stereochemical reaction cycle

$$(+)\text{-}(R)\text{-}\mathbf{1} \xleftarrow{R} (-)\text{-}(R)\text{-}\mathbf{4} \xrightarrow{R} (-)\text{-}(R)\text{-}\mathbf{5} \xrightarrow{R} (-)\text{-}(R)\text{-}\mathbf{6} \xrightarrow{I} (+)\text{-}(R)\text{-}\mathbf{1}$$

This four-reaction cycle contains four chiromers, three retentions, and one inversion. Unlike the cycle of Chart I, the presence of a ligand metathesis and one inversion makes this reaction cycle podal. All chiromers contain tolyl and oxygen as common ligands and therefore the cycle is diligostatic.[2]

Todd R. Williams, Robert E. Booms, Donald J. Cram*
Contribution No. 2873, Department of Chemistry
University of California at Los Angeles
Los Angeles, California 90024
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The Isotopic Rearrangement of *n*-Propyl-α-^{13}C-benzene

Sir:

In 1964 Roberts, Khalaf, and Greene[1] proposed that the isotopic rearrangement of α or β ^{14}C-labeled *n*-propylbenzene in the presence of aluminum chloride involves diphenylpropanes as key intermediates. Later in the same year, Farcasiu[2] suggested that the isotopic rearrangement and the small amount of isomerization of isopropylbenzene that also occur result from a mechanism in which diphenylhexyl cations ($C_{18}H_{21}^+$) are produced, rearrange, and undergo fission in a way similar to the "bimolecular mechanism" proposed by Karabatsos for the isotopic rearrangements of ^{13}C- and ^{14}C-labeled *tert*-pentyl cations.[3,4]

Both of these mechanisms provide explanations for an important aspect of the *n*-propylbenzene isotopic rearrangement; the lack of rearrangement of the isotopic carbon to the γ position. In both of them the intermediate carbonium ions which lead to equilibration of the isotope between the α and β positions can be secondary, whereas primary carbonium ions are required to put the isotope into the γ position.[5]

An obvious and elegant test to distinguish between these two mechanisms would be to use ^{13}C as the labeling isotope. The diphenylhexyl cation mechanism would allow the formation of dilabeled and unlabeled as well as monolabeled molecules of *n*-propylbenzene, whereas only monolabeled isotopic isomers could result from rearrangement *via* diphenylpropane intermediates. Analysis of the reaction products by mass spectroscopy should thus allow an assessment of the extent of rearrangement *via* bimolecular processes.

n-Propyl-α-^{13}C-benzene was synthesized from Ba-$^{13}CO_3$ by a procedure analogous to the one described previously for *n*-propyl-α-^{14}C-benzene.[6] The isotopic analysis of ^{13}C-labeled molecules was obtained by mass spectrometry through comparison of the intensities of the p and p + 1 ions. The spectrum of unenriched *n*-propylbenzene at 70 eV exhibits a strong parent peak, p (*m/e* 120), and a small p + 1 peak (*m/e* 121) which corresponds to the expected distribution of molecules containing ^{13}C from natural abundance. The enriched *n*-propyl-α-^{13}C-benzene exhibited a much higher ratio of *m/e* 121:120 peaks, of course (Table I). The formation of dilabeled and unlabeled molecules from monolabeled molecules of *n*-propylbenzene upon reaction with $AlCl_3$ could be detected easily by a significant change in the ratio of the *m/e* 121:120 peaks.

Table I. Mass Spectroscopic Analysis of *n*-Propyl-^{13}C-benzene before and after Reaction with Aluminum Chloride

	m/e 120 ($C_9H_{12}^+$), %	*m/e* 121 ($C_8{}^{13}CH_{12}^+$), %	*m/e* 91 ($C_7H_7^+$), %	*m/e* 92 ($C_6{}^{13}CH_7^+$), %
		Before Reaction		
Expt 1	67.7	32.3	67.7	32.3
Expt 2	83.1	16.9	82.1	17.9
		After Reaction		
Expt 1	67.7	32.3	82.5	17.5
Expt 2	83.3	16.7	90.8	9.2

The distribution of the label in the fragment ions of the mass spectrum could be used to locate the position of the label within the parent molecule, if there were no scrambling of carbon atoms preceding the primary fragmentation. Cleavage of the bond between the α and β carbon atoms does indeed produce the tropylium ion ($C_7H_7^+$, *m/e* 91, and $C_6{}^{13}CH_7^+$, *m/e* 92, base peak) without scrambling the label, thereby making possible determination of the amount of ^{13}C in the α position before and after the isotopic rearrangement.

