Unsaturated halides, even those leading to the highly stable allyl cations, are also difficult precursors. Here, even if some ion were formed from the initial quantity of halide, further amounts would invariably lead to polymerization since addition of cation to the double bond is usually faster than ionization. Such ions have, until now, been prepared in other ways.[3,4]

We report here a new method which permits the preparation of pure samples of easily rearranged carbonium ions and which also allows the use of unsaturated halides as precursors of allyl cations. The key idea is the formation of molecular beams of the reactants through introduction *via* nozzles into a highly evacuated chamber. These beams impinge on a surface cooled with liquid nitrogen to form an intimate solid mixture of the reagents. Only a faint haze of material is deposited on the sides of the chamber. This shows that the pressure is low enough to avoid collisions in the gas phase.

The apparatus used in this technique is shown in Figure 1. Flasks of SbF_5 and the appropriate organic chloride precursor (attached through a short length of capillary tubing if the chloride is very volatile) are attached to the side arms of the apparatus and degassed thoroughly. The chamber is cooled and evacuated and the transfer is begun. The rate of introduction of the two reagents is controlled through the temperatures of the flasks containing the reagents. Where the volatility of the halide requires it, a capillary can be interposed between the halide flask and the rest of the apparatus. The reactants meet only on the bottom of the central chamber. The reaction may occur then or on later warming to $-120°$. SO_2ClF or another solvent may be distilled in from the vacuum line to decrease the viscosity of the ion sample. Mixing can be facilitated by sealing a glass-covered magnetic stirring bar in the central chamber. The solution is decanted while at $-100°$ or lower to nmr tubes which are attached to the side of the chamber.

sec-Butyl cation was prepared using this method by the simultaneous distillation over 30 min of 2.5 g of SbF_5 at 3° and 0.1 ml of *sec*-butyl chloride at $-28°$ through a 40 × 0.5 mm capillary tube. Following preparation of the ion 1.5 ml of SO_2ClF was added by distillation and the solution was warmed to $-100°$ and then transferred to the nmr tubes which were sealed off and removed. Integration of the 100-MHz nmr spectrum showed 95% unrearranged *sec*-butyl cation and 5% rearranged ion as the *tert*-butyl cation to be compared with the 75% *sec*-butyl cation and 25% *tert*-butyl cation resulting from the best previous preparation.

When 100 mg of 3-chloro-3-methylbut-1-ene and 8 g of SbF_5 were introduced into the apparatus, a solution was obtained with a spectrum which we assigned to 1,1-dimethylallyl cation. A complex group of peaks was observed between τ 1.5 and 2.1 assigned to the allyl protons and a singlet of twice the area at τ 6.4 ascribed to the methyls. The presence of such a small chemical shift between the inside and outside allylic protons is not unexpected, since most of the charge must be on the tertiary center rather than the primary carbon. The observation of a single peak for the two methyl groups can mean either that the chemical shift difference happens to be zero or that there is a low barrier for rotation around the bond to the vinyl group. This would again be not unexpected if the charge were localized on the tertiary carbon. No change in the spectrum was observed from -90 to $+80°$. We could not ascertain from the observed spectrum whether the vinyl protons form an ABC multiplet or an A_2B multiplet.

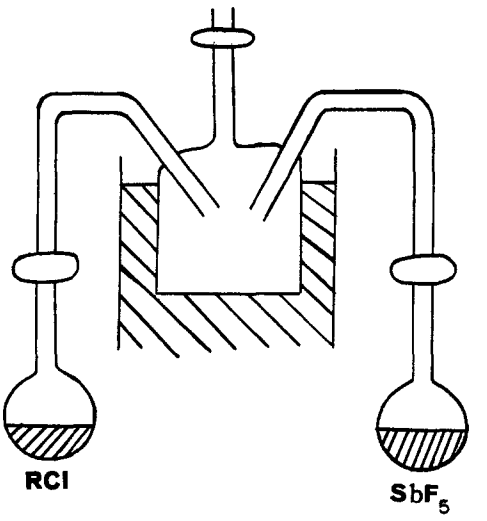

Figure 1.

