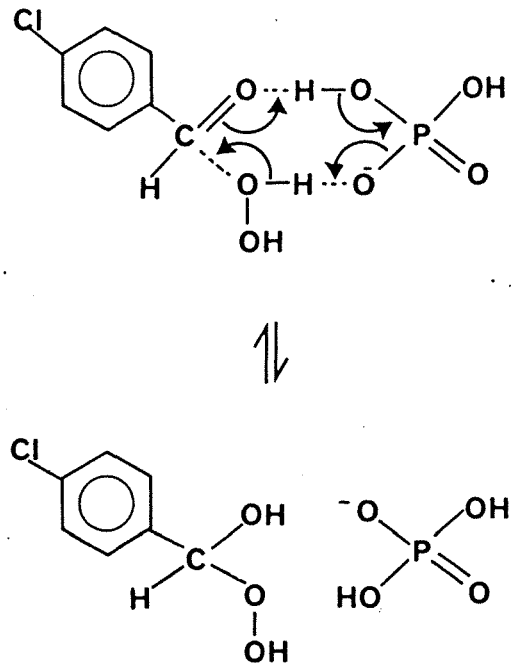
$$CO_2 + H_2O \underset{k-1}{\overset{k}{\rightleftharpoons}} HCO_3^- + H^+$$

Gibbons and Edsall[8] have measured the rate of hydration of CO_2 in the absence of carbonic anhydrase by a spectrophotometric indicator technique, using a stopped-flow apparatus. The hydration constant k calculated from initial rates increases with monohydrogen phosphate (**6**) concentration. Their results strongly support the observation of Roughton and Booth that **6** catalyses the reaction. Pocker and Reaugh[9] also reported significant catalysis of the retro reaction, *i.e.*, dehydration of bicarbonate, by **6**. However, using the same indicator method and apparatus employed by Gibbons and Edsall, Ho and Sturtevant[10] found no significant monohydrogen phosphate catalysis of the hydration reaction.

Hydration of acetaldehyde is a reversible, general acid-general base catalyzed reaction. Pocker and Meany[11] have observed that catalysis of this reaction by monohydrogen phosphate (**6**) is about 10 times greater than that by imidazole (**7**) or diethylmalonate dianion even though the three bases are of comparable strength. Similarly, the rate constant for dihydrogen phosphate (**8**) is about 10 times and 40 times greater than those for diethylmalonate monoanion and the imidazolium ion, respectively. Hence, **6** is believed to participate in the hydration reaction by a mechanism involving simultaneous general base and general acid catalysis. The solvent deuterium isotope effects observed in this reaction are also in agreement with a mechanism involving a rate-determining proton or deuteron transfer step.



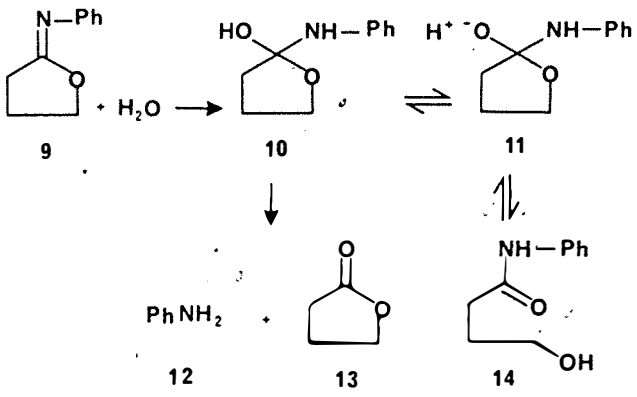
The formation of *p*-chlorobenzaldehyde-hydrogen peroxide hemiacetal is a reversible, general base catalyzed reaction. The kinetic data obtained by Sander and Jencks[12] suggest that dihydrogen phosphate (**8**) catalyzed this reaction *via* concerted acid-base catalysis, although its contribution is small. Dimethyl phosphate anion, which is incapable of abstracting and donating a proton simultaneously, actually causes a decrease in reaction rate.



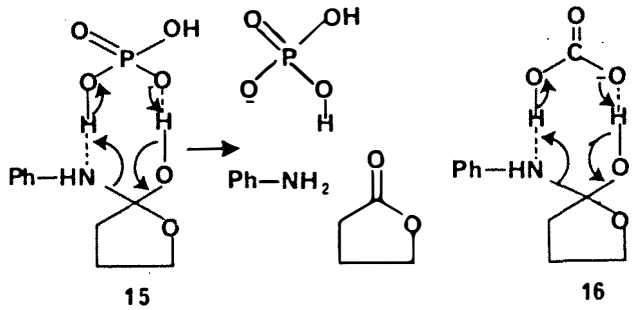
The mechanisms of the reactions of formaldehyde with semicarbazide and with urea have been studied by Glutz and Zollinger.[13] The greater catalytic activities of bicarbonate and dihydrogen phosphate (8) than expected from the Brønsted relationship suggest a tautomeric, bifunctional catalysis by these two reagents.

Breakdown of a Tetrahedral Intermediate in the Hydrolysis of Imidates and Imidazolinium Chloride

Hydrolysis of the cyclic imidate 2-N-phenyliminotetrahydrofuran (9) produces aniline (12) and butyrolactone (13) in acidic, and γ-hydroxybutyranilide (14) in alkaline solution. The transient, but kinetically demonstrable intermediary tetrahedral adduct 10 (and its zwitterionic equivalent 11) has been proposed to account for the pH-dependent shift of product distribution.[14]



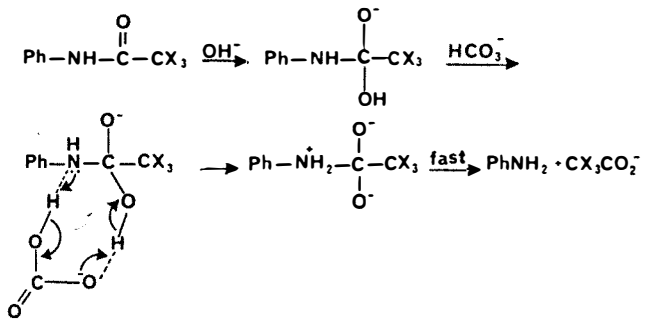
Phosphate or bicarbonate buffers direct the breakdown of 10 in favor of aniline (12) formation at the expense of the yield of 14, without affecting the hydrolytic rate of 9. It is postulated that the oxyacid anions act as bifunctional acid-base catalysts by promoting a concerted proton transfer in the neutral intermediate 10. The transition states for the catalysis by dihydrogen phosphate (8) and bicarbonate are depicted as 15 and 16. Analogous structures may be drawn for monohydrogen phosphate (6), monophenyl phosphate, arsenate, and carboxylic acids, which are similarly effective catalysts.



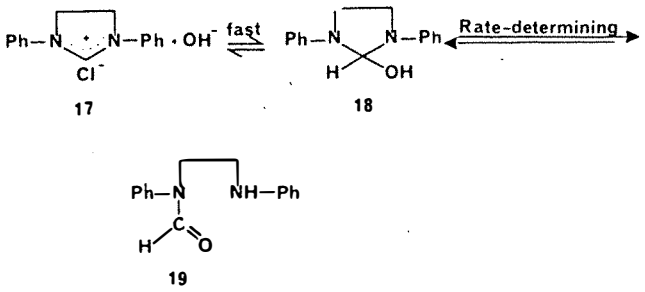
In contrast to the hydrolysis of 9, the rate of hydrolysis of 14 in weakly alkaline media is accelerated by phosphate and bicarbonate buffers.[15] It has been shown that intramolecular nucleophilic participation of the γ-hydroxyl group of 14 leads to the formation of 10 (the same neutral, tetrahedral intermediate in the hydrolysis of 9), which is then converted to products by phosphate or bicarbonate ions *via* tautomeric catalysis.

Hydrolysis of acyclic imidate esters seems to follow the same mechanism proposed for the cyclic imidate 9. Bifunctional catalysts (phosphates, bicarbonate, acetic acid) divert the breakdown of the intermediary tetrahedral adducts from the formation of amides to the production of amines (and esters).[16]

Hydrolysis of trifluoro- and trichloroacetanilide also involves a tetrahedral intermediate and the rate is greatly enhanced by dihydrogen phosphate (8) and bicarbonate ions.[17] At pH lower than the pK_a of the anilides, the rate constants for both substrates are about 40 times greater than the rate constants obtained in the absence of the catalysts. The bicarbonate and phosphate ions exert their catalytic activity by shuttling protons in the intermediate.

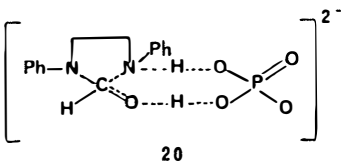


The hydrolysis of the amidine 1,3-diphenyl-2-imidazolinium chloride (17) to N-(2-anilinoethyl)formanilide (19) is a model for the reaction of formyl derivatives of tetrahydrofolic acid. Compound 17 reacts with one molecule of water to form an intermediary, tetrahedral adduct 18. The rate-determining breakdown of 18 to 19 is a general base catalyzed process, and is also catalyzed by bifunctional acid-base catalysts, such as monohydrogen phosphate (6) and bicarbonate.[18] The transition state for the concerted two-proton transfer catalyzed by 6 is expressed by 20.



17 18

19



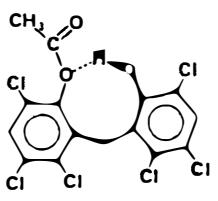
20

Hydrolysis of Esters

Bell-shaped pH-k_{obsd} profiles have been obtained for the hydrolysis of four systems of esters: (a) 6- and 8-quinolyl hydrogen glutarates and succinates; (b) *o*- and *p*-carboxyphenyl succinates; (c) methyl γ- and β-resorcylates; and (d) phenyl 4- and 6-substituted salicylates. These bell-shaped profiles have been interpreted as resulting from intramolecular bifunctional catalysis. Recently the hydrolysis of these four ester systems has been critically re-examined by Maugh and Bruice.[19] However, these authors could find evidence for the participation of only one functional group in the hydrolytic reaction in all cases.

While Maugh and Bruice[19] concluded that there were no evident examples in the literature for intramolecular general acid-general base or nucleophilic-general acid catalysis of ester hydrolysis in water, two seemingly authentic cases of intramolecular bifunctional catalysis of ester hydrolysis were reported at about the same time.[20,21]

The first case was presented by Higuchi and co-workers.[20] At pH <8, hexachlorophene monoacetate (**21**) undergoes hydrolysis in aqueous solution approximately 500 times faster than the diacetate **22**. The 500-fold enhancement in rate represents an intramolecular, monofunctional general acid catalysis. Chemical evidence has indicated that the hydrolysis of the monosuccinate **23** proceeds by intramolecular nucleophilic catalysis, *i.e.*, attack by the free carboxylate at the ester carbonyl carbon atom. An additional intramolecular, monofunctional nucleophilic catalysis is reflected by a much greater hydrolytic rate of the monosuccinate **23** than that of the monoacetate **22** (3×10^4-fold increase at pH <5). The approximately 1.5×10^7-fold increase in hydrolytic rate of the monosuccinate **23** over that of the diacetate **22**, therefore, represents a simultaneous bifunctional catalysis.



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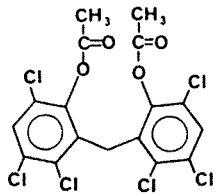

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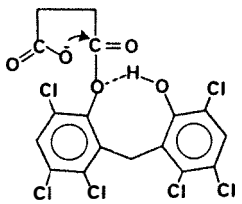
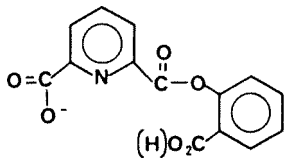


23

The second case is the hydrolysis of the salicylic acid ester of pyridine-2,6-dicarboxylic acid (**24**) in the presence of a metal ion (Ni^{2+}) in water which has been studied by Breslow and McAllister[21] as a carboxypeptidase model. Here also, intramolecular bifunctional catalysis by a combination of an internally bound metal ion (Ni^{2+}, bound by the pyridinecarboxylate group) and a neighboring carboxylate was realized. The two function simultaneously but their interaction is only "semi-cooperative." Therefore, the overall bifunctional catalysis is relatively modest.



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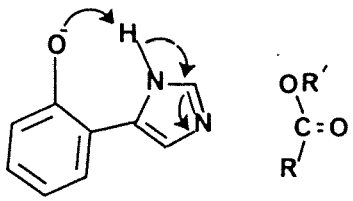
Another type of bifunctional catalysis *via* a "push-push" mechanism has been described by Overberger and Shen.[22] For the solvolysis of *p*-nitrophenyl acetate (**25**) and *p*-nitrophenyl toluate (**26**) in 30% *n*-PrOH-H_2O at pH 10.02 and 26°, the rate constants have been estimated to be 137.4 l. mol^{-1} min^{-1} for **25** and 6.39 l. mol^{-1} min^{-1} for **26** when catalyzed by 4(5)-(2′-hydroxyphenyl)imidazole (**27**), compared to the corresponding rate constants 5.24 l. mol^{-1} min^{-1} and 0.11 l. mol^{-1} min^{-1} by simple nucleophilic imidazole catalysis. The intramolecular catalysis, as depicted by the following scheme, is attributed to the favored geometry between the phenoxide ion (at pH 10.02) and imidazole in **27**. An imidazole-catalyzed imidazole catalysis



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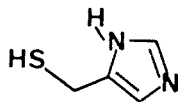

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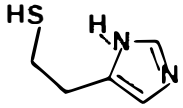


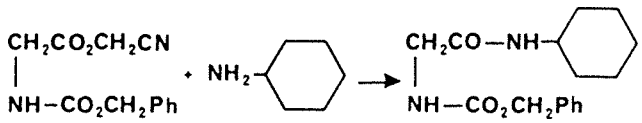
29

for the solvolysis of 25 in the presence of poly-[23] and oligo-4(5)-vinylimidazole[24] is also observed.

Similar cooperative catalysis involving a sulfhydryl group and an imidazole ring has been described.[25,26] 4(5)-Mercaptomethyl-(28), 4(5)-mercaptoethylimidazole (29), and certain peptides having an imidazole and a thiol group are effective catalysts for the hydrolysis of 25. The activation energy of hydrolysis is lowered the most by the two strongest catalysts 28 and 29.

Aminolysis and Related Acyl Transfer Reactions. Application to Amide/Peptide Syntheses without Racemization.

Aminolysis of esters is one of the common methods for the preparation of amides and peptides. However, it is normally not a rapid reaction, although acid (*e.g.*, NH_4Cl) and base (*e.g.*, CH_3ONa) catalysis of this reaction has been reported. On the other hand, this reaction is greatly accelerated by certain tautomeric bifunctional reagents, even with "low-energy" or sterically hindered esters, or with both esters and amines of comparatively large molecular weights.[27,28] All of the useful catalysts are aromatic heterocyclic compounds having a $-\overset{OH}{\underset{|}{C}}=N-$, a $-\overset{SH}{\underset{|}{C}}=N-$, or a $-\overset{HN-}{\underset{|}{C}}=N-$ group* which can undergo tautomerism, such as 2-hydroxypyridine (2), 2-mercaptopyridine, 2-hydroxyquinoline, pyrazole (30), 4-bromopyrazole, 5-hydroxy-3-methylpyrazole, 2-hydroxy-4,6-dimethylpyrimidine, 4-hydroxy-2-methylprimidine, 3,6-dihydroxypyridazine, 1,2,3-triazole, and 1,2,4-triazole (31). Tables 1 and 2 show the effectiveness of some of these reagents in catalyzing aminolysis reactions.



32 33

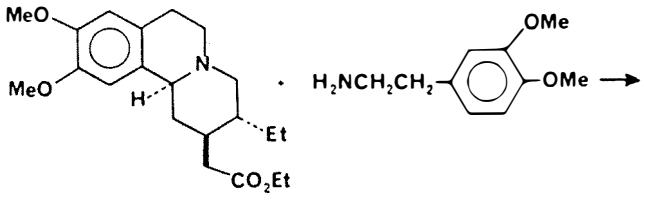
Table 1. Catalyzed reaction of 32 with 33 in acetronitrile at 20° for 30 minutes (Ref. 27)

Catalyst	Extent of Reaction (%)
2-Hydroxypyridine	61
Pyrazole	63
4-Bromopyrazole	73
1,2,4-Triazole	63

Table 2. Catalyzed reaction of 34 and 35 at 169° for 5 hours (Ref. 28)

Catalyst	Yield of 36 (%)
2-Hydroxypyridine	87
2-Hydroxyquinoline	43
5-Hydroxy-3-methylpyrazole	79
2-Hydroxy-4,6-dimethylpyrimidine	83
4-Hydroxy-2-methylpyrimidine	66
3,6-Dihydroxypyridazine	63
1,2,4-Triazole	44

* Editor's note: See page 14 for an example of our ability to locate structurally related chemicals.



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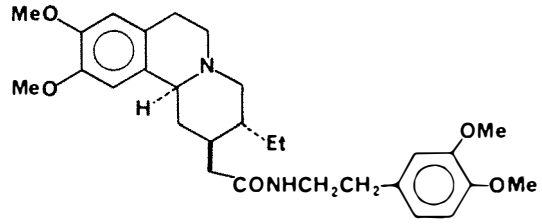

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The effect of 2-hydroxypyridine (or 2-pyridone) on the reaction of a variety of amines with some normally not very reactive ("low-energy") esters has been studied by Openshaw and Whittaker,[28] and their results (partially shown in Table 3) indicate that 2-hydroxypyridine (2) is a generally applicable catalyst for the reaction of strongly basic amines with esters. One advantage of using 2 in amide or peptide syntheses over the other catalysts is the ease of its removal from the product due to its high solubility in water.