Samples of *n*-propyl-α-^{13}C-benzene (500 mg, 32.3% monolabeled and 498 mg, 16.9% monolabeled) were treated with aluminum chloride in the presence of benzene and a trace of water (1.0:0.5:6.0:0.1 molar ratios, respectively) at reflux for 7 hr, duplicating the conditions previously reported[1] to give equilibration of the isotope between the α and β positions. The work-up, involving decomposition of the reaction mixture with water, was carried out as before. Preparative glpc resulted in 60–67% recovery of *n*-propylbenzene.

The quantitative mass spectral data were obtained with a CEC 21-110 mass spectrometer with an ionizing voltage of 70 eV. Peak intensities were measured by means of a potentiometric recorder. The data are presented in Table I. The natural abundance of ^{13}C, 1.1% per carbon atom, was subtracted from each of the observed values.

As may be seen from the first two columns of Table I, there was no significant change in the ratio of the *m/e* 120:121 peaks before and after reaction, showing that no unlabeled or dilabeled *n*-propylbenzene molecules were produced. The change in the ratio of the *m/e* 91:92 peaks before and after reaction (columns three and four) was indicative of the expected isotopic rearrangement; in experiment 1, 54.3% (17.5/32.3 × 100) and in experiment 2, 51.4% (9.2/17.9 × 100) of the ^{13}C remained in the α position after reaction.

^{13}C and ^1H nmr spectroscopy was also used to confirm ^{13}C label position among the α, β, and λ positions,

(1) R. M. Roberts, A. A. Khalaf, and R. N. Greene, *J. Amer. Chem. Soc.*, **86**, 2864 (1964).
(2) D. Farcasiu, *Rev. Chim. (Bucharest)*, **10**, 457 (1965).
(3) G. J. Karabatsos, F. M. Vane, and S. Meyerson, *J. Amer. Chem. Soc.*, **83**, 4297 (1961).
(4) G. J. Karabatsos and F. M. Vane, *ibid.*, **85**, 79 (1963).
(5) One deficiency of the "bimolecular mechanism" is the fact that t predicts the rearrangement of *n*-propylbenzene to isopropylbenzene as easily (*i.e.*, *via* secondary and tertiary carbonium ions) as the isotopic rearrangement, in disagreement with experimental facts. The mechanism involving diphenylpropane intermediates allows for rearrangement to isopropylbenzene only *via* primary carbonium ions, which would not be expected to be facile, in accord with experimental facts.
(6) R. M. Roberts and J. E. Douglass, *J. Org. Chem.*, **28**, 1225 (1963).

but the analysis was less accurate than that by mass spectrometry (± <5% *vs.* ± <2%). The ^{13}C nmr spectrum of the isotopic label-rearranged *n*-propylbenzene (experiment 1) was obtained with a Varian DP60-IL (modified) spectrometer using CAT of 2163 scans. Two overlapping triplets were observed corresponding to the α and β carbon atoms, at 56.8 and 43.5 ppm, respectively, calculated using an external methyl iodide reference. Integration by peak weights indicated that the ^{13}C label was distributed 53% at the α position and 47% at the β position. The proton nmr spectrum of the same sample (experiment 1) displayed a small side band upfield from the γ-methyl absorption which was a result of ^{13}C in natural abundance at the γ carbon, and which did not increase after the isotopic rearrangement (Varian HA-100 spectrometer).

These results show that during the reaction of *n*-propylbenzene with aluminum chloride in the presence of excess benzene and a trace of water at reflux, a process takes place which scrambles the α and β positions, but not the γ position of the recovered compound. It is unlikely that this rearrangement involves diphenylhexyl cations as intermediates, since such intermediates could easily rearrange to produce dilabeled and unlabeled propylbenzene molecules by shifts and intermediates fully equivalent to those required to scramble the α and β positions.