When 1-chloro-3-methylbut-2-ene was used as a precursor, the same peaks were present but in addition a set of extraneous peaks could be observed. We believe that these are due to a dimer formed by attack of a molecule of cation on a neighboring molecule of the precursor before it has a chance to ionize. This hypothesis was supported by observing that using a very high ratio of SbF_5 to chloride led to lower quantities of extraneous peaks. This isomer would be expected to be more susceptible to attack by another cation, since it has a secondary–tertiary double bond in contrast to the primary–secondary double bond of the tertiary chloride.

Thus far, cyclopentenyl,[5] cyclopentadienyl,[6] and dimethylisobutyl[7] carbonium ions have been prepared by this method for the first time. It is likely that many additional interesting cations which have not previously been available could be prepared in this way.

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Enzymatic Preparation of an Optically Active Phosphotriester, Asymmetric Only at Phosphorus

Sir:

In this communication we describe the first preparation of an optically active triester of phosphoric acid,

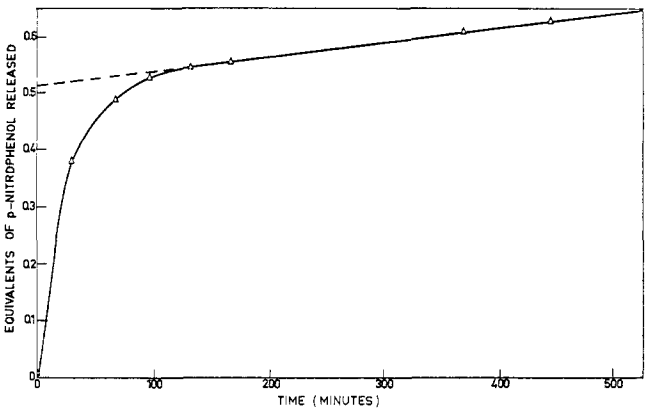

Figure 1. Release of *p*-nitrophenol from MBNP during hydrolysis at pH 8.08, 38°, catalyzed by horse serum.

asymmetric at the phosphorus atom. Such esters promise to be of special value in determining the steric courses and kinetics of reactions of phosphoric acid esters, and thus for evaluating any influence of pseudorotation on their chemistry. The stereospecificity of a wide variety of enzymes can also be studied using optically active phosphotriesters, which may lead to an understanding of the biological relationships between these enzymes.

The asymmetric triester methyl *n*-butyl *p*-nitrophenyl phosphate (MBNP) was synthesized from di-*p*-nitrophenyl hydrogen phosphate (prepared by the method of Moffatt and Khorana[1]) as follows. Di-*p*-nitrophenyl hydrogen phosphate (95.5 g), suspended in 2.5 l. of ether at 15°, was treated with diazomethane (13.08 g in 400 ml of ether) added dropwise over 2.5 hr with vigorous stirring. The mixture was then stirred at room temperature for 2.5 hr and evaporated to dryness under vacuum, and the residue was recrystallized from ethanol. The yield of methyl di-*p*-nitrophenyl phosphate was 79.0 g (79.4% of theory) with mp 140.2–141.5°, lit.[1] 142–143°. Unreacted di-*p*-nitrophenyl hydrogen phosphate was recovered from the mother liquor of recrystallization, dried, and methylated as before, yielding a further 9.61 g of methyl di-*p*-nitrophenyl phosphate, mp 139–141°. The overall yield (88.6 g) was 89.1% of theory. Alkaline hydrolysis[1] of this triester, followed by chromatography on Dowex 50W-X8 cation-exchange resin in the H^+ form, yielded methyl *p*-nitrophenyl hydrogen phosphate. The diester was decolorized in benzene with cellulose and then recrystallized consecutively from benzene, acetonitrile, and chloroform. The final yield was 46.9 g (80.8%) with mp 121–122.5°, lit.[1] mp 123.5–124.5°. The methyl *p*-nitrophenyl hydrogen phosphate (46.96 g) in 700 ml of dry ether was butylated by the dropwise addition of diazobutane (17.7 g in 350 ml of ether[2]) at 0° over 2 hr. The suspended diester completely dissolved during the reaction; the resulting pale yellow solution was washed at room temperature with 0.05 *M* $NaHCO_3$ and water and then dried over Na_2SO_4. The solvent was evaporated, finally at the water pump at 100°; the sample was then purified by molecular distillation at 150°, 10^{-4}–10^{-5} Torr, being collected as a pale yellow oil, yield 48.5 g (83.3%). A middle fraction of the distillate released 96.1% of the expected quantity of *p*-nitrophenol on alkaline hydrolysis and contained 3.3% by weight of free *p*-nitrophenol, which was eliminated by passing a $CHCl_3$ solution through a column of activated alumina (Woelm). The ultraviolet spectrum has $\lambda_{max}^{CH_3CN}$ at 272.5 (ε 9650) and 212.2 (ε 8120). The nmr spectrum ($CDCl_3$) contains peaks at τ 9.07 (triplet, 3 H), 8.59 (multiplet, 2 H), 8.33 (multiplet, 2 H), 6.11 (doublet, 3 H), 5.80 (quadruplet, 2 H), and 2.17 (aromatic quadruplet, 4 H). *Anal.* Calcd for $C_{11}H_{16}O_6NP$: C, 45.68; H, 5.58; N, 4.84; P, 10.71. Found: C, 45.83; H, 5.68; N, 4.48; P, 10.8.