Table 3. 2-Hydroxypyridine catalyzed aminolysis of esters

Ester	Amine	Amide uncatalyzed yield (%)	Amide catalyzed yield (%)
Butyl acetate	Cyclohexylamine	trace	42.5
Butyl acetate	Benzylamine	1.95	58.5
Ethyl butyrate	Benzylamine	1.7	22.0
Ethyl benzoate	α-Phenylethylamine	0.36	11.5
Ethyl salicylate	Benzylamine	38.6	48
Ethyl *m*-hydroxybenzoate	Benzylamine	4.6	6.7
Ethyl *p*-hydroxybenzoate	Benzylamine	—	trace
Ethyl butyrate	Aniline	0.55	3.7

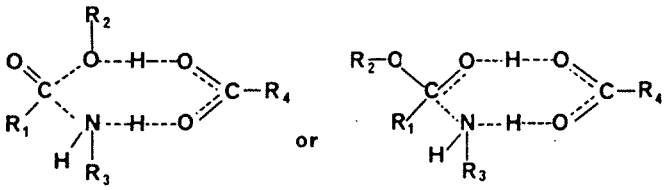
Partial racemization frequently occurs in peptide syntheses. Therefore, Beyerman and co-workers[29] have examined the possible influence of several representative bifunctional catalysts on racemization in aminolysis of peptide esters, such as cyanomethyl, *p*-nitrophenyl, thiophenyl, and vinyl esters. None of the catalysts investigated [*i.e.*, 2-hydroxypyridine (2), pyrazole (30), 4-bromopyrazole, 4-nitropyrazole, and 1,2,4-triazole (31)] caused any detectable racemization except 1,2,3-triazole which led to a small extent of racemization in one case. Imidazole, while accelerating aminolysis of esters to some extent, is not a tautomeric bifunctional catalyst. It was found to cause considerable racemization in several cases.

Recently, Rony[2] re-examined the catalysis of (a) mutarotation of tetramethylglucose, (b) *n*-butylamine aminolysis of *p*-nitrophenyl acetate (25) by 2-pyridone (3), pyrazole (30), and 1,2,4-triazole (31), and concluded that these catalysts are acting in a similar fashion (*i.e.*, tautomeric bifunctional catalysis) in all three reaction systems.

Aminolysis of esters is also catalyzed by carboxylic acids, but not by phenols. Studying the kinetics of the aminolysis reaction of N-benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester with glycine *t*-butyl ester in dioxane, Nakamizo[30] has established the order of catalytic effectiveness of some carboxylic acids and 2-pyridone (3).

$$Me_3CCO_2H > AcOH > \text{2-pyridone} > CH_2ClCO_2H$$

He has shown that carboxylic acids do not act as simple general acid catalysts but are truly bifunctional catalysts. The following eight-membered cyclic transition states are proposed for the catalytic mechanism. The kinetic data for trimethylacetic acid and acetic acid suggest that the proton-abstracting ability of the catalyst in the transition complex plays a more important role in the catalysis.



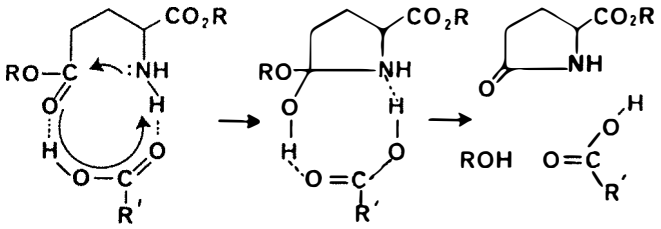
Methoxyaminolysis of substituted phenyl acetates in water is likewise catalyzed by carboxylic acids.[31] The catalytic constants are independent of the acid strength of the catalyst (Table 4).

Table 4. pK_a-independent catalysis of methoxyaminolysis of *p*-nitrophenyl acetate.

Carboxylic acid	pK_a	Catalytic constant k_3, M^{-2} min^{-1}
Chloroacetic acid	2.86	0.52
Methoxyacetic acid	3.53	0.36
Formic acid	3.75	0.38
2-Chloropropionic acid	3.98	0.46
Acetic acid	4.76	0.42
Propionic acid	4.87	0.45

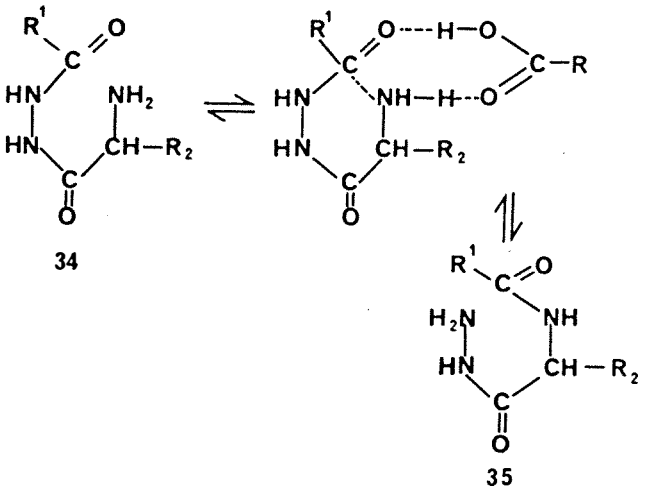
The independence of catalysis upon the acid strength of the carboxylic acids is rationalized in the following manner. Since carboxylic acids act as tautomeric bifunctional catalysis, a polar substituent in the carboxylic acid will affect the proton-donating power of the hydroxyl group and the proton-abstracting power of the carbonyl oxygen atom in opposite fashion. Consequently, there is no net change in the overall effects of the polar substituent.

Lactam formation from glutamic acid diesters is another example of aminolysis subject to bifunctional catalysis by carboxylic acids.[32] Acylation of anilines with acetic anhydride, benzoic anhydride, or benzoyl chloride in benzene has also been shown to be catalyzed by carboxylic acids.[33,34] The catalytic effect decreases in the order:



$$CCl_3CO_2H > CH_2ClCO_2H > PhCO_2H > AcOH$$

The reversible rearrangement of N-acyl-N′-α-aminoacylhydrazines (34) to N-acyl-α-amino acid hydrazides (35), is an intramolecular acyl transfer reaction, and is catalyzed by carboxylic acids. Trimethylacetic, propionic, and acetic acid are the most effective catalysts. The rearrangement reaction is also accelerated by sulfuric and phosphoric acid, but not by boric acid or other acidic compounds. The catalysis by carboxylic acids, H_2SO_4, and H_3PO_4 is attributed to their capability of tautomerism.[35]

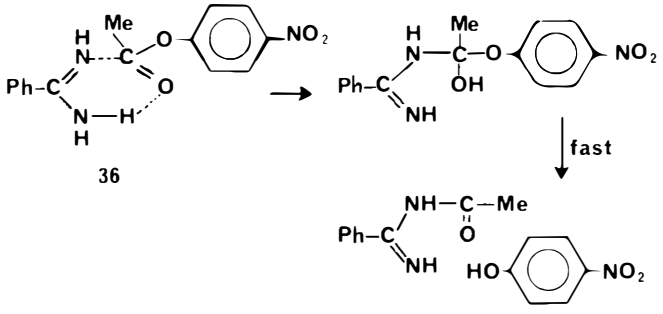


34 35

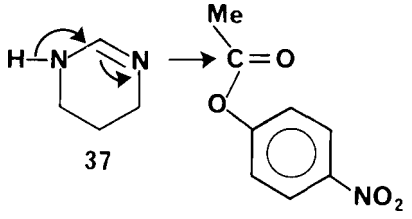
It is interesting that while tropolone is a weak catalyst, 2-pyridone (3) has no effect on this rearrangement reaction. Potassium bicarbonate and sodium carbonate are able to induce the rearrangement of 34 to 35 in aqueous solutions.[36] However, instead of being bifunctional catalysts, these two compounds perhaps act as monofunctional, general base catalysts in this case.

Benzamidine (36) has a basicity comparable to that of *n*-butylamine, and yet reacts with *p*-nitrophenyl acetate (25) in chlorobenzene at a rate 1.5×10^4 times faster. It is tempting to relate the unusual reactivity with its bifunctional nature. Thus, benzamidine (36) was suggested to be a bifunctional nucleophile which could react in a concerted

process to form a neutral tetrahedral intermediate.[37] However, the cyclic amidine 1,4,5,6-tetrahydropyrimidine (**37**),



whose geometry prohibits bifunctional catalysis for nucleophilic attack by the imine nitrogen, reacts 46 times faster with **25** than does benzamidine.[38] Therefore, the previous suggestion that benzamidinolysis involved bifunctional catalysis was rejected.

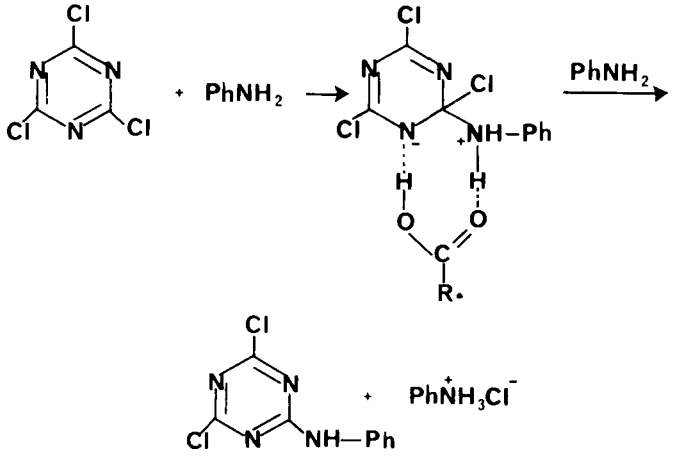


Nucleophilic Aromatic Substitutions

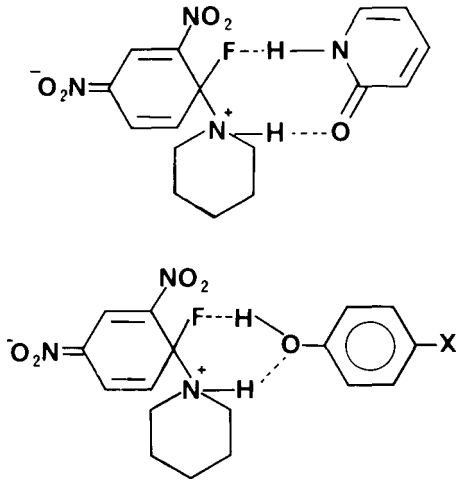
There have been a few cases of bifunctional catalysis involved in nucleophilic substitution reactions. For example, the reaction of cyanuric chloride with aniline in benzene is catalyzed by 2-pyridone and by carboxylic acids.[39] The catalytic effects, which bear no direct relationship with the acid strength, decrease in the order:

2-pyridone > AcOH > $ClCH_2CO_2H$ > $PhCO_2H$ >> CCl_3CO_2H

This reaction is not catalyzed by 4-pyridone or by phenols.



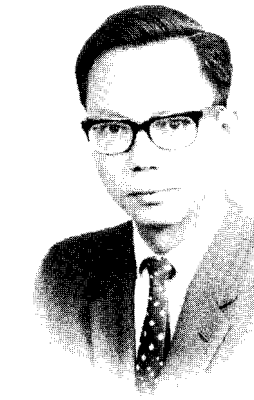
2-Pyridone also catalyzed the displacement of the fluorine in 1-fluoro-2,4-dinitrobenzene (**38**) by piperidine (**39**) in benzene, whereas N-methyl-2-pyridone has no effect.[40] In



contrast to the reaction of cyanuric chloride with aniline, the reaction of **38** and **39** is accelerated by phenols, which exert the bifunctional catalytic action in the sense of **40**.[41] The catalysis is independent of the nature of the para-substituent of the phenol, and therefore, is independent of the acid strength of the phenols. However, anisole has no effect on the rate of the reaction.

Heterogeneous Bifunctional Catalysis

Finally, one class of bifunctional catalysts used in petroleum "reforming" should be mentioned. These catalysts contain a metal which catalyzes hydrogenation-dehydrogenation reactions, and a support with acid sites which catalyzes skeletal isomerization.[42] Unlike all of the bifunctional catalysts discussed above, the two components of these metal-acid support type catalysts, *e.g.*, Pt-SiO_2/Al_2O_3 exert their action *independently* and at *different sites* in the catalyst.



Dr. J. P. Li

Appendix. Summary of Bifunctionally Catalyzed Reactions

Reaction	Catalysts
Mutarotation of Tetramethyl- and Tetraacetylglucose	2-Pyridone 2-Hydroxyquinolines 2-Aminopyridine Carboxylic acids Pyrazole 1,2,4-Triazole Picric acid Ammonium phenoxide Reversed ionic micelles
Hydration of CO_2 and Dehydration of Carbonic Acid	Phosphates Bicarbonate Other inorganic oxyacid anions
Addition of H_2O and H_2O_2 to Aldehydes	Phosphates
Hydrolysis of Imidates and Imidazolinium Compounds	Phosphates Bicarbonate Arsenate Carboxylic acids
Hydrolysis of Esters	4(5)-(2′-hydroxyphenyl)imidazole 4(5)-Mercaptomethylimidazole 4(5)-Mercaptoethylimidazole
Aminolysis of Esters	2-Pyridone 2-Hydroxyquinolines 2-Mercaptopyridine Pyrazoles Hydroxypyrimidines 3,6-Dihydroxypyridazine 1,2,3-Triazole 1,2,4-Triazole Carboxylic acids Tropolone
Nucleophilic Substitution	2-Pyridone Carboxylic acids Phenols

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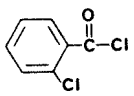
JUST RELEASED

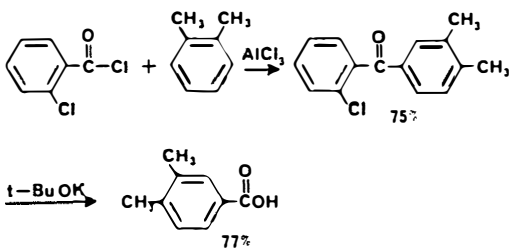
New Uses and Applications

Preparation of aromatic acids

o-Chlorobenzoyl chloride



Aromatic compounds can be 2-chlorobenzoylated and then cleaved by a t-butoxide water reagent to yield pure acids. Thus o-xylene is converted to 3,4-dimethylbenzoic acid in high yield.



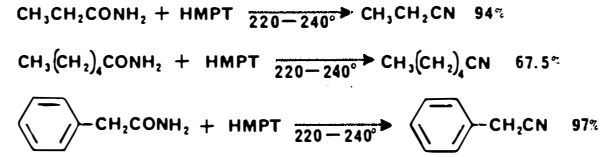
M. Derenberg and P. Hodge, *Tetrahedron Letters*, 3825 (1971).

Dehydrating agent

Hexamethylphosphoramide (HMPT)

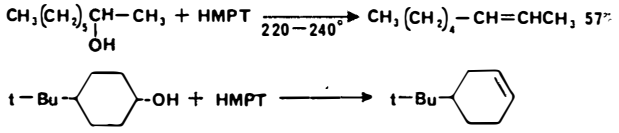
$[(CH_3)_2N]_3P{=}O$

Nitriles from amides (1)



HMPT is advantageous in the preparation of sensitive nitriles because neither it nor the reaction products are highly acidic.

Olefins from alcohols (2)



R. Monson and H. Priest, *Can. J. Chem.*, 49, 2897.
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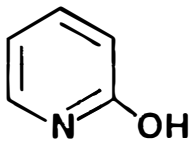
Better Computer Searches From Aldrich

Aldrich Now Has Two Computer Search Systems: A Structural Fragment Search and a WLN Search



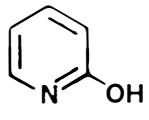
We have selected the fragment shown above to demonstrate our recently expanded computer searching capabilities. It is one of the kind of structures acting as a bifunctional catalyst, and as the article on page 69 describes, the two functional groups must be adjacent to one another to achieve this property. We have been able to locate groups of chemicals using our fragment coding system[1] for several years, but we were limited in cases where ring order or multiple groups were needed. We now have added a second routine based on Wiswesser Line-Formula Chemical Notation (WLN for short). The coding of a chemical by WLN describes all of the atoms and their attachment completely and specifically. Using our WLN searching program, we asked for a 6-membered nitrogen heterocycle with a hydroxyl group on an adjacent carbon atom. The complete results are shown on the next page. The search did produce compounds with one, two, or three nitrogen atoms in the ring. Each molecule has at least one hydroxyl group next to a ring nitrogen atom. Several examples are shown below, to illustrate how our computer can locate other potential bifunctional catalysts.

The new WLN routine has not displaced our fragment based searching because the WLN search is considerably slower. Our fragment search program also has the advantage that various groups or elements may be excluded. In addition, the search may be done on the basis of a specific molecular weight or a selected molecular weight range. This gives our staff a choice of systems. We can provide the greatest selectivity utilizing the most efficient system depending upon the individual request. For example, our phenethylamine search is practical only by our fragment system. Many complex molecules, such as morphine, contain this fragment within a complex ring system, and it is next to impossible to locate by a Wiswesser Line Notation based search, but can easily be done by the fragment search.

The computer searching service is available at no charge.

If you wish a computer search for a group of structurally related chemicals, please write our Technical Services Department. A sketch of the desired fragment will assist us in processing your request.

[1] W. F. Buth, *Aldrichimica acta*, 1, 3 (1968).



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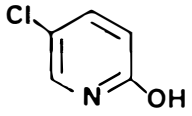

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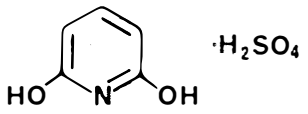

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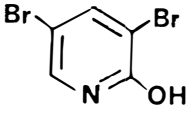

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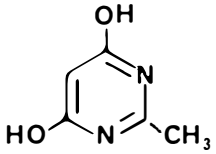

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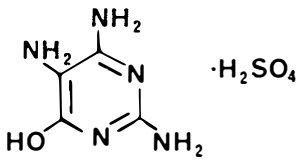

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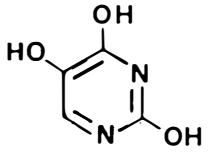

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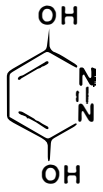

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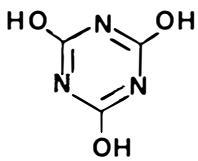

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ABOUT THE COVER

When we first looked at the large painting (oil on wood, 42 x 32 inches) reproduced on our cover, we couldn't help wondering: was this meant to be an advertisement for our liquid crystals which have been used on women's breasts for early signs of breast-cancer? To us, the old men looked like gynecologists in consultation, yet our chemist-collector told us that the painting depicts an apocryphal story which was a favorite of baroque artists, of Susannah being propositioned by two lecherous, old priests. Vulgarity, like beauty, must be in the eye of the beholder: to us the beautifully painted men look positively saintly, and the girl expectant rather than aghast.