The mechanism involving diphenylpropane intermediates[1] offers a satisfactory explanation of the data, since only monolabeled *n*-propylbenzene can result from this process. The positive effect of solvent benzene concentration on the rate of scrambling the α and β positions and the slow rate at which *n*-propylbenzene is isomerized to isopropylbenzene can also be seen as logical consequences of the diphenylpropane mechanism.

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Royston M. Roberts,* Thomas L. Gibson[7]
Department of Chemistry, University of Texas at Austin
Austin, Texas 78712
Received August 21, 1971

Orientation Effects in Reactions of Acyl Chymotrypsins

Sir:

An important component of the catalytic efficiency of enzymes is their ability to juxtapose reacting groups in configurations which maximize reactivity.[1] It is qualitatively clear that the distance between reacting atoms is not the only determinant of reactivity—the orientation of the reacting groups is also important. Opinions differ as to the importance of this effect.[2] Progress in this area has been impeded by a lack of unambiguous examples of the effect of orientation. We present here data concerning the reactivity of certain acyl chymotrypsins which provide clear evidence for the operation of an orientation effect.

Acyl chymotrypsins can react with nucleophiles other than water, such as methanol[3–5] and amines.[4–7] Hydroxylamine[4–6] and methoxylamine[7] are effective nucleophiles toward acyl enzymes derived from nonspecific substrates, but they are very ineffective nucleophiles toward acyl enzymes derived from specific substrates.

Ammonia, the nitrogen nucleophile which is structurally most like water, has not been studied extensively. Ammonia and primary amines are quite reactive toward furoyl chymotrypsin,[5] but no parallel study of acyl enzymes derived from specific substrates has been reported. We have measured the reactivity of ammonia toward *N*-acetyl-L-tryptophanyl chymotrypsin by two independent methods. The lack of reactivity of ammonia in this case is in striking contrast to its reactivity toward furoyl chymotrypsin and provides evidence for very specific geometric constraints in the hydrolysis of acyl chymotrypsins.

The reactivity of ammonia toward *N*-acetyl-L-tryptophanyl chymotrypsin was measured by hydrolysis of *N*-acetyl-L-tryptophanamide at pH 9.43 in the presence of 0.003–0.1 *M* ammonia containing 8.5 atom % ^{15}N. The reaction was stopped after 10–50% hydrolysis and the remaining starting material was isolated and analyzed[8] for ^{15}N. Incorporation of the heavy isotope into the amide never exceeds 0.005 atom %. Because of this small incorporation and because of the presence of a substantial isotope effect on the reaction[8,9] it is impossible to make an accurate estimate of the exchange rate. It is clear that ammonia is no more than four times as reactive as water.[10]

Because of the lack of precision of the isotopic method we also measured the reactivity of ammonia by another method. *N*-Acetyl-L-tryptophan methyl ester was hydrolyzed with chymotrypsin at pH 10.0 in the presence of 0.10 *M* ammonia. After completion of the hydrolysis, the product mixture was analyzed by quantitative column chromatography for the presence of *N*-acetyl-L-tryptophanamide.[11] Only a very small amount of amide was formed. The reactivity of ammonia toward this acyl chymotrypsin is approximately sixfold less than that of water after correction for protonation of ammonia.[10]

From these studies and from previous studies of aminolysis of chymotrypsins a very significant fact emerges. The nucleophilicity of ammonia toward acyl

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(9) M. H. O'Leary and M. D. Kluetz, *ibid.*, **92**, 6089 (1970).

(10) This figure was calculated by incorporating 55.5 *M* for the water concentration and considering the pK_a of ammonia to be 9.25.

(11) A DEAE-cellulose column was used. The analysis was made by Ehrlich assay on concentrated effluent fractions. In some experiments a small amount of *N*-acetyl-L-tryptophanamide was added before the enzymatic hydrolysis in order to check the reliability of the procedure. The expected increase in yield of amide was observed. Under the conditions of our experiments an insignificant fraction of the amide formed would be hydrolyzed.