In view of the reported occurrence of an enzyme in mammalian serum which hydrolyzes phosphotriesters,[3] we studied the hydrolysis of MBNP catalyzed by horse serum, as follows. Two stoppered spectrophotometer cells were filled with Tris–HCl buffer (pH 8.08, 0.098 *M*, 3.05 ml) containing CH_3CN (2.6% v/v) and serum (6.6% v/v) and in the test cell MBNP (0.20 m*M*); these were equilibrated at 38°. Release of *p*-nitrophenol from the triester was followed by periodic measurements of absorbance at 400 nm in a Cary 14 spectrophotometer. As indicated in Figure 1, an initial fast reaction released 51.6% of the total *p*-nitrophenol, and this was followed by a much slower, apparently zero order, increase in absorbance. Addition of fresh triester solution to the test cell after the initial fast release of *p*-nitrophenol resulted in a renewed vigorous release of *p*-nitrophenol, showing that the component of horse serum responsible for the hydrolysis had not been irreversibly denatured or inhibited by the products of hydrolysis. This result supported our expectation that the triester would exist in two enantiomeric forms and indicated that horse serum contained an enzyme capable of stereoselectively catalyzing hydrolysis of one of the enantiomers.

Beef serum was shown to contain a similar stereoselective phosphotriesterase and was used to prepare small quantities of optically active MBNP. Typically, two flasks containing Tris–HCl buffer (pH 8.0, 0.10 *M*, 200 ml), $CaCl_2$ (50 m*M*), EDTA (0.5 m*M*), CH_3CN (4% v/v), beef serum (12.5% v/v), and in the test flask MBNP (50 mg) were incubated at 38°; the serum had previously been passed through a Sephadex G-25 column to remove free lipids. The reaction flasks were stirred, and their A_{400} checked periodically to follow the release of *p*-nitrophenol. Once this had reached the slow stage, CH_3CN (22.5 ml) was added to each flask, followed by concentrated $HClO_4$ (7.0 ml). The mixtures were then cooled to 0° and centrifuged to remove precipitated protein and the supernatants adjusted to pH 8.5 by addition of 5 *M* NaOH and then extracted each with 5 × 30 ml of cyclohexane. The extracts were evaporated after drying (Na_2SO_4) and redissolved in identical volumes of CH_3CN. Of the MBNP remaining when $HClO_4$ was added to the hydrolysis flask, approximately 69% was recovered in the CH_3CN solution. Two independently prepared test solutions contained MBNP with $[\alpha]^{25}D$ +8.48 ± 0.05° (*c* 0.278 and 0.716, acetonitrile). The concentration of MBNP in these solutions was assayed by alkaline hydrolysis. The control solution showed no optical activity.

The optical purity of MBNP solutions was assayed

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using beef serum phosphotriesterase to hydrolyze any residual (−)-MBNP in an aliquot and following the hydrolysis at 400 nm. The MBNP (50–70 μg) was equilibrated in Tris–HCl buffer (3.10 ml) containing $CaCl_2$, EDTA, and CH_3CN (1.61% v/v), with partially purified phosphotriesterase. Residual (−)-MBNP was hydrolyzed in a pseudo-first-order reaction, concurrent with slow, apparently zero-order, hydrolysis of the (+)-MBNP by the enzyme. Adding a trace of racemic MBNP (1 μg) to such a reaction mixture already containing the optically active MBNP confirmed that the first-order reaction was hydrolysis of (−)-MBNP. In this case, a first-order increase in A_{400}, corresponding to hydrolysis of one-half of the added (±)-MBNP, occurred, with a rate constant the same as that for the reaction of the enzyme with a sample of optically active MBNP. These assays showed that one of the preparations of (+)-MBNP described above contained 0.50% (−)-MBNP and the other contained 0.04%.