Our chemist is skeptical about expertises which often accompany paintings. 'A good painting,' he says, 'does not need one, and a bad painting is not improved by a piece of paper which alleges that the painting is by the wrong artist.' This painting came with an expertise attributing it to Pieter Lastman, Rembrandt's teacher. Our chemist believes that it is Dutch, ca. 1630, but just does not know who painted it, though he hopes to find out, perhaps with the help of a reader. If you think you know, please write to us: a painter this good deserves to have his work identified.

ALDRICHIMICA ACTA

Volume 5, Number 2
1972

Published by
ALDRICH CHEMICAL COMPANY, INC.
Milwaukee, Wisconsin

Editor, Kathleen D. Ryan

Deuterium Analysis and Organic Chemistry

James R. Cox, Jr. and M. Robert Willcott III
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I. INTRODUCTION

Clarification of many intriguing details of the mechanisms of organic and inorganic reactions has resulted from the appropriate utilization of isotopically substituted compounds. Among the labels in common use deuterium is perhaps most prevalent, owing in part to its ubiquitous occurrence in organic molecules, in part to its low cost, ready availability and ease of handling, and in part to the wide range of analytical techniques which are available for it. Since some of the applications are keystone, the chemistry tends to be well known. In our experience the analytical aspects of the problem are frequently overlooked. In this brief review we wish to discuss first the more common analytical methods for deuterium, and then to cite some problems in organic chemistry which have been studied by deuterium labeling, subverting the chemistry to the analytical features. There will be no attempt at a comprehensive coverage of deuterium; the futility of such an attempt is indicated by the fact that a recent monograph which itself made no claim to comprehensive coverage contained more than one thousand literature citations.[1] We have selected cases which are interesting to us for any of a variety of reasons, so this document is essentially a personal one.

II. ANALYSIS

A. DEFINITIONS

At first sight, the specification of the amount of deuterium label present in a molecule is no problem at all. However, it is our experience that the definitions of purity can be confusing unless carefully spelled out. For that reason, we remind the reader of these definitions:

$$\text{Atom \% deuterium} = \frac{\#\text{D atoms in the molecule}}{\#(\text{H} + \text{D})\text{ atoms}} \times 100$$

[Max value = 100%]

$$\text{Mol \% deuterium} = \frac{\#\text{ moles component}}{\text{total }\#\text{ moles in mixture}} \times 100$$

[Total mol % (D + H) = 100%]

It is also possible to discuss the atom % label at a particular position in the molecule, and the atom % label in a functional group (e.g., methyl). In every case, the prudent worker will set down his definitions so the reader will have no trouble following the specification of purity.

The units mol % are a more precise description of the material in question, the units atom % less demanding, although on occasion the two values can be identical. Six hypothetical compounds and mixtures of compounds are shown in Figure 1, together with acceptable alternative descriptions of their composition. Note for case 3 that 33.3 atom % D is scarcely descriptive while the 50 mol % values are precise. Entries 5 and 6 show at least two different ways of having 99 atom % deuterium in methylene chloride. Again the mol % specification describes the mixture accurately.

The same problems occur in the specification of solvent purity. For instance, dimethylsulfoxide-d_6, 99 atom % D, is a mixture of unknown precise composition of several deuterated species. Assuming that all of the protium were in the species C_2HD_5SO, 99 atom % D purity corresponds to only

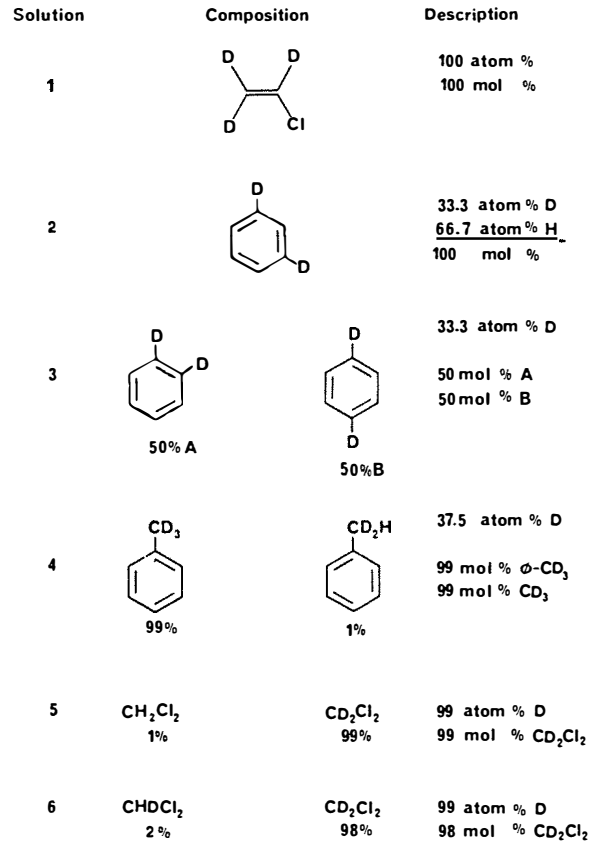


Figure 1.

94.0 mol % C_2D_6SO. To achieve 99 mol % purity requires achievement of a minimum of 99.83 atom % D. Although this complication seldom afflicts nmr solvent users, it can be of major import, say, in the study of relative reaction rates of deuterated and isotopically normal species. To allow solution of problems such as these, as well as to accommodate the high sensitivity spectrometers being introduced, some years ago Diaprep Incorporated* developed the technology required to produce reagents and solvents of ultra-high isotopic purity.

B. WATER ANALYSIS BY DENSITY METHODS

Prior to the advent of modern instrumental techniques, deuterium content of a sample was assayed by determining the deuterium content of the water formed on combustion of the sample. The analytical methods relied upon the fact that 100 mol % D_2O has a density approximately 10% greater than that of 100 mol % H_2O and that the densities of mixtures of intermediate composition fall on a smooth curve between these extremes. Provided that the sample can be combusted completely and that chemically pure water of representative isotopic composition can be isolated from the combustion mixture in sufficient quantity to perform a density analysis, this method can then be applied to the determination of the atom % deuterium content of the sample. Chemical purification of the water is frequently difficult; even gases dissolved in the sample and the ^{18}O content of the sample are among errors which must be reckoned with.

*Diaprep Incorporated is a wholly owned subsidiary of Aldrich Chemical Company, Inc.

The problem of the analysis of heavy water is considered in detail in reference 2, a remarkable summary of the technology for heavy water analysis developed as part of the Manhattan Project. Although much of this work is experimentally demanding but intellectually sterile, ultimately several ingenious microanalytical techniques were developed which permit an accurate determination of density to be made on drops of approximately one μl volume by measuring the rate at which the drop falls through a carefully prepared liquid medium which varies in density along the path of the drop. Suitable density gradients may be constructed from heterogeneous mixtures of readily available organic solvents. Accuracy of 10 ppm is readily attainable; since in practice three significant figures permit the specification of the isotopic content to a precision satisfactory for chemical studies, the method is satisfactory but tedious.

C. INFRARED SPECTROSCOPY

Isotope effects in the infrared are the subject of a recent monograph[3] which deals at length with deuterium and includes a number of examples of other isotopes such as ^{13}C, ^{15}N, and ^{18}O. Oriented toward the application of ir to determination of detailed structural parameters, it provides a leading reference to much of the literature which deals with isotope effects but gives minimal treatment to the analytical aspects of the problem.

The most commonly noted result of deuteration of a carbon-containing compound in the infrared spectrum is the removal of the C-H stretch bands from the 3000 cm^{-1} region to C-D stretch bands in the 2200 cm^{-1} region. If these stretching modes are treated as simple harmonic oscillators, then it can be shown that the frequency ratio ν_D/ν_H is given by the square root of the reduced masses of the oscillator. In mathematical formalism it is

$$\nu_D/\nu_H = \left[\frac{M + 2}{2M + 2}\right]^{1/2}$$

where M is the mass of the atom bearing hydrogen. This fraction has the value 0.72–0.74 for most of the RH and RD pairs of interest to organic chemistry. In other words, it is the deuterium that causes most of the change and not the heavy atom to which it is attached. This theoretical feature is also observed in practice. Other absorptions in the molecule have to be treated heuristically, since the computational power necessary to solve a force field problem for the undeuterated material and then to predict the changes in the finger-print region for deuterated material is still too great to allow anyone to undertake the computation for any except very small molecules. Indeed, a strong caveat should be attached to any simplistic assignment of C-H or C-D stretching frequencies, as they very often turn out to be coupled to other vibrational modes, or to be superimposed on overtones of other modes. One dramatic example of this effect is apparent in the 2000-3000 cm^{-1} region of the spectra of CH_3NO_2 and CD_3NO_2, Figure 2; the strong, residual absorption around 3000 cm^{-1} in the deuterated compound cannot be due to C-H stretching. Indeed, most of the absorption in that region of the protio compound probably is not.

Extinction coefficients in infrared spectroscopy are generally difficult to ascertain. Every extinction coefficient has to be determined individually so there does not seem to be a

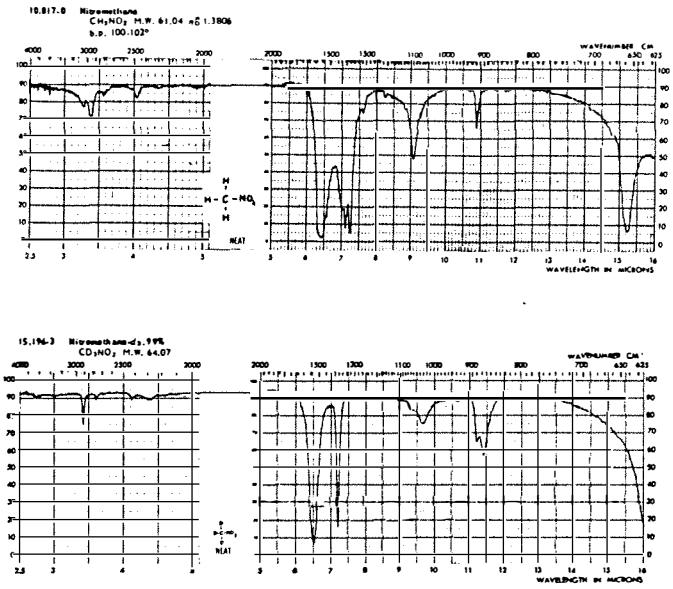


Figure 2.

reliable *a priori* quantitative analysis based on the ir. However, in this problem as in many other analytical techniques it is possible to calibrate the spectrum and to use these calibrated spectra for routine determination of isotopic purity. Moreover, chemical impurities are likely to show up somewhere else in the spectrum. The ir method, using suitable standards, is routinely applied for quality control in Diaprep's solvent manufacturing processes.

Kreevoy and Straub[4] and Crespi and Katz[5] have described methods based upon absorptions in the near ir for determination of deuterium content of partially deuterated water which are remarkably insensitive to the presence of solutes, and which are therefore suitable for biological applications without tedious isolation of the water.

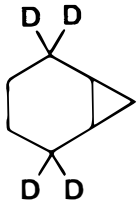
D. OPTICAL ROTATORY DISPERSION

If deuterium is introduced into a position in a chiral molecule remote from the chiral center, it will alter the optical rotatory dispersion behavior by an isotope effect. These effects are usually small and are therefore difficult to utilize for analytical purposes, since they require measurement of small differences in large numbers. If a deuterium atom is introduced at a prochiral methylene, the molecule typically possesses a small rotation, but since the undeuterated material possesses zero rotation, any optical activity properly measured may be attributed to the presence of the isotope. Since deuteration is seldom complete in such a system, the specification of purity now requires a statement about both the mol % of deuterated material and the optical purity of the mixture of deuterated species. Determination of optical purity (the ratio of the observed rotation of the sample to the rotation of the pure enantiomer, which is equivalent to the excess of the one enantiomer over the other), a difficult problem at best, presents even greater challenges in chiral deuterium compounds. Methods complementary to normal resolution procedures, such as enzymatic processes and stereospecific eliminations, are frequently employed. This area has been admirably reviewed.[6,7]

E. MASS SPECTROSCOPY

In principle, mass spectroscopy can in many instances give a detailed mol % composition of deuterated substrates. The mass spectroscopic data can be forced to give the atom % composition with some computational effort. Even though the superficial details of the mass spectrometer experiment are well known, several subtleties merit mention, many of which Biemann has outlined and discussed, including numerous examples.[8] There seem to be an inordinate number of opportunities for error in the experiment, but with suitable care most of the errors can be minimized.

The difficulties start with sample purification and continue throughout the analysis. Isotopic fractionation occurs to a greater or lesser extent in all separation methods. The only complete safeguard is to avoid isotopic fractionation altogether: by utilizing all of the compound in question. If one, in fact, attempts this, impurities may interfere seriously with the determination. The middle course between the risks of overpurification and underpurification may be narrow. An example of this problem was encountered during the attempted vpc purification at the University of Houston of nominal norcarane-d_4, 1. The symmetrical, well resolved



(1)

vpc peak was rich in norcarane-d_5 in the leading edge and rich in -d_3 in the trailing edge.[9] Mol % changes as great as 25% were noted when no special precautions were taken. Similar problems exist with partially deuterated benzene and other hydrocarbons. Furthermore, fractionation of isotopic label might even occur in the inlet system of the mass spectrometer. This last problem is almost beyond the operator's control, but it must be kept in mind.

If all sources of error have been eliminated, then the high resolution mass spectrum should display in the parent region one peak at each mass corresponding to each molecular species present, in the ratio of intensities corresponding to the mol fractions of each mass species in the mixture. This ideal condition is difficult, or perhaps impossible, to achieve, but it can be approached closely enough to make the data useful. Since the ions created by bombardment with electrons in the mass spectrometer are species which contain excess energy, they tend to undergo reactions including intermolecular processes and intramolecular reorganizations. These reactions may be minimized, but not eliminated, by ionization of the parent compound at the lowest voltage which is sufficient to remove an electron from the parent molecule (appearance potential). Operation of the spectrometer at the appearance potential decreases the sensitivity, and makes analytical precision difficult to obtain. Furthermore, deuterium substitution may alter the appearance potential or the ionization cross section.

Increasing the ionizing voltage creates ions in more highly excited electronic states which undergo fragmentations more rapidly and therefore tend to shorten the lifetime of the parent ion, resulting in an apparent loss of the parent ion from the spectrum. Among the fragmentation reactions, loss of hydrogen is a frequent event which occurs in a decidedly nonrandom fashion. If extensive fragmentation is occurring, the parent region may then give only a qualitative estimate of extent of labeling. For the same reason, it is dangerous to attempt isotopic analysis on a portion of the spectrum which has resulted from a fragmentation, but much useful structural information is contained in the fragmentation pattern.[8]

Even if a well resolved parent ion region can be obtained, numerical analysis of the spectrum may be complicated. For instance, there are several peaks in the parent region of a molecule due to the presence in nature of the isotopes ^{13}C, ^{2}H, ^{18}O, and halides other than fluorine. Although isobaric species actually differ sufficiently in mass to be resolvable under high resolution conditions, in fact operation of a mass spectrometer under high resolution conditions presents demands beyond the range of most laboratories. In the low resolution spectrum the fine structure of the parent peaks due to isobaric species is not resolved. For instance, since ^{13}C occurs in natural abundance at about 1.1% of the carbon atoms present in a sample, in methane a parent ion of mass 17 is present at 1.1 mol % in addition to the ion of mass 16, and in benzene a species of mass 79 is present at about 6.6 mol %. These species would not be resolved under low resolution from monodeuterated benzene, and the observation of, say, 15% of a mass 79 species in a partially monodeuterated benzene implies that the degree of deuteration is actually only 8.4%. The numerical analysis of a low resolution spectrum of a partially deuterated, reasonably large molecule rapidly becomes a difficult problem.

Finally, it should be noted that the mass spectrometer need not discriminate amongst isomeric species which have the same mass. A mixture of the three isomeric dideuterobenzenes (Table 1, entries 2 and 3) would not be distinguishable from a pure sample of any one of them by mass spectrometry.

F. NUCLEAR MAGNETIC RESONANCE

The masking of hydrogen resonances by the substitution of deuterium has played a major role in establishing the protocol for analysis of deuterated materials by proton nuclear magnetic resonance spectroscopy. However, this technique is only one of three distinct experimental arrangements for studying deuterium. Deuteron nmr, a more technically demanding and therefore less used technique, has the advantage of permitting direct observation of the deuterium atoms, but the experimental techniques are difficult.[10] Very recently high resolution carbon-13 nmr has become an experimental reality and offers a new and exciting prospect for deuterium analysis of partially deuterated molecules.[11]

Detection of deuterium in a molecule by proton nmr has usually been accomplished by observing that signals are absent from the spectrum which would have been present if protons were in the molecule at the positions occupied by deuterium. In the absence of unusual complications the nmr signal intensity is proportional to the number of protons present, and can be determined by integration of the spectrum. A partially deuterated material can be assayed by proton nmr by correctly evaluating the integral. If the proton substituted by deuterium gives a well-resolved signal then it is possible to determine the mol % composition of the mixture. More usually, measurements done in this way give more reliable information about atom % purity (i.e. average D content) than about mol % purity. Substantial additional information is provided by the proton-deuteron coupling constants (*ca.* 1/6 as large as proton-proton coupling constants) and by the distinct splitting pattern due to deuterium's quadrupole moment (spin =1).

This quadrupole coupling is manifest in the proton region as three lines of equal intensity for each deuteron that possesses a measurable coupling constant. For instance CH_2D groups appear as 1:1:1 triplets with approximately a 2 Hz coupling. There are also deuterium isotope effects on the proton resonances which routinely cause an upfield shift for hydrogens bonded to the same carbon as deuterium.[12] The maximum magnitude of the isotope shift is only 0.01-0.03 ppm (i.e. 1-3 Hz at 100 MHz) but it does provide supplementary analytical data. It is easier to observe the isotope shifts when the quadrupolar and dipolar coupling of the deuterium nucleus is removed by heteronuclear spin decoupling. Frequently, partially deuterated materials give a much sharper proton spectrum when deuterium is decoupled.

Proton nmr is a useful tool for determining the isotopic purity of highly deuterated materials, such as nmr solvents. Clearly, the higher the purity of the solvent, the fewer interfering proton peaks exist. A convenient method of assay is to obtain the spectrum of the solvent, add a carefully measured aliquot of the protonated solvent, and obtain integrals on both solutions. Addition of successive aliquots can be used to produce further integrals and a plot of signal intensity vs. added material can be made. Extrapolation of the straight line to zero added standard then provides a value for the atom per cent purity at the beginning of the experiment. Diaprep produces a group of solvents of isotopic purity > 99.95 atom % D. In these solvents the proton peak is difficult to detect even by high sensitivity spectrometers, and they are, therefore, suitable for the most exacting requirements.

The sensitivity of the deuteron nmr experiment is lessened by the fact that the gyromagnetic ratio of the deuteron is approximately 1/6 of that of the proton, thereby creating a smaller population in the excited state in the Boltzmann distribution. The isotope is quadrupolar and has a somewhat shorter relaxation time, which leads to a broadened intrinsic line width. These factors taken together cause spectrometers' sensitivity to the deuteron to be greatly reduced relative to the proton. It is at present possible especially using Fourier transform techniques to obtain deuterium spectra on samples of 100-500 mg. In spite of the difficulties of the experiment, deuterium nmr spectra offer unsuspected advantages to the user. First, deuterium-deuterium coupling constants are nearly unobservable since they are 1/36 the magnitude of corresponding proton-proton coupling constants. This reduction produces simple spectra which appear almost as a bar graph. The complex spin-spin patterns which haunt interpretation of proton nmr are eliminated. Unfortunately, the physics which makes the coupling constants small compresses the chemical shift scale by a factor of 6 so that many deuterium atoms resonate so close to each other on the frequency scale that the effective resolution of various signals is difficult.

Stothers and Nikon[11] have found a spectacular method to obtain detailed information about deuterium bound to carbon in organic molecules by observing particular features of the ^{13}C nmr spectrum. Now that high resolution carbon spectra are routinely available they have studied a number of partially deuterated bicyclic systems and made the following key observations. The carbon atom to which deuterium is attached (C_α) is shifted by an isotope effect of about 0.5 ppm. The β carbon is shifted by 0.10-0.15 ppm and the γ carbon is sensibly unaffected. The coupling constants provide an interesting contrast. The directly bonded ^{13}C-D coupling is 1/6 the magnitude of the ^{13}C-H coupling. The three bond coupling D-C_β is nil, but the four bond coupling D-C_γ becomes appreciable (see Table I). Furthermore, the long range coupling displays most of the characteristics identified with dihedral angles in the Karplus equation. One other useful feature is that protons can be decoupled from ^{13}C producing a single resonance for each proton-bearing carbon. The deuterium is still coupled, producing triplets of equal intensity (i.e., 1:1:1) for carbons which bear a single deuteron. All of these features taken together provide a method of obtaining detailed isotopic composition from the integrated intensities of the proton-carbon signals vs. the deuterium-carbon signals. This technique may well become the method of choice for analyzing deuterium in organic molecules in the immediate future.

TABLE I. DEUTERIUM SUBSTITUTION EFFECTS ON ^{13}C NMR SPECTRA

	Isotope Shift	Coupling ^{13}C-D
C_α	0.5 ppm	20-40 Hz
C_β	0.15 ppm	Nil
C_γ	Nil	2-5 Hz

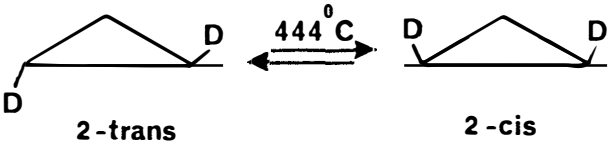
III. APPLICATIONS

A. CHLOROFORM

The analysis of deuterochloroform provides an interesting example of the application of several of these techniques to organic analysis. Since chloroform tends to undergo decomposition upon attempted gas chromatography this method is not routinely suitable for analysis, even though the volatilities of protiochloroform and deuterochloroform are sufficiently different that they should be separable under analytical conditions by glc. The combustion of chloroform, followed by purification and analysis of water formed thereby represents a sufficiently difficult chemical problem that this method is not suitable. Chloroform is a rather dense substance due to the high percentage of chlorine in it; the contribution of the hydrogen isotope to the density makes little change; so methods based upon the density of chloroform are not suitable either. The complicated distribution of chlorine isotopes at natural abundance produces a parent region characterized by numerous peaks, and without tedious numerical analysis, the mass spectrum is useless for analysis of deuterium content. The authors do not know a really suitable method for the analysis of very low concentrations of deuterium, although it is possible that the direct analysis by deuterium nmr would suffice. At intermediate concentrations the nmr integral method using suitable reference materials is satisfactory. At high deuterium concentrations when the proton signal has become small and the signal to noise ratio in the nmr has therefore become low, it is possible to take advantage of highly specific differences in the ir spectra of protiochloroform and deuterochloroform in order to effect the analysis. Deuterochloroform possesses absorption in the 3000 cm^{-1} region, but contrary to intuitive assumption, most of this absorption is not attributable to C-H stretch. Under good conditions, the residual C-H stretch can be resolved partially as a shoulder on the main band, but calibration for the purpose of quantification is difficult, and this region is, then, not ideally suited for ir analysis. However, careful inspection of the ir spectra of the two compounds reveals that isotopically normal chloroform possesses an ir absorption at about 1220 cm^{-1} which is totally absent from deuterochloroform.[13a] By careful calibration of the instrument and the cells this band can then be made the basis for a convenient, quantitative determination which is capable of detecting one part of $CHCl_3$ in ten thousand parts of $CDCl_3$.[13b]

B. CYCLOPROPANE AND DERIVATIVES

Rabinovitch, Schlag, and Wiberg [14,15] pioneered the use of deuterium to explore thermolysis reactions[16] when they observed the interconversion of *cis*- and *trans*-1,2-dideuteriocyclopropane (2-*cis* and -*trans*). The two isomers were



synthesized from cyclopropene by stereospecific hydrogenation reactions developed by Rabinovitch and Looney.[17] A striking difference in the spectroscopy of the two compounds was that the *cis* isomer exhibited an intense infrared absorption at 11.83μ, while the *trans* isomer did not. The infrared spectra of *cis*- and *trans*-dideuteriocyclopropane and of other interesting deuterated cyclopropanes can be found in Schlag's Ph.D. thesis.[18] Empirical Beer-Lambert law constants were obtained for various authentic *cis-trans* mixtures, and the 11.83μ ir band was then used to monitor the mixtures of cyclopropane-d_2 after separation by gas chromatography from propylene-d_2. The authors estimate an absolute accuracy of the analytical method of *ca.* 0.5%. Furthermore, mass spectroscopic analysis verified the identical isotopic composition of the mixture before and after pyrolysis. Any intermolecular process was thereby ruled out. The kinetic description of cyclopropane pyrolysis is beyond dispute, but the interpretation of the experimental results is still unsettled.[19] Two major mechanistic descriptions of this reaction invoke either a process with an intermediate diradical (two step) or with no intermediate (concerted).

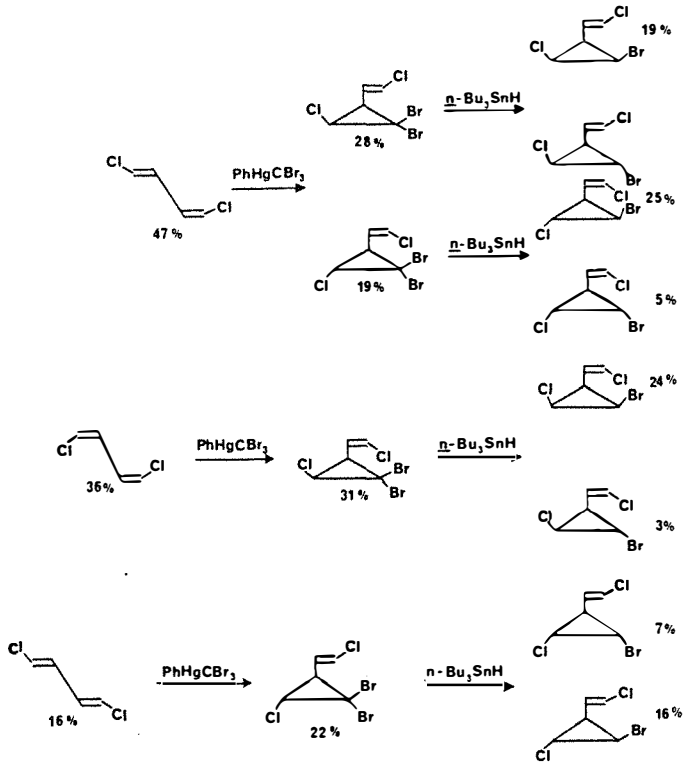
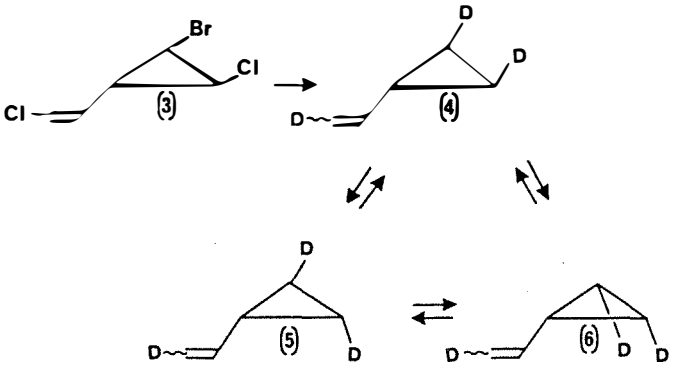


Figure 3. Synthesis of the Precursor of Trideuterovinylcyclopropane 3

The time history (kinetics) relating four different 1-vinyl-2,3-dideuteriocyclopropanes provides another insight into the cyclopropane pyrolysis mechanism. The kinetics can be used to distinguish one path (the diradical) as the more nearly correct description of the pyrolysis of this optimally labeled substrate. Stereospecific synthesis of any of these four compounds seemed tedious until the chance observation that reduction of a mixture of the dichlorobromovinylcyclopropane isomers, 3, (as derived from synthesis in Fig. 3 led to the single dideuteriocyclopropane, 4.[20,21] In this



specific instance, nmr provided an informative analytical method, after recognition that the cyclopropyl absorption bands were complicated by the deuterium quadrupole coupling (see Fig. 4). The effect of the deuterium nuclei is striking. The spectrum of the starting material decoupled (Fig. 5) is easy to interpret as a typical first order splitting pattern. Likewise the two spectra of the remaining materials were predictable from known *cis* and *trans* cyclopropyl coupling constants and chemical shifts. The vinylcyclopropane thermolysis mixture resulting from compound 4 consists of three compounds (4, 5, and 6 respectively). Its composition could be continuously monitored by integration of the high resolution deuterium decoupled proton nmr spectra, and by taking account of the splitting patterns. A histogram summarizing this set of predictions for the resonances at 0.64 and 0.32δ are shown in Fig. 6. In this way the rate of appearance of both new compounds was shown to be identical and the case for the diradical intermediate was fortified. Mass spectroscopy was used to ascertain the isotopic composition of all starting material and products. The invariance of the mass spectra was taken as evidence that no intermolecular events occurred.

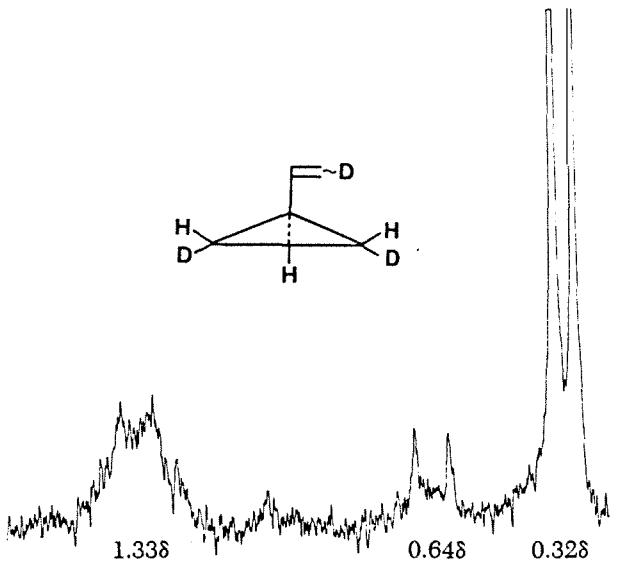


Figure 4. 100 MHz NMR Spectra of Cyclopropyl Hydrogens in Compound 4 (250 Hz Sweep Width)

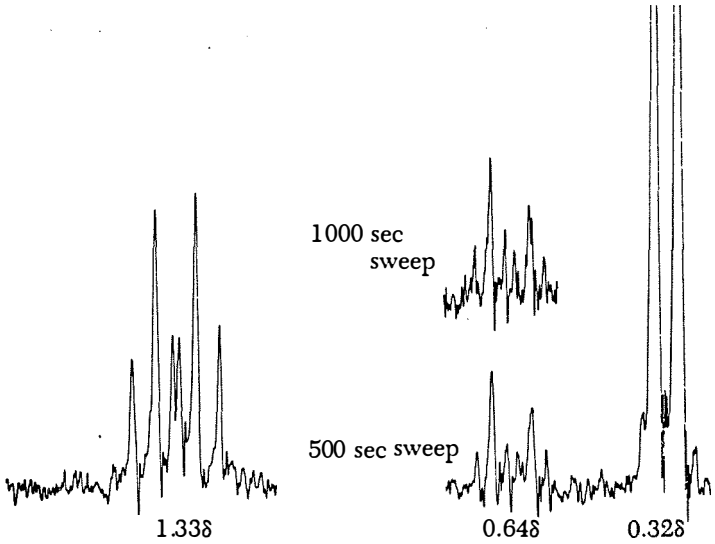


Figure 5. Deuterium Decoupled 100 MHz NMR Spectra of Cyclopropyl Hydrogens in Compound 4 (250 Hz Sweep Width)

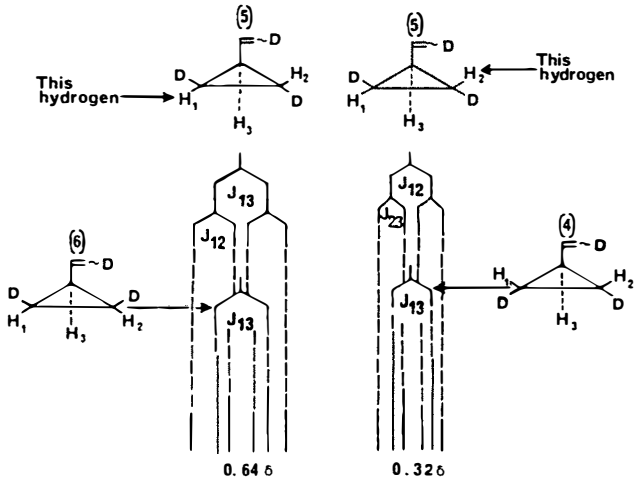


Figure 6. Splitting Patterns of Cyclopropyl Hydrogens in a 1:2:1 Mixture of Compounds 4, 5, and 6 Deuterium Decoupled

C. THUJENE

Thujene, 7, a more highly substituted, optically active, vinylcyclopropane, also undergoes a degenerate vinylcyclopropane rearrangement. Like the other cyclopropyl derivatives which we have already discussed, rearrangement of this compound can be adequately studied if a deuterium label is present. Doering and Schmidt[22] have recorded a comprehensive experimental and historical account of the thujene rearrangement. This paper, containing details which are of necessity omitted in this review, should be consulted as a model of how to realize the maximum amount of detail from a deuterium labeling experiment. In brief, Doering and Schmidt consider four mechanisms (A, B, C, and D) which are described in terms of the observed properties of the pyrolysis of trideuterothujene, 8 (Fig. 7). Path A is the simple enantiomerization of the

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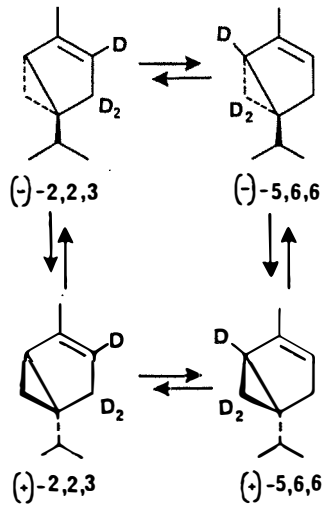
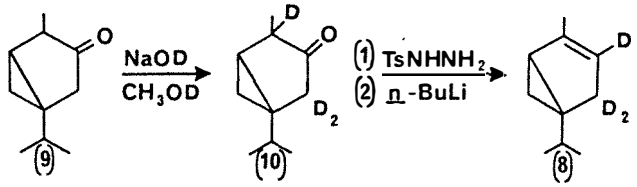


Figure 7. Vinylcyclopropane Interconversions of Trideuterothujene 8

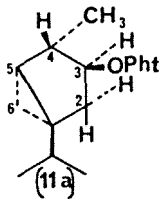
starting thujene [(−)-2,2,3 to (+)-2,2,3; no 5,6,6 product], and path B is deuterium scrambling without enantiomerization [(−)-2,2,3 to (−)-5,6,6]. Both of these descriptions can be discounted immediately by Doering and Lambert's[23] earlier observation that thujene undergoes racemization and mixing of the deuterium label concurrently. Path D is that which involves a symmetrical, planar, high-energy intermediate which, once formed, escapes to all four possible thujenes at equal rates. Path C is distinguished by having a high-energy asymmetrical intermediate which retains the conformation of thujene and produces the enantiomerized and deuterium scrambled product [*i.e.*, (−)-2,2,3 to (+)-5,6,6]. Both paths C and D require that the rate of racemization be twice the rate of the deuterium scrambling. Doering points out that the only way it is possible to distinguish between the two pathways is to stop the reaction early and analyze the (+) and (−)-thujene separately for the position of the deuterium nuclei. This then sets the analytical problem.