At present we are studying the stereospecific inhibition of some serine hydrolases by MBNP and the stereochemistry of this and other asymmetric phosphotriesters.

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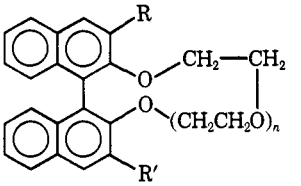
Complete Optical Resolution by Differential Complexation in Solution between a Chiral Cyclic Polyether and an α-Amino Acid[1]

Sir:

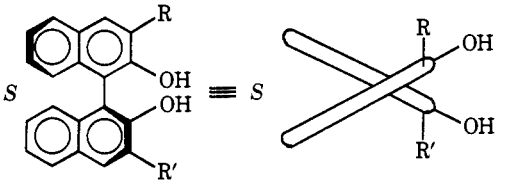
Complexation–decomplexation reactions between organic entities are necessary stages in enzyme-catalyzed reactions. At the active site, the host and guest parts of the complex are highly ordered. Studies of structured molecular complexes[2] between organic compounds in solution not involving enzymes have centered on cyclodextrins as host molecules[2a–f] and on catalysis or inhibition of reaction rates through complexation.[2a–h] Most optical resolutions involve differences in formation rates or in energies of crystal lattices.[2m–o,s,t,z] Others involve solid–liquid[2n–r,v] or gas–liquid chromatography[2n–r,v] or dialysis.[2w] Racemate distribution between water and optically active liquids[2u,x] gave a maximum optical purity of 2 ± 0.9%.[2u] One full resolution by countercurrent extraction has appeared.[2y]

We report the first optical resolution of a racemate by differential complexation in liquid–liquid chromatography to give optically pure enantiomers. The host molecule was designed for configurational differentiation in complexation in solution, and the relative configurations of the more stable diastereomeric complexes were predicted from examination of molecular models in advance of experiment.

The previous paper reported racemates **1–5** and the facts that acids **1**, **2**, and **4** complexed valine better than acid **3** or ester **5**.[3] Optically pure acids (*S*)-**1**,[4a,b] (*S*)-**3**,[4a,b] and (*R*)-**4**[4a,b] now have been prepared (comparable yields)[3] from optically pure (*S*)- or (*R*)-**6**.[5] Reduction of (*S*)-**6** and (*R*)-**6** gave (*S*)-**7**[4a,b] (79%) and (*R*)-**7** (77%),[4a,b] respectively. Diazomethane and (*R*)-**6** gave (76%) the dimethyl ester (*R*)-**8**[4a,b] mp 243–245°,



1, $n = 4$; R = R′ = $CH_2OCH_2CO_2H$
2, $n = 4$; R = H; R′ = $CH_2OCH_2CO_2H$
3, $n = 3$; R = R′ = $CH_2OCH_2CO_2H$
4, $n = 5$; R = R′ = $CH_2OCH_2CO_2H$
5, $n = 4$; R = R′ = $CH_2OCH_2CO_2CH_3$



	R	mp°C	$[\alpha]^{25}_{578}$	(*c* 1.1)
(*S*)-**6**	CO_2H	> 285	−198°	$(CH)_5N$
(*R*)-**6**	CO_2H	> 285	+195°	$(CH)_5N$
(*S*)-**7**	CH_2OH	190–193	−63.5°	$(CH_2)_4O$
(*R*)-**7**	CH_2OH	192–195	+64.1°	$(CH_2)_4O$

$[\alpha]^{25}D$ +172°,[4d] reported[6a] mp 239–240°, $[\alpha]^{25}D$ +159°.[4d] Reduction of (*R*)-**8** gave (*R*)-**7** (63%), mp 195–196°, $[\alpha]^{25}_{578}$ +63.8°.[4d] The absolute configura-

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