The starting material, (−)-2,2,3-trideuterothujene, was prepared via an unambiguous synthesis from thujone, 9.



(9) (10) (8)

The properties were checked by optical rotation, nmr, and ir. A large number of changes which occurred in the ir spectrum are noted in the paper. Resolution of the thujens formed on pyrolysis was accomplished indirectly by hydrocarbon of the mixture, formation of isothujyl phthalates, and separation of diastereomeric salts of the



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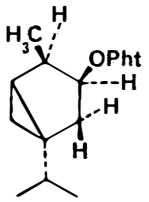
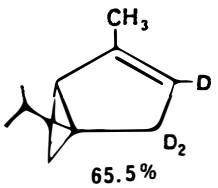
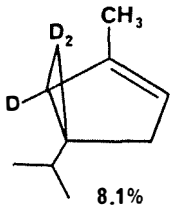


(11b)

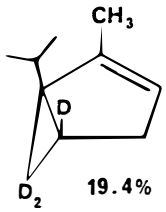
phthalates. The optically pure phthalates, 11, gave 100 MHz spectra in which H-3, H-4 + 2α, H-2β and H-6α + 6β occur in distinct, integrable spectral regions. Appropriate algebraic manipulations in which all important nmr variables were explicitly considered led to the result shown in the figure below for 35% conversion of (−)-2,2,3-trideuterothujene.



65.5%



8.1%



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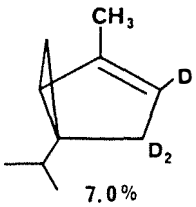
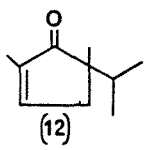


7.0%

Doering and Schmidt concluded from this that the mechanism is "the combination of two processes involving the opening of the three-membered ring to an intermediate consisting of two independent radicals."[22]

D. THUJONE

Thujone, 9, the terpene precursor of thujene, illustrates how deuterium can be used to resolve structural ambiguities as well as to probe mechanism. A reinvestigation of the rearrangement of thujone at 265° revealed *inter alia* two products of unknown structure.[24,25] A combination of chemical and spectroscopic evidence was sufficient to reduce the structural problem to that of determining to which of four isomeric cyclopentenones, 12-15, each product



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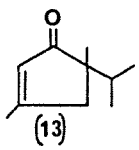

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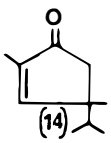

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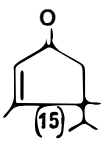
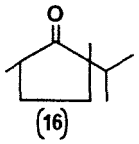


(15)

corresponded. Tentative assignments of structures were made by observing that MeOD/MeO$^-$ exchange caused no alteration in the nmr spectrum of one isomer (12) but complete loss of the nmr signal at the 2.1δ for the other isomer (14). A completely convincing ancillary experiment was performed by reducing each of the compounds to a cyclopentanone, 16-19, and exchanging the enolizable hydrogens for deuterium. Note that the saturated ketones exchange 1, 2, 3 and 4 protons, respectively. Carefully purified, deuterium exchanged ketones, assayed by mass spectroscopy, contained one and three deuterium atoms. This certified that 12 and 14 were proper structures for the thujone products. A possible mechanism for these intriguing transformations can be proposed from the results



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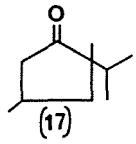

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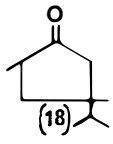

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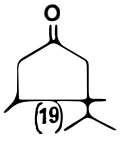


(19)

of pyrolyzing thujone-d_3 (10) and analyzing the two cyclopentenones by integration of the nmr signals.[26] The results of this integration and an acceptable mechanism are shown in Figure 8. It consists of a series of precedented enolizations, vinylcyclopropane rearrangements, cyclopropyl ring breaking reactions, and hydrogen migrations.

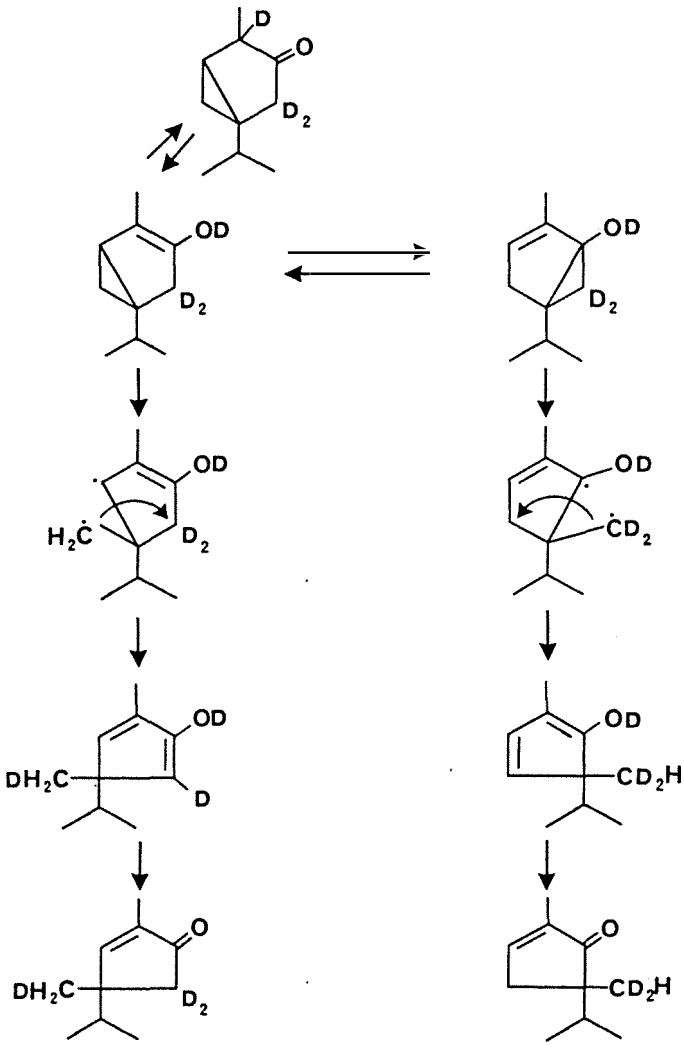
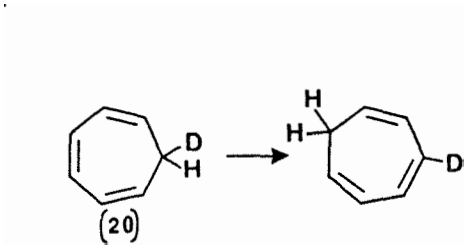


Figure 8. A Mechanism for the Thermolysis of Thujone

E. INTRAMOLECULAR HYDROGEN MIGRATIONS

Deuterium transfers, and obviously hydrogen transfers, in intramolecular processes were observed even before they were popularized by the codification of orbital symmetry rules for concerted reactions.[27] Indeed, some of these earlier observations provided an impetus to study the sigmatropic hydrogen transfer reaction in a theoretical framework. Two such examples are recorded in this section.

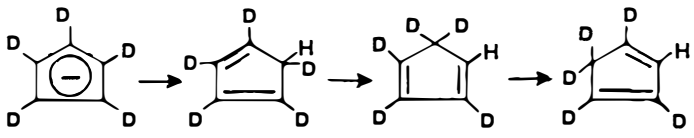
Kloosterzeil (1963) prepared 7-deuterocycloheptatriene, **20**, by reduction of the tropylium ion.[28] The compound was 93.9 mol % D by mass spectroscopy. The deuterium was found to be at the 7 position by nmr. The methylene peak at 2.2δ was of intensity 1.06, and showed a deuterium



(20)

coupling constant of *ca.* 2 Hz and an upfield isotope shift of 0.034 ppm. Heating this tropylidene between 150-200° caused sizeable changes in the nmr spectrum but no changes in the mass spectrum. Comparison of the intensities of the nmr signals for H-1,6, H-2,5 and H-3,4 as functions of reaction time revealed that the process was a [1,5] hydrogen shift. No other simple combination of kinetic expressions for other hydrogen shifts (e.g., [1,3], [1,6]) will reproduce the behavior of the system. Even though this reaction is frequently cited an as example of deuterium migration, it should be noted that the first event which must occur to give rise to an observable reaction is hydrogen migration.

Several months later Roth extended the observations of Mironov on hydrogen shifts in cyclopentadienes to cyclopentadiene-d_5, **21**.[29] This substance was prepared by quenching perdeuteropentadienyl sodium with H_2O. The nmr spectrum of the freshly prepared cyclopentadiene exhibited signals at 6.68, 6.53, and 2.92δ in the ratio



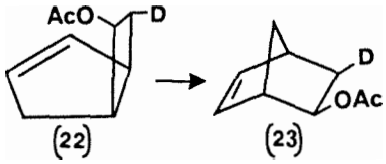
(21)

6:6:88. On heating to 50° the single hydrogen atom was dispersed through the nmr spectrum until all of the peak areas were equal. The dispersal was not random but the proton accumulated first in the 6.53δ peak and was subsequently found at 6.68δ. The unambiguous asignment of the 6.53δ peak to protons 1 and 4 was also carried out by Roth.

The mass spectrum was invariant with the extent of reaction, thus verifying the intramolecularity. Once again the only consistent interpretation of these experimental data is that a rapid [1,5] sigmatropic hydrogen shift takes place.

F. CARBON MIGRATION

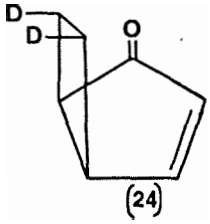
Berson and Nelson employed a multi-step stereospecific synthesis to prepare the 3-*exo*-deutero-2-*endo*-acetoxybi-



(22) (23)

cycloheptene, **22**.[30] The stereochemical integrity of the deuterium was guaranteed by nmr, the *exo* stereochemistry by synthesis, and the presence of 1.0 D atom per molecule by mass spectroscopy. When this compound was heated at 307° it rearranged to *exo*-3-deutero-*exo*-norbornenylacetate, **23**. The correctness of structural formula **23** was assured from previous experiments using unlabeled materials. The *exo* deuterium label was determined by the absence of a proton nmr resonance at 1.33δ. This region was assigned to the 3-*exo*-hydrogen in the parent material by both chemical shift and coupling constant correlations. The coupling patterns in the deuterium labeled material changed predictably. Finally, the nmr spectrum of the thermolysis product was superimposable with the spectrum of an authentic sample of **23**. The authors estimated that as little as 5% of *endo*-3-deuterium label would have been detected in this way. The rather large number of precautions in the analysis were dictated by the conclusion that the configuration at the migrating carbon was inverted during [1,3] sigmatropic rearrangement. This carefully documented case is now accepted as one of the prime examples of the predictive power of the control of organic reactions by orbital symmetry.

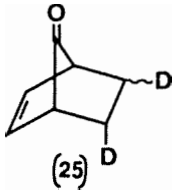
A photochemical [1,3] sigmatropic migration experiment, similar in concept, was carried out on the dideuterobicycloheptenone, **24**.[31] Cargill and Sears prepared this com-



(24)

pound by stereospecific diimide reduction of the cyclobutene. The *exo*-deuterium was assured by the nmr experiment and the deuterium content of 87 mol % $C_7H_6D_2O$ was

measured by mass spectroscopy. The dideutero-7-ketonorbornene, 25, produced on photolysis exhibited a deuterium



(25)

decoupled 100 MHz proton nmr spectrum of sufficient detail to permit a stereochemical analysis. The symmetry of this molecule is such that the *endo* and *exo* resonances occur in pairs and are distinct in chemical shift. Integration of the spectrum provides an *exo-endo* ratio of 2.5, a result which obtains if the stereochemistry at the migrating group is randomized. Furthermore, the simplified spectrum produced by deuterium decoupling showed no *cis-endo* dideutero coupling pattern and strengthened the conclusions about the loss of stereochemistry during a [1,3] sigmatropic photochemical rearrangement.

IV. ENVOI

The range of application of deuterium to the solution of chemical and biochemical problems is limited only by the ingenuity of the investigator. In this brief account we have attempted to indicate how some basic analytical techniques have been cleverly adapted to particular systems of current interest, resulting in the elucidation of details of molecular structure and reaction mechanism which were hitherto obscure. Many important areas have been omitted for want of space, in particular kinetic isotope effects and the complex problems encountered in the study of the metabolism of biological materials. Since deuterium may be used relatively safely as a tracer in biological systems, even in massive amounts, one may expect this area to furnish many future examples of ingenious applications. Here, as in the cases we have cited, the imaginative union of synthetic and analytical techniques will probably be the key to success.

FOOTNOTES

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9. Generally deuterated hydrocarbons are more volatile than their protio analogs, while deuterated hydrogen bonded molecules, such as water, are less volatile than their protio analogs.
10. See, for example, W. R. Moore, P. D. Mogolesko and D. D. Traficante, *J. Amer. Chem. Soc.*, 94, 4753 (1972).
11. J. B. Stothers and A. Nikon have kindly provided experimental details in advance of publication (*J. Amer. Chem. Soc.*). *See also* D. Lauer, E. L. Motell, D. D. Traficante and G. E. Maciel, *J. Amer. Chem. Soc.*, **94**, 5335 (1972).
12. H. Batiz-Hernandez and R. A. Bernheim in "Progress in Nuclear Resonance Spectroscopy," V. 3, J. W. Emsley, J. Feeney and L. H. Sutcliffe, eds., Pergamon, New York, 1967, p. 63.

13a. See ref. 3, pp. 68–70.

13b. Details of this method will be made available to persons who wish to assay their deuterochloroform. We are grateful to Prof. Addison Ault for helpful discussions of the method.

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16. Prior to the advent of commercial high resolution nmr spectrometers (1953-54) only scattered investigations had foreshadowed the power of the deuterium tracer technique. It is noteworthy, however, that a kinetic isotope effect on reaction rate had been first demonstrated much earlier by Westheimer and Nicolaides in their landmark investigation of the mechanism of chromic acid oxidation of 2-propanol. F. H. Westheimer and N. Nicolaides, *J. Amer. Chem. Soc.*, **71**, 25 (1949).
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The Determination of Optical and Enantiomeric Purity by Nuclear Magnetic Resonance Spectroscopy (NMR)

John Campbell, Aldrich Chemical Company, Inc.

INTRODUCTION

Optical purity of a substance is defined as the ratio of its specific rotation $[\alpha]$ to the specific rotation of the pure enantiomer $[A]$.[1] Enantiomeric purity measures the excess of one enantiomer over the other and is equal to the magnitude of the optical purity.[1]

$$\text{Optical purity} = \frac{\text{specific rotation of substance}}{\text{specific rotation of enantiomer}} = \frac{[\alpha]}{[A]}$$

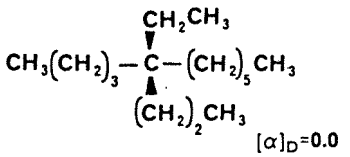
$$\text{Enantiomeric purity} = \frac{E_+ - E_-}{E_+ + E_-} = 2E_+ - 1$$

(E_+ and E_- are the mole fractions of the enantiomers)

Clearly, the measurement of optical purity requires the specific rotation of the pure enantiomer or absolute rotation, $[A]$, in addition to the easily measured value of the specific rotation, $[\alpha]$, of the optically active compound. Alternately, enantiomeric purity and hence optical purity can be determined by a direct measurement of the proportions of the enantiomers present in a mixture. In some cases, absolute rotations may be predicted from structural considerations.[2] However, such correlations have limited utility and absolute rotations are most commonly obtained experimentally.

The classical approach to the determination of optical purity usually involves a total resolution, or separation, of enantiomers to obtain their absolute rotations. This separation of enantiomers (E_+, E_-) requires the reaction of an optically impure compound with an optically pure resolving reagent (R_+) to give a mixture of diastereomers (E_+R_+, E_-R_+). The diastereomers then can be separated by some technique such as fractional crystallization as long as a careful choice of resolving reagent has been made to maximize differences in their physical characteristics.

Aside from the experimental difficulties of choosing the proper resolving reagent and of separating the mixture of diastereomers (E_+R_+, E_-R_+), it is often difficult to determine when resolution is complete. Resolution is usually regarded as complete when the physical characteristics of the diastereomers are unchanged by further purification, or when the two enantiomers are obtained in equal states of optical purity. In some cases, however, such criteria have led to erroneous conclusions.[3] Furthermore, the optical purity of substances with very low absolute rotations, such as chiral deuterated compounds, cannot be determined accurately by polarimetry (e.g., **1**[4] and **2**[1]).



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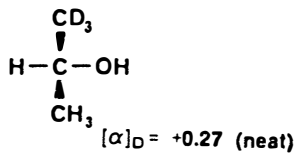


2

Another method of determining the optical purity of a substance for which absolute rotations have not been measured involves relating the compound under study to another compound of known optical purity through a sequence of stereospecific reactions. If, however, the degree of stereoselectivity of the reaction sequence is not known, then the accuracy of the optical purity determination is somewhat restricted.

Since the accurate measurement of optical purity is of critical importance in many reaction mechanism studies, a less tedious method which does not involve total resolution of enantiomers is desirable.

Recently developed methods of total resolution include chromatographic separation of enantiomers on optically active substrates[5] and chromatographic separation of diastereomers, on achiral substrates,[6] derived from the reaction of an optically pure resolving agent with the mixture of enantiomers. Other techniques, which do not require separation of enantiomers or diastereomers, are the isotope dilution, calorimetric and nuclear magnetic resonance spectroscopy (nmr) methods.[1] Perhaps the least time consuming and most convenient methods are those utilizing nmr.

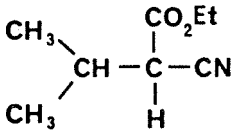
NMR METHODS

Diastereotopic nuclei, or nuclei which cannot be interchanged by a symmetry operation,[1] exists in diastereotopic environments in either chiral or achiral solvents. In principle, they are anisochronous and should have different chemical shifts and different coupling constants. For example, the methyl groups of **3** show a chemical shift difference of 0.037 ppm in carbon tetrachloride.[7] In contrast, enantiotopic nuclei are equally screened in achiral solvents and are isochronous. However, in chiral solvents or in achiral solvents containing a chiral complexing reagent these nuclei become diastereotopic. The above symmetry relationships have been used in the nmr method to determine optical purity by measuring the diastereomer ratios derived from the reaction of the mixture of enantiomers with an optically pure reagent, and also by measuring enantiomer ratios in chiral solvents or in achiral solvents containing a chiral complexing reagent.

CHIRAL DERIVATISING REAGENTS

The nmr determination of enantiomeric purity by integration of diastereomeric nuclei requires that no racemization or optical fractionation occurs during the derivatisation of the compound under study. In principle, any optically pure resolving agent which fulfills these requirements is applicable. However, in practice, it must produce diastereomers with a sufficient chemical shift difference between diastereotopic nuclei to permit their accurate integration. In addition, the spectral patterns of the diastereotopic nuclei should be simple (*e.g.*, doublets or singlets) in order to improve integration accuracy.

The diastereotopic nuclei in the derivative which have different chemical shifts may arise from part of the structure of the original compound being studied or from part of the derivatising reagent. For example, the acid chloride of (*R*)-*O*-methylmandelic acid (**4**) has been used to determine optical purities of **2**,[8] and **5**,[9] as well as other amines and alcohols.[1,9]



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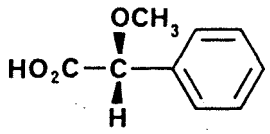

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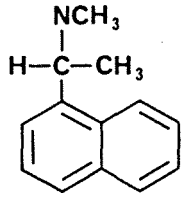

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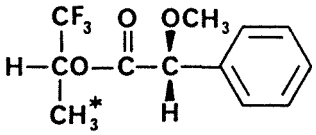

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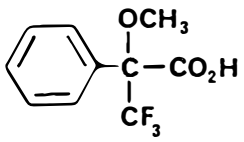
Reaction of the acid chloride of **4** with an alcohol yields an ester such as **6**. In the case of **6** the optical purity of the original alcohol was determined by integration of the diastereotopic methyl (CH_3^*) resonances which show a chemical shift difference of 0.12 ppm in a 10% solution in carbon tetrachloride.[10] The methoxyl resonances of derivatives such as **6** are often used to determine the optical purity of the precursor alcohol or amines.[10,11] However, in some compounds, the methoxyl resonances may not show any readily observable chemical shift difference in a particular solvent. In these instances, a change of solvent may increase chemical shift differences; for example, in benzene-d_6 the chemical shift difference of the methoxyl resonances of **6** is increased to 0.025 ppm.[10] In cases where only one methoxyl resonance is observed for compounds such as **6** it is advisable to prepare this derivative using racemic *O*-methylmandelic acid. Examination of the nmr spectrum of this material will prevent the possibility of mistaking accidental overlap of resonances with the presence of only one diastereomer of **6** which would be produced only from optically pure precursor alcohol.[11] (*R*)-*O*-methylmandelic acid (**4**) has also been used to determine the optical purity of a series of secondary carbinols and the method generally gives results as accurate as those obtained by vapor phase chromatography or polarimetry.[10] However, in some instances, racemization was observed during the preparation of the diastereomeric esters, especially in the case of sterically hindered alcohols.[11]

Mosher[12] has introduced a new reagent, α-methoxy-α-trifluoromethylphenylacetic acid (**7**) which circumvents the problems of racemization which have been encountered in some applications of *O*-methylmandelic acid. Mosher's reagent generally exhibits excellent separation of diastereotopic fluorine and proton resonances, marked stability to racemization of derivatives under severe conditions, and the presence of a trifluoromethyl group which permits the use of fluorine nmr. Furthermore, optical purity determinations with this reagent require as little as 20 mg of sample. This reagent has been applied to the determination of optical purity of a series of secondary amines and alcohols,[12] phenylethylene glycol[13] and phenyltrimethylsilylcarbinol.[14]

Typical chemical shift differences of diastereotopic nuclei are shown in Table 1. A comparison of observed enantiomeric purities with the actual values (Table 1) indicates the high accuracy of the nmr method.



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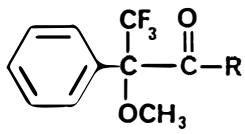

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Table I. Chemical shift differences of diastereotopic nuclei of 8 and enantiomeric purities of derivatives of type 8 (Ref. 12)

	Enantiomeric purity		Chemical shift differences of diastereotopic nuclei (ppm)		
	Actual	Found	CF_3	OCH_3	CH_3
$PhCH(OH)CF_3$	45.2	45.5	0.5	0.16	—
$CH_3CH(OH)CF_3$	75.8	75	0.27	0.05	0.16
$CH_3CH(OH)$-*t*-C_4H_9	7.8	7.5	0.20	0.05	0.07
$CH_3CH(OH)C_2H_5$	82.4	82.0	0.07	0.03	0.10
$PhCH(NH_2)CH_3$	42.4	42.4	0.23	—	0.07

Mikolajczyk also has reported that diastereomeric α-phenylethylamine salts of chiral phosphorus thioacids have different proton[15(a)] and phosphorus[15(b)] nmr spectra. Sufficiently large chemical shift differences were observed to permit the determination of enantiomeric purity using either the proton or phosphorus spectrum.

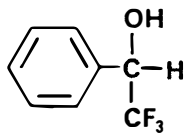
CHIRAL SOLVENTS

In addition to these derivatising reagents, optically active solvents have also been used in the nmr method to determine enantiomeric purity. When a racemic solute (E_+,E_-) is dissolved in a racemic solvent (S_+,S_-), transient diastereotopic solvates are formed. Since solute-solvent interactions are averaged on the nmr time scale the diastereomeric interactions in the two enantiomeric solvate sets, E_+S_+ — E_+S_- and E_-S_- — E_-S_+ produce only one set of solute signals. However, in an optically active solvent (S_+) only one set of diastereomeric interactions (E_+S_+ and E_-S_+) exists and enantiotopic nuclei of the solute then are unequally screened. These nuclei, in principle, should show different chemical shifts, and integration of their resonances then would provide a direct measure of the enantiomeric purity of the solute.

The chemical shift difference of enantiotopic nuclei in a chiral solvent depends on various experimental considerations, such as type and optical purity of the solvent and temperature.

The optically active solvents which cause enantiotopic solute nuclei to show the greatest chemical shift difference are those which contain a group of high diamagnetic anisotropy near the solvent asymmetric center and which interact with or solvate strongly the solute. Furthermore, the solvent should be optically pure since any decrease in solvent optical purity is reflected by a decrease in the chemical shift difference of the enantiotopic solute nuclei. Lowering the temperature at which the spectra are observed also may increase the enantiomeric chemical shift differences.[19]

Two widely used solvents are 2,2,2-trifluoro-1-phenylethanol[16] (9) and α-(1-naphthyl)ethylamine[17] (10).



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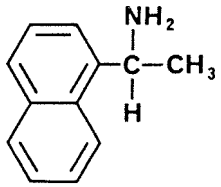

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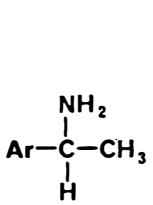
Solvent 9 is somewhat more useful than 10 because proton nmr spectra obtained in 9 are less cluttered by solvent resonances. It has been used to determine optical purities of aromatic amines,[18] sulfoxides,[19] α-amino esters,[20] sulfinamides, sulfinates, sulfites, thiosulfinates, phosphine oxides,[20,21] and amine oxides.[21] Solvent 10 has been used for optical purity determination of aromatic alcohols[23] and α-hydroxy acids.[24] In the case of α-amino esters,[20] optical purities observed by nmr agree with a high degree of accuracy with those determined by other methods even though the observed chemical shift differences of enantiotopic nuclei are generally small (less than 0.10 ppm).

In addition to the measurement of optical purities, correlations of absolute configuration with the relative chemical shift of enantiotopic nuclei in optically active solvents have been used to predict absolute configurations in a series of closely related compounds.[18-23] For example, spectra of aromatic amines of type 11 in S(+)-2,2,2-trifluoro-1-phenylethanol show the methine proton of the S enantiomer at lower field than the corresponding resonance of the R enantiomer.[18] The relative chemical shifts were rationalized in terms of solvates 12 and 13. On the basis of this model



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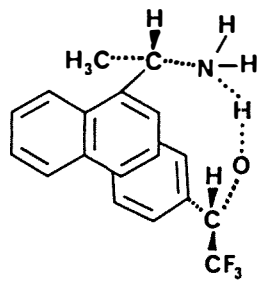

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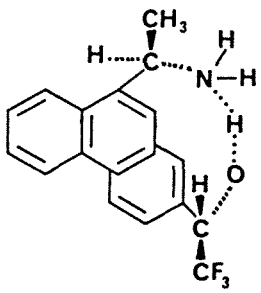

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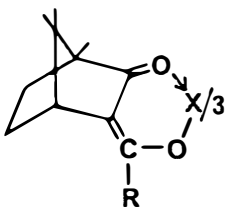
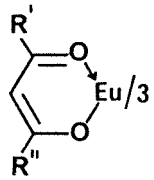
it was predicted that the methine peak of S-11 would be shielded more than that of R-11. Therefore, the S configuration was assigned to aromatic amines of type 11 which exhibited the lower field methine resonance in S(+)-2,2,2-trifluoro-1-phenylethanol.

These correlations of enantiomer chemical shifts with structure have been extended to aromatic alcohols,[23(a)] α-hydroxy acids,[24] amine oxides,[22] sulfoxides[19] and α-amino esters.[20] In the case of (−)-N-methyl-N-ethylaniline oxide the prediction of configuration was confirmed by X-ray analysis.[22] However, as noted by the authors,[23(a,b)] this model is naive and ignores many solute-solvent interactions. Nevertheless, it may be useful for the determination of absolute configuration of a series of closely related compounds as long as some have known absolute configurations.

CHIRAL LANTHANIDE SHIFT REAGENTS

Since the formation of diastereotopic solvates of a racemic compound in a chiral solvent enables the determination of optical purity by nmr, the possibility also exists that any chiral reagent which will complex strongly with the substrate could be used in the nmr method to measure enantiomer ratios. For example, Anet and Cram[25] observed that sulfoxides complex so strongly with chiral alcohols that optical purity measurements could be performed in a dilute solution using an achiral solvent. Since lanthanide shift reagents complex strongly with a variety of functional groups and induce remarkable changes in chemical shifts,[26] an optically active lanthanide shift reagent might be expected to effect different chemical shift changes for enantiomeric substrates.





14 R = *t*-C_4H_9, X = Eu
15 R = CF_3, X = Eu or Pr
16 R = n-C_3F_7, X = Eu or Pr

17 R′ and R″ are bulky groups derived from terpenes.

Recently, the chiral lanthanide shift reagents 14,[27] 15,[28] 16[28,29] and 17[30] have been prepared and indeed induce different effects on the spectra of enantiomers. For instance, a solution of 15 (Eu) and 1,2-dimethyl-*exo*-2-norbornanol (0.7:1 molar ratio) in carbon tetrachloride show a remarkable 0.5 ppm difference for one set of enantiotopic methyl groups[28] (see Table II for additional examples). The chiral lanthanide shift reagents are excellent choices for the determination of optical purity because the spectra are not obscured by ligand resonances, the chemical shift differences observed are generally large, and a wide range of compounds can be studied. For example, alcohols, amines, epoxides, ketones, esters, and sulfoxides show useful chemical shift differences between enantiotopic nuclei. The reagents 15(Eu) and 16(Eu) are the most useful as lanthanide shift reagents[26] and also as indicators of optical purity since they effect the greatest chemical shift difference between enantiotopic substrate nuclei and provide spectra of high resolution.

Table II. Chemical shift differences ($\Delta\Delta\delta$) of Enantiomers in the presence of **15** (Eu) (Ref. 28).

Compound	Proton	$\Delta\Delta\delta$ (PPM)*
2-Octanol	α-CH_3	0.11
	1-CH_3	0.37
1,2-Dimethyl-*endo*-2-norbornanol	2-CH_3	0.33
1-Phenylacetate	-CO_2CCH_3	0.18
3,3-Dimethyl-2-aminobutane	α-CH_3	0.28
cis-β-Methylstyrene oxide	β-CH_3	0.27
1-Methyl-2-norbornanone	1-CH_3	0.17

* Molar ratio of **15** (Eu) to substrate 0.6. These are *not* the optimum $\Delta\Delta\delta$ values.

In summary the optical or enantiomeric purity of a substance is an important quantity which often cannot be measured easily by classical means. The nmr methods provide a relatively convenient and simple way to determine enantiomeric purity which does not require a total resolution. Although enantiomeric purity can be determined in the nmr methods by using chiral derivatising reagents, solvents and lanthanide shift reagents, the shift reagents will likely have the widest applicability. In any case the nmr methods provide convenient alternatives to other classical methods.

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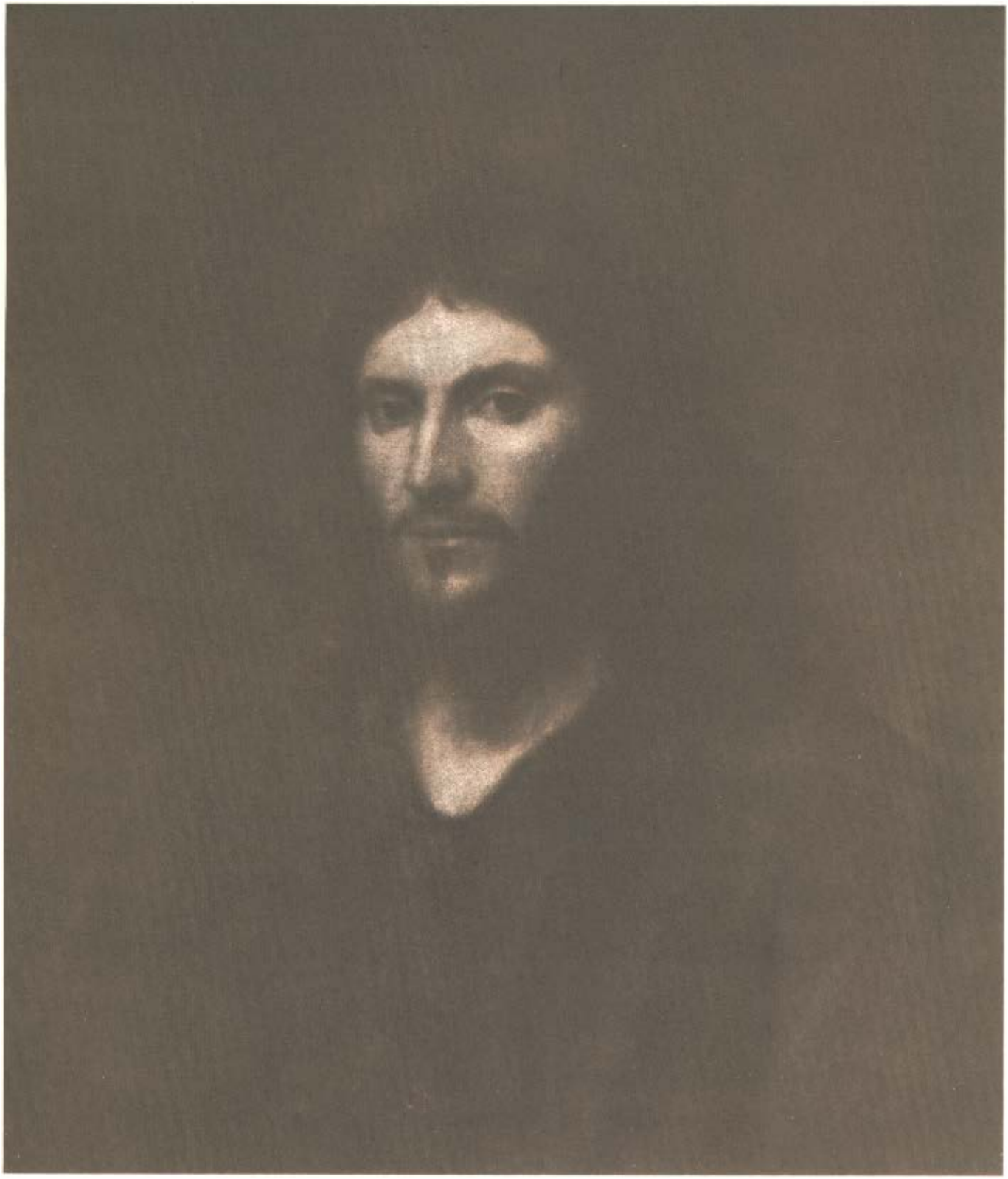

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ABOUT THE COVER

Our chemist-collector seldom buys paintings depicting flowers or landscapes, clearly preferring to look at people. The portrait you see here (oil on canvas, 25½ x 21 inches) came up at auction in Lucerne a few years ago, and was then called Italian, ca. 1700, which was not surprising, because it was so dirty that you could not tell what it was. Our chemist believes that it was painted by one of Rembrandt's last students, Johann Ulrich Mair, who had come to Rembrandt in the 1650's, and then returned to his native Augsburg.

Probably this is a portrait of a young Jew who lived near Rembrandt's house – almost certainly done as a study of Jesus. This student looks to us like a man with a probing mind, perhaps a mystic, certainly a man in search of God – much closer to our idea of Jesus than many sweet baroque idealizations. Man was created in God's image, Genesis tells us, and a really good portrait attests to this.

ALDRICHIMICA ACTA

Volume 5, Number 3
1972

Published by
ALDRICH CHEMICAL COMPANY, INC.
Milwaukee, Wisconsin

Editor, Kathleen D. Ryan

ALDRICH CHEMICAL COMPANY, INC.
CRAFTSMEN IN CHEMISTRY

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A Biologic Assimilation of Inorganic Energy

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There is at least one highly visible flux of energy from the physical world into the world of living organisms. This is the flux of electromagnetic energy that is continuously emitted by the sun, then captured and assimilated at the surface of the earth by the photosynthetic processes of certain living cells. When sunlight is available, a green, highly diverse world of algae and plants transforms physical energy into chemical energy at rates sufficient, not only for their own existence, but also for the growth of another entirely dependent world. That is the world of heterotrophic* organisms, including man himself. That is the world for which photosynthesis by photoautotrophs provides the essential foodstuff through an organic synthesis of gigantic proportions.

Yet, if the flow of visible light from the sun were to be interrupted, still another form of life exists which could also accomplish all the basic chemical syntheses typical of autotrophy - the chemoautotrophic organisms. These were discovered in the final decades of the nineteenth century by Sergius Winogradsky. Admittedly, this is only a world of inconspicuous bacteria multiplying in the dark by division. Yet, it is an autotrophic* world with functions, such as the cycling of nitrogen, which are essential to many other organisms. Most of all, chemoautotrophic* bacteria are autotrophs. They can live without any requirement for preformed organic food, being in that respect similar to photoautotrophs.* Unlike photoautotrophs, however, they do not thrive on the energy from quanta of visible light, but rather lead an existence based on the oxidation of simple inorganic compounds ranging from ferrous, sulfide, ammonia, and nitrite ions to hydrogen and even carbon monoxide gas. All chemoautotrophs share biochemical mechanisms by which they can specifically release and transform the chemical energy of inorganic compounds, some of which are so poisonous that they appear to be incompatible with life. For example, some species can assimilate the energy of explosive mixtures of hydrogen and oxygen gas, others utilize the energy of the oxidation of ammonia to nitrous and to nitric acid, or of sulfides and thiosulfates to sulfuric acid. In all these cases, the microorganisms harness inorganic energy in such a way that it leads to a reduction of carbon dioxide as the first and crucial step in the synthesis of complex organic carbon compounds. Although these organisms are both fascinating thermodynamic machines, and intriguing sites of chemical synthesis, it was left to recent years to uncover the basic chemical mechanisms involved, and to provide insight into the interesting process of the biologic assimilation of inorganic energy.

How do chemoautotrophs accomplish this astonishing feat? How, for instance, does *Nitrobacter**, the nitrite oxidizer, handle the extremely toxic and highly reactive nitrite ion in such a way that the energy yielded from its oxidation can be utilized in the organic fixation and reduction of carbon dioxide? One may answer this question by asserting that *Nitrobacter* has ingeniously borrowed biochemical mechanisms from other organisms. These have been modified somewhat and are protected extremely well in order to meet the cells' specific needs. In all fairness to natural history, however, one should also be willing to acknowledge the possibility that the converse postulate is true, i.e., that chemoautotrophs were the inventors and the other organisms the modifiers and the borrowers.

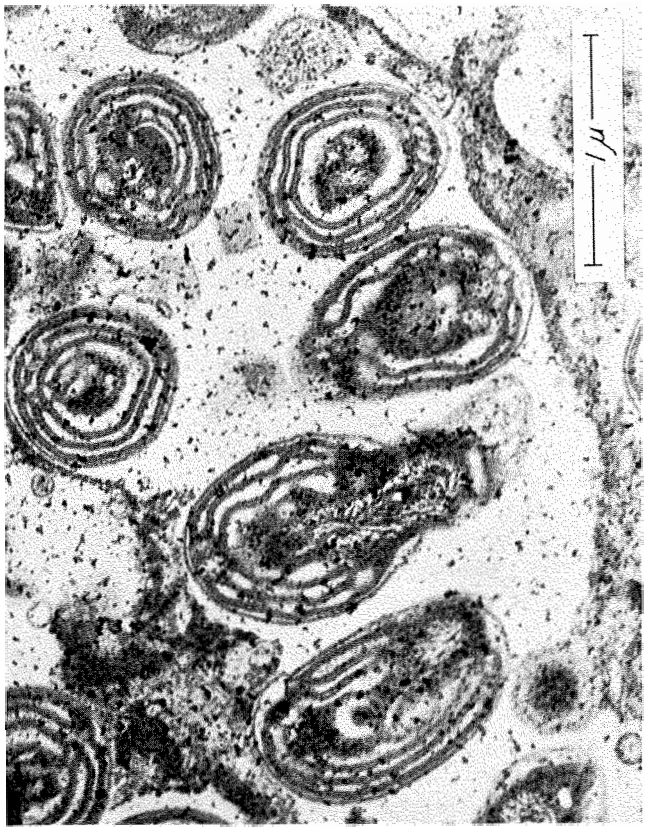

Fig. 1. Electron-micrograph of an ultra-thin section of Nitrobacter.

A glance at an electron-micrograph of an ultra-thin section of *Nitrobacter* (Fig. 1) may be a first step in qualifying the preceding statements. Surprisingly enough, it is the cell's gross structure that helps protect it from its poisonous

* For further definition of terms, see Biochemical Glossary on page 37.

inorganic nutrient. Several double layered membranes completely envelop the interior of the cell. To these membranes are bound all biocatalysts which are necessary for the oxidation of the nitrite ion. As the nitrite diffuses from the surroundings into the cell's interior, it must pass sequentially through one membrane layer after another, each well equipped to convert nitrite rapidly into the much less reactive and less toxic nitrate.

While structure combined with catalytic activity can explain the cells' extraordinary resistance to nitrite, it fails to explain the chemistry of this ion's oxidation or the mechanisms by which the cell harnesses the energy released. An answer to these questions was suggested in 1916 by Otto Meyerhof[1]. He recognized the similarities between the oxidation of nitrite by *Nitrobacter* and the respiratory process in heterotrophic cells. In addition, a thermodynamic efficiency of the biologic oxidation of an inorganic compound, first measured calorimetrically by Meyerhof, was found to be rather poor. Despite those early and careful studies, most of the underlying chemistry, and particularly, the cooperative nature of the associated catalytic events, remained poorly understood.

As more and more enzymes and coenzymes were discovered, and their function as biocatalysts recognized, this knowledge was also applied to chemoautotrophs. In *Nitrobacter*, for instance, Lees and Simpson[2] found that cytochromes, the electron transporting enzymes of cell respiration, were present and were somehow associated with the oxidation of nitrite. These studies also confirmed the stoichiometry: $NO_2^- + \frac{1}{2} O_2 = NO_3^-$ of the reaction established earlier by Meyerhof and they explained the observed sensitivity of *Nitrobacter* to various inhibitors of cell respiration. In addition, it was later shown that these cytochromes are bound to the very membranes that envelop the cell (Fig. 1). After cell rupture, cytochromes could be isolated along with the membranes, and in subsequent spectroscopic studies some of the conditions under which the individual cytochromes undergo reversible reduction and oxidation were elucidated. In fact, sophisticated spectroscopy applied to the participating cytochromes and other redox catalysts became one of the most important research tools for unraveling the puzzle of the biologic assimilation of inorganic energy.

Two discoveries in the early 60s advanced our understanding of this process considerably. Aleem and Nason[3] showed in 1960 that the enzymatic oxidation of nitrite not only involves electron-transport catalyzed by cytochromes, but that it is also coupled to the formation of adenosine triphosphate, the key compound in cellular energy-transfer. Our laboratory reported in 1963[4] that the cell-free oxidation of nitrite is coupled to a reduction of diphosphopyridine nucleotide, one of the hydrogen transferring coenzymes. However, while Aleem and Nason's observation was thermodynamically feasible, ours was not. The standard reduction potential of the pyridine nucleotides with a value of -0.32 V was much too negative to permit pyridine nucleotide reduction by nitrite with a reduction potential of +0.4 V. Still, the diphosphopyridine nucleotide was only reduced by nitrite in the presence of oxygen, never in its absence. Furthermore, it appeared that in order to reduce the pyridine nucleotide by nitrite, the latter must also be oxidized by molecular oxygen.

This formidable, thermodynamic puzzle of the assimilation of inorganic energy is not unique for *Nitrobacter*. In fact, it is shared by all chemoautotrophs utilizing inorganic substrates which by themselves cannot reduce pyridine nucleotides. Aleem and co-workers[5] showed that the reduced form of mammalian cytochrome* c could reduce pyridine nucleotides anaerobically when added to homogenates of *Nitrobacter* or *Ferrobacillus* (the iron oxidizer). Yet, this reduction occurred only if adenosine triphosphate was also present. Furthermore, our laboratory observed[6] that *Nitrobacter*, the nitrite oxidizer, became a nitrate reducer when oxygen was withdrawn and nitrate made available. Using the isolated membranes, it was shown that only DPN·H reduced nitrate anaerobically, and moreover, virtually all cytochromes which participated in the oxidation of nitrite were now found to be catalytically active in the reduction of nitrate. In addition, an anaerobic formation of adenosine triphosphate from inorganic phosphate and adenosine diphosphate occurring in conjunction with the reduction of nitrate by reduced diphosphopyridine nucleotide was observed.

This reaction was interesting for more than just one reason. First of all, it linked nitrate, the product of the nitrite oxidation, with the oxidation of reduced pyridine nucleotides. In this respect, the reaction was opposite from that observed earlier, *i.e.,* the reduction of DPN by nitrite. Secondly, the nitrate reduction also produced ATP which, as shown earlier, was required in the anaerobic reduction of pyridine nucleotides by reduced mammalian cytochrome c. Moreover, the quantitative extent, or the rate of the enzymatic reduction of nitrate, was impressive and was comparable only with the rates of nitrite oxidation.

Another biochemical possibility gained, therefore, an increased significance. That is, the observed reduction of nitrate by reduced pyridine nucleotide could represent a reversal of the reaction sequence which, while proceeding in a forward direction, results in the oxidation of nitrite. Furthermore, the formation of ATP, coupled to this nitrate reduction, could actually serve as the principal mechanism for supplying the energy needed in the forward reaction, thereby overcoming the formidable thermodynamic barrier. This would then permit an oxidation of nitrite by DPN. This assumption received substantial support from observations by Chance and Hagihara[7] and by Klingenberg and Schollmeyer[8]. Both groups reported an energy-linked reduction of pyridine nucleotides in mitochondria, the respiratory organelles of heterotrophic cells, while oxidizing succinic acid. In this case, it was also an ATP utilization that made the thermodynamically unfeasible pyridine nucleotide reduction by succinate possible by reversing part of the respiratory chain. Furthermore, the ATP essential to this reaction was formed through the enzymatic oxidation of succinate by molecular oxygen occurring simultaneously with pyridine nucleotide reduction. Evidently, this scheme could be applied easily to the oxidation of nitrite by *Nitrobacter*.

Indeed, this microorganism seems to have all the essential elements of a functional energy-linked electron transport system. It has a membrane-bound sequence of electron transport

* For definition of terms, see Biochemical Glossary on page 37.

enzymes such as cytochromes, as well as flavoproteins and pyridine nucleotides; all of which are capable of undergoing reversible oxidations and reductions while transporting electrons. In addition, this enzymatic system is linked to the nitrite ion which it can oxidize and the nitrate ion which it can reduce; provided, of course, suitable oxidants such as oxygen, or reductants such as DPN·H are available. The application of the concept of energy-linked electron transport to the assimilation of the inorganic energy of the nitrite oxidation by *Nitrobacter* requires, however, that nitrite reduce at least one cytochrome directly. Consequently, molecular oxygen should not be essential as a direct oxidant. It should be replaceable by another suitable electron acceptor. If so, this would prove the initial step of the oxidation of nitrite to be:

$$NO_2^- + H_2O \rightarrow NO_3^- + 2\ e$$

where a suitable electron acceptor, for instance, a cytochrome, would undergo a simultaneous reduction.

$$2\ e + 2\ \text{cytochrome (Fe III)} \rightleftharpoons 2\ \text{cytochrome (Fe II)}.$$

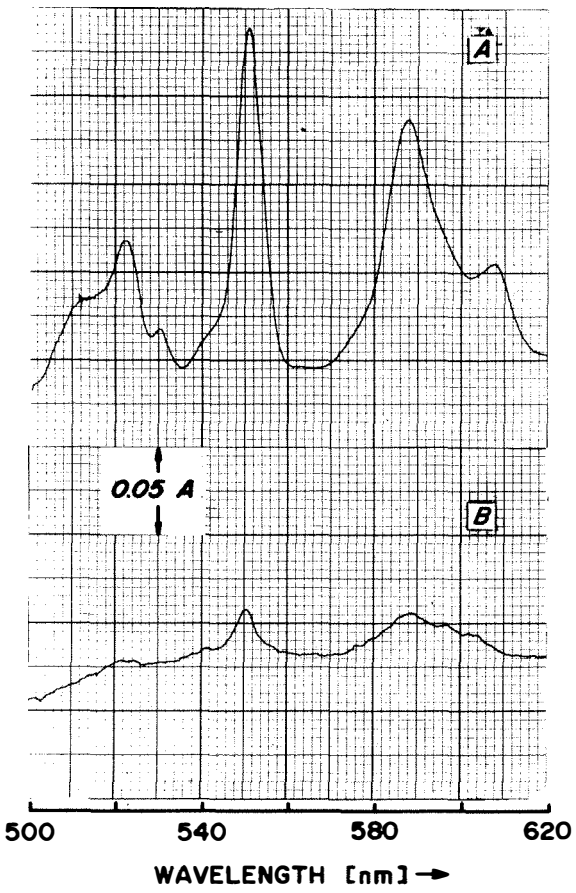

Fig. 2. Low-temperature difference spectrum of Nitrobacter cytochromes reduced by nitrite (top) and reoxidized by chlorate (bottom). Beginning from the right margin (top), the α-bands of three reduced cytochromes are clearly distinguishable. Their disappearance (bottom) indicates the reoxidation of these cytochromes.

Lees and Quastel[9] had shown earlier that *Nitrobacter* could reduce chlorate. In 1963 and 1964, we observed in our laboratory that in the absence of oxygen, chlorate could also oxidize nitrite enzymatically with cell-free membrane preparations from this organism. We further demonstrated a simultaneous reoxidation of cytochromes previously reduced by nitrite (Fig. 2). Indeed, these observations provided the first experimental evidence validating the above equations. They also suggested that nitrate, the oxidation product of the reaction, did not derive its additional oxygen atom from gaseous or molecular oxygen but obtained it from water. This was confirmed shortly thereafter by Aleem[10], who showed that only when nitrite was oxidized enzymatically in water labeled with the isotope ^{18}O, did this isotope appear in the nitrate formed. This incorporation did not occur when the ^{18}O label was provided only in the gaseous oxygen.

Apparently, the first molecular event by which the living cell first harnesses the inorganic energy of the nitrite oxidation consists of the reduction of a cytochrome. Thus, it had to be determined which of the three known cytochromes of *Nitrobacter* was the first to react with nitrite and what events followed. Kinetic dual-wavelength spectroscopy of the formation of the α-absorption bands of the cytochromes revealed that nitrite reduced the cytochrome exhibiting an α-band at a wavelength of 590 nm faster than the one with a band at 552 nm[11]. This indicated that the 'cytochrome 590' might be the first to oxidize nitrite while undergoing reduction itself. Further, it suggested a possible reaction sequence, i.e., the 'cytochrome 552' reoxidizing 'cytochrome 590'. However, the indirect reduction of 'cytochrome 552' by nitrite seemed to be a somewhat more complicated reaction. Admittedly, this cytochrome is chemically similar to the reduced mammalian cytochrome with which Aleem *et al.* were able to reduce pyridine nucleotides anaerobically in the presence of cell-free *Nitrobacter* preparations *and* ATP as mentioned earlier. Yet it appeared from other experimental data that the reduction of 'cytochrome 552' required not only reduced 'cytochrome 590', but also ATP. This evidence was obtained by two independent lines of experimentation[11]. First, ferricyanide was used as an electron acceptor. Like chlorate, ferricyanide permits the anaerobic oxidation of nitrite without forming the highly reactive chlorite, however. In fact, it can be shown that ferricyanide can be reduced by nitrite with *Nitrobacter* membranes as an enzyme source. Furthermore, this anaerobic reduction is not affected by the presence of 5×10^{-4} M 2,4-dibromophenol. Since dibromophenol is an uncoupling agent of oxidative phosphorylation, it uncouples both the formation and also the utilization of ATP from electron-transport reactions without affecting them as such. Consequently, no ATP utilizing steps were involved in the reduction of ferricyanide by nitrite which would be inhibited otherwise by the presence of the uncoupling agent. However, when oxygen replaced ferricyanide as an oxidant of nitrite, 5×10^{-4} M 2,4-dibromophenol inhibited nitrite oxidation strongly, thus indicating the participation of at least one ATP utilizing reaction step in this particular case. The second experimental approach consisted of a detailed analysis of various reduction states of 'cytochrome 552', as shown in Fig. 3, which suggested this cytochrome as a prime candidate for a first ATP requiring step in the indirect oxidation of nitrite by oxygen. In addition, this

appeared to be a step which was either bypassed or not utilized in the anaerobic reduction of ferricyanide by nitrite.

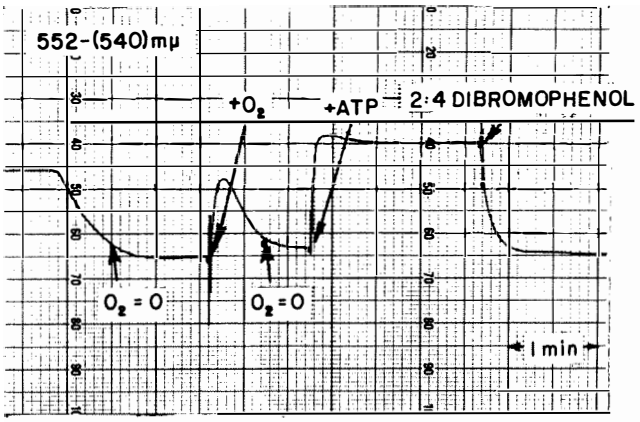

Fig. 3. The energy-linked response of 'cytochrome 552'. Dual-wavelength spectroscopy of membrane preparations provides a quantitative analysis of this cytochrome's reduction state under various conditions.

The recording of Fig. 3 depicts quantitatively the oxidation and reduction of the 'cytochrome 552' still bound to the isolated membranes of *Nitrobacter*. The means of observation and measurement is dual-wavelength spectroscopy. The cytochrome is first reduced by an addition of nitrite to a suspension of isolated membrane. This produced the lead trace at the left edge of the recording. When oxygen is depleted, reoxidation of the cytochrome occurs, as indicated by a downward deflection of the trace. This in itself is a curious response to the presence and to the depletion of an oxidant. If a small amount of oxygen is again added, an immediate but transient reduction of the cytochrome occurs that lasts only as long as the added oxygen. However, if a small amount of oxygen is added together with an excess of ATP, the reduction of the cytochrome occurs again immediately, but now it also persists anaerobically. Yet, even in the presence of ATP, the reduced cytochrome can be reoxidized when a trace of oxygen is added together with a 2 x 10^{-4} M final concentration of the uncoupling reagent 2,4-dibromophenol. Not only can ATP no longer maintain the reduced state of 'cytochrome 552', but also the addition of oxygen is no longer capable of reducing this cytochrome. Quite to the contrary, it now results in its oxidation.

Indeed, this is a rather confusing series of observations, yet they shed considerable light on the means by which nature can assimilate inorganic energy. Energy packages derived from inorganic matter, and too small by themselves, are supplemented by energy derived from the hydrolysis of ATP. Thus, some of the crucial energetic barriers presented at various steps of cytochrome reduction, for instance the reduction of 'cytochrome 552', are overcome. As in the instance of our example, *Nitrobacter*, once the reduction of this particular cytochrome is accomplished, a dividing point in the road is reached where the electron transport is split into two new pathways. The reduced 'cytochrome 552' has apparently a choice between two oxidative mechanisms. It can elect to become oxidized by molecular oxygen as terminal oxidant and electron acceptor. This enzymatic pathway involves other cytochromes and ATP *generating* steps. Reduced 'cytochrome 552' can, however, also elect to become oxidized by DPN as terminal oxidant and electron acceptor. This too involves a sequence of enzymatic reactions. However, these reactions *consume* ATP in order to overcome the additional energetic barriers in a way similar to that shown in Fig. 3 for 'cytochrome 552'.

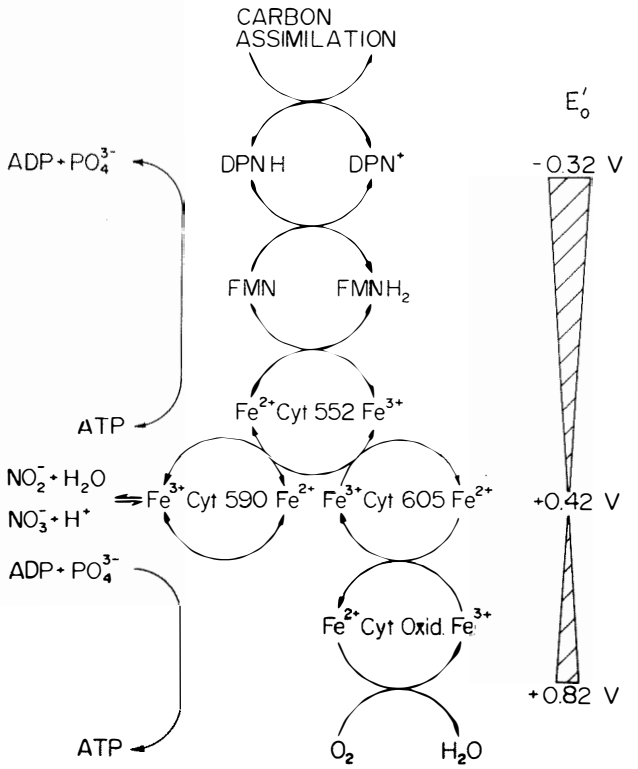

Fig. 4. Diagrammatic presentation of the enzymic mechanisms of the assimilation of inorganic energy.

Looking at the diagrammatic presentation (Fig. 4) of some of the known chemical and enzymatic occurrences just described, one can easily envision how this system for the assimilation of inorganic energy becomes functional. The supply of substrates and the demand for them due to cellular synthetic requirements seems to determine the molecular events. For instance, with nitrite and oxygen available, nitrite will be oxidized to form ATP and, of course, also nitrate. With DPN and ATP available, nitrite can also be oxidized to form DPN·H and, in addition, ADP. The branches of the oxidative pathways of nitrite can, therefore, fulfill the requirements of the organism for the assimilation of carbon dioxide, i. e., produce DPN · H and ATP. While the downhill ATP forming electron transport system can satisfy this ATP requirement directly, it can also satisfy the requirement for DPN · H indirectly by sustaining the ATP utilizing uphill electron transport energetically.

The presence of two alternate pathways for nitrite oxidation should also result in a variable stoichiometry for the oxidation of nitrite by molecular oxygen. Indeed, it was shown earlier that an increase in the carbon dioxide partial pressure increases the NO_2^-/O_2 ratio[12]. We now know that this is an immediate result of a higher DPN·H utilization in the autotrophic assimilation of carbon dioxide. In fact, the stoichiometry observed by Meyerhof ($NO_2^- + \frac{1}{2} O_2 = NO_3^-$) is valid only under the particular conditions of his experiment, that is, in the absence of carbon dioxide which was absorbed by alkali hydroxide. However, at the time the increase in the NO_2^-/O_2 ratio with increasing CO_2 partial pressure was noticed, this observation was difficult to understand, and consequently it was interpreted incorrectly at first. Only the later findings, which have just been described, made it possible to explain these results adequately.

Indeed, this is the very intriguing system which can assimilate and utilize inorganic energy at an astonishing thermodynamic efficiency. Our laboratory determined this efficiency for *Nitrobacter* recently by direct calorimetry[13]. We found it to be an overall efficiency of 34 percent, thus placing the process of the biologic assimilation of inorganic energy in the company of some of the best thermodynamic efficiencies known today.

*BIOCHEMICAL GLOSSARY

Adenosine Triphosphate - ATP: The purine nucleotide formed by the phosphorylation of adenosine diphosphate (ADP). The reversal of this reaction, the dephosphorylation of ATP either by hydrolysis or by transfer of the terminal phosphoryl group is an important chemical mechanism for preservation and utilization and for conversion and transfer of energy in cells.

Autotrophs: Self-feeding organisms and cells utilizing carbon dioxide as the sole source of carbon. *Photoautotrophs* use light and *chemoautotrophs* chemical oxidation-reduction reactions as energy sources.

Cytochromes: Iron-containing proteins which catalyze electron-transfer by reversible changes in the valence of iron, Fe(II) $\rightleftharpoons$ Fe(III). The iron is contained in the porphyrin ring of the heme component of these enzymes. The reduced forms of cytochromes show three typical absorption bands (α-, β-, and γ-bands) in the visible light region which can serve in their identification. Various cytochromes have also different but characteristic standard reduction potentials. Cytochromes can function catalytically as enzymes, or can serve as the substrates of other cytochromes, thereby forming an enzymatic chain.

Heterotrophs: Cells and organisms which feed on other cells. They utilize more complex and more reduced forms of carbon from their environment.

Nitrobacter: A species of nitrifying, chemoautotrophic soil bacteria which oxidize nitrite to nitrate and, in conjunction with *Nitrosomonas*, constitute a part of the natural nitrogen cycle.

Pyridine nucleotides: The heat-stable coenzymes of enzymatic oxidation-reduction reactions which involve both hydrogen and electron transfer. A synonym for *diphosphopyridine nucleotide* is nicotinamide adenine dinucleotide and abbreviations used for the oxidized and reduced forms are: DPN and DPN·H or NAD and NAD·H.

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L. A. Kiesow

The Methadone Story

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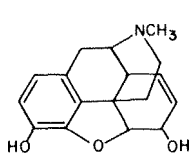
Abstract -

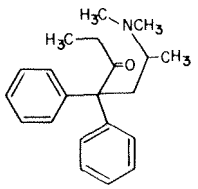
Methadone, a synthetic analgesic, topologically similar to morphine and with a similar pharmacologic profile was confiscated from Germany in the spring of 1945. Craving for methadone does not match that experienced for morphine or heroin and withdrawal symptoms, although prolonged, are much milder than with morphine or heroin. Consequently its principal use in the United States to 1965 was for the alleviation of heroin withdrawal sickness. Since that time, it has been used as a substitute for heroin in so-called maintenance programs begun by Dole and Nyswander of New York City. Its virtues are high oral effectiveness, long duration of action and blocking of the thrill normally given by heroin. At present 45 - 50 thousand former heroin addicts are participating in the programs. Many are now useful members of society. Longer-acting methadone derivatives (e.g., acetylmethadols) hold promise of being superior to methadone.

In the spring of 1945, a team of scientists headed by Dr. Ervin C. Kleiderer of Eli Lilly and Company was sent by the United States government to study the pharmaceutical activities at the I. G. Farbenindustrie plant, Hochst am Main, Germany. Among the interesting products disclosed in the so-called Kleiderer Report that followed[1] was 6-dimethylamino-4,4-diphenyl-3-heptanone (amidone, methadone, II), a potent analgesic initially synthesized by Bockmuhl and Ehrhart[2] of Germany and first tested for spasmolytic properties. Since that time, methadone researches have been numerous, and a plethora of papers have been published on the chemistry and pharmacology of this drug and on literally thousands of analogs and derivatives.[3,4]

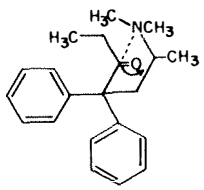
Although on casual examination, methadone's structural resemblance to morphine (I) appears remote, an inspection of molecular models reveals striking similarities in rigidity and topology.[5,6] More recently, Beckett[7] suggested that interaction of the free electron pair of the nitrogen and the slightly positive carbonyl carbon of methadone might assist in the formation of a favorable conformation (III) for a biological receptor that would also accommodate morphine and pethidine. One might consider, too, a conformation (IV) which would result from intramolecular bonding between the (protonated) nitrogen (at physiological pH)and the oxygen function.[4b] In any event, methadone and morphine do have in common, chemically, a quaternary carbon, a phenyl group attached to this carbon, and a tertiary amino group two carbons removed.



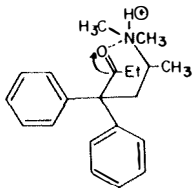
MORPHINE, I



METHADONE, II



III



IV

Fig. 1

The synthesis of methadone (Fig. 2) is readily achieved in two steps by condensation of diphenylacetonitrile (V) and 2-chloro-N,N-dimethylpropylamine (VI) ($NaNH_2$[2,4] or KOH[8] as carbanion-forming reagent) followed by reaction of one (VII) of the two isomeric nitriles thus formed with ethylmagnesium bromide. The other nitrile (VIII) leads to isomethadone (IX), also an effective pain-relieving compound.

It has been amply demonstrated that the pharmacologic profile of methadone closely parallels that of morphine with some *important* time-action differences and *clearly superior oral*

effectiveness for methadone.[9,10] In an individual dependent on large daily doses of morphine, methadone can be substituted and the dose rapidly reduced with days elapsing before the patient is aware that either has taken place. Impending abstinence phenomena can be avoided by administration of small *oral* doses of methadone. Given a choice, some former addicts prefer methadone (for its prolonged effect) to morphine or heroin, but some reverse this choice later because of the greater peak thrill which morphine and heroin provide.[9,10,11]

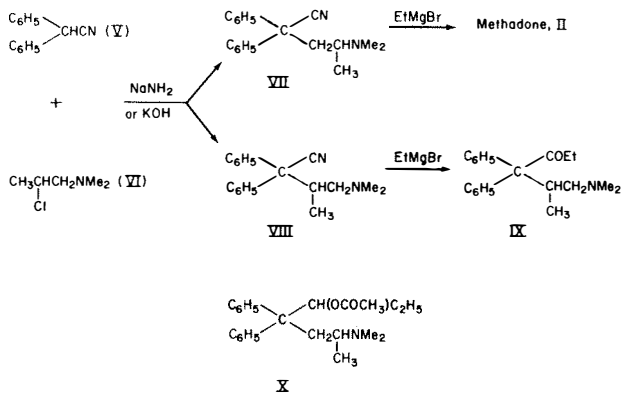


Fig. 2

Chronic administration of methadone causes rapid development of tolerance and cross-tolerance to heroin, morphine and other morphine-like agents (but not to barbiturates and stimulants [9,10,11]) and of a physical dependence of the morphine type. On abrupt withdrawal, abstinence signs are not evident for about 48 hours. These signs never reach severe intensity and disappear more slowly than with a morphine dependence. Thus, orally administered methadone has long been used to minimize withdrawal sickness in narcotics addiction.

About 1964, Drs. Vincent Dole and Marie Nyswander, a husband-wife medical team of New York City, recognizing the inadequacies of narcotics-addiction treatment and cognizant of the differences in pharmacologic properties of the large battery of 'narcotic' drugs at a physician's disposal, conceived and began a program of 'maintenance* therapy' with a few *selected* heroin addicts. Their philosophy[12] was based on the belief that *rehabilitation* of a drug-dependent individual should be given priority over *elimination* of drugs. Their choice of methadone was out of consideration not only for the published pharmacologic properties of available agents, but also for actual clinical trial of some of these agents in a small group of patients during a two-year period.[10,12]

*For a statement on the concept of maintenance, see Wld. Hlth. Org. Techn. Rep. Ser., 1970, No. 460, p. 20, ff. and references therein.

The original protocols and rationale[13] emphasized the importance of having available a long-acting orally effective drug (methadone) and plausible reasons for failure of the limited trial with 'maintenance' [with relatively short-acting, parenterally (sometimes self-) administered morphine] from 1919-1923. Using methadone,[13] patients can be stabilized by a single, daily, oral dose. Maintaining patients on methadone was said to be [13] 'no more difficult than maintaining diabetics with oral hypoglycemic agents.' It was further stated[13] that methadone 'appears to relieve narcotic hunger and thus free the patient for other interests as well as protect him against readdiction to heroin by establishing a pharmacological block.'

The program was developed in three phases.[13] 1. Stabilization of addict patients with methadone hydrochloride, administered orally, in an unlocked hospital ward during a period of about six weeks. The patients are given a complete medical workup, psychiatric evaluation, review of family and housing problems and job-placement study. 2. Discharge of patients to outpatient care, these patients returning every day for methadone medication. 3. Return of the patients to society. These patients still receive methadone medication (cf. references 10, 13 for details of these three phases).

Dr. Everette L. May

During the approximately eight years since its inception in New York City, the methadone program has proliferated throughout the United States and Canada. It is estimated that in the United States alone, 300 Investigational New Drug (IND) licenses have been issued by the Food and Drug Administration (which jointly with the Bureau of Narcotics and Dangerous Drugs exercise controls over the program) for this modality of treatment of narcotics addiction[14]; there are presently about 50,000 patients in the program.* No reliable figures are available on what percentage of this restricted group of patient addicts have been returned as useful members to society or what has been the impact of the methadone program on crime and economics. The consensus seems to be that overall, the results are favorable. Also, not yet known is what will happen if and when the 'rehabilitated', methadone-dependent individual is deprived of this 'crutch'. Will there be recidivism to the same extent as with other treatment methods or is the methadone-treated patient truly cured?

As for the future, it is likely that methadone maintenance programs will continue to grow; political leaders have called for rapid expansion in New York City alone to a caseload of 25,000. Better drugs for this type of treatment are being sought. Already, a methadone derivative, alpha-(+)-3-acetoxy-6-dimethylamino-4,4-diphenylheptane (alpha-acetyl-methadol),[15] a longer-acting, also orally effective drug has been tried with marked success.[16] More recently[17] the *levo* isomer has been under study. Others are no doubt in the chemical and pharmacologic 'pipelines'.

*For a current medical report *cf.* Medical World News (a McGraw-Hill publication) March 17, 1972, p. 53.

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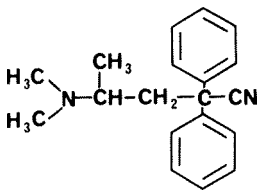
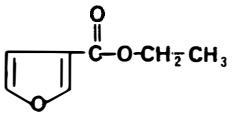
Pharmaceutical intermediate



4-Dimethylamino-2,2-diphenylpentanenitrile
(methadone nitrile)

Precursor of methadone hydrochloride, a drug used in the treatment of heroin addiction.

Medicinal building block



Ethyl 3-furoate

Used in the synthesis of 4-ipomeanol and other naturally occurring ring compounds.[1]

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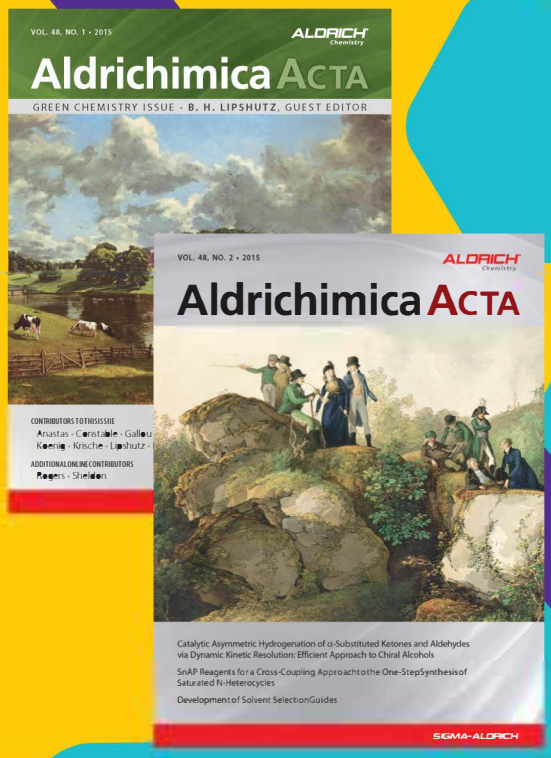

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