**Table II. Chlorides from Alcohols Using PPTC**

$$ROH \xrightarrow[CHCl_3]{PhPCl_4} RCl$$

| R | conditions | % yield |
|---|---|---|
| *n*-hexyl | 25 °C, 6 h | 77 |
| benzyl | 25 °C, 6 h | 100 |
| *s*-pentyl | 50 °C, 12 h | 80 |
| *i*-pentyl | 25 °C, 12 h | 44 |
| neopentyl | 25 °C, 12 h | 55 |
| (R)-(−)-2-octyl | 25 °C, 5 min | 85 (94% ee inversion) |
| *tert*-butyl | 50 °C, 12 h | 97 |
| norbornyl | 25 °C, 12 h | 60 |
| cyclopropylmethyl | 0 °C, 5 min | 98 |

distilled to give the desired product in good yield (Table II).

With this discovery, we have broadened the scope of reactivity of PPTC. We feel that continued studies on PPTC itself and other similar organophosphorus compounds could potentially contribute a variety of new synthetic methodologies to the organic chemist.

## Experimental Section

All products were identified by comparison to authentic samples. A typical procedure is given for the preparations of the aryl chlorides listed in Table I. The chlorides listed in Table II were prepared in the same manner using the conditions specified in Table II.

**General Procedure.** Chlorine gas (10 g, 142 mM) is bubbled into phenylphosphorus dichloride (18.6 mL, 142 mM) at such a rate as to maintain the reaction temperature at 70–80 °C. After the chlorine addition is complete, the resulting molten PPTC is a clear yellow liquid. Phenol (13.4 g, 142 mM) is added on one portion, and the reaction mixture is heated to 160 °C overnight. The cooled reaction mixture is then poured onto crushed ice/water (200 mL) and neutralized with 50% aqueous sodium hydroxide. After extraction with ether, the combined ether extracts are dried and distilled to give chlorobenzene (12.1 g, 76%) as a colorless liquid.

**Registry No.** PPTC, 4895-65-2; $PhPCl_2$, 644-97-3; PhOH, 108-95-2; *p*-$BrC_6H_4OH$, 106-41-2; *m*-$HOC_6H_4OMe$, 150-19-6; *p*-$HOC_6H_4F$, 371-41-5; *m*-$HOC_6H_4Me$, 108-39-4; $Me(CH_2)_5OH$, 111-27-3; $PhCH_2OH$, 100-51-6; $Me(CH_2)_2CH(Me)OH$, 6032-29-7; $HOCH_2CH_2CHMe_2$, 123-51-3; $HOCH_2C(Me)_3$, 75-84-3; (*R*)-$MeCH(OH)(CH_2)_5Me$, 5978-70-1; t-BuOH, 75-65-0; PhCl, 108-90-7; *p*-$ClC_6H_4Br$, 106-39-8; *m*-$ClC_6H_4OMe$, 2845-89-8; *p*-$ClC_6H_4F$, 352-33-0; *m*-$ClC_6H_4Me$, 108-41-8; $Me(CH_2)_5Cl$, 544-10-5; $PhCH_2Cl$, 100-44-7; $MeCH_2CH_2CH(Me)Cl$, 29593-35-9; $ClCH_2CH_2CHMe_2$, 107-84-6; $ClCH_2C(Me)_3$, 753-89-9; (*R*)-$Me(CH_2)_5CH(Cl)Me$, 18651-57-5; t-BuCl, 507-20-0; *m*-$HOC_6H_4Cl$, 108-43-0; *m*-$HOC_6H_4CF_3$, 98-17-9; *m*-$ClC_6H_4Cl$, 541-73-1; *m*-$ClC_6H_4CF_3$, 98-15-7; 2,4-dichorophenol, 120-83-2; α-naphthol, 90-15-3; bicyclo[2.2.1]heptan-1-ol, 51566-98-4; cyclopropylmethyl alcohol, 2516-33-8; 1,2,4-trichlorobenzene, 120-82-1; 1-chloronaphthalene, 90-13-1; 1-chlorobicyclo[2.2.1]heptane, 765-67-3; cyclopropylmethyl chloride, 5911-08-0.

---

# Precursors to Carbon-13-Labeled Reactive Intermediates: Preparation and NMR Characterization of Two Double Label Isomers of Methyl Cyclopropene-3-carboxylate

P. C. Myhre,* Claudia Tata Maxey, Deborah C. Bebout, Stephen H. Swedberg, and Barry L. Petersen

*Department of Chemistry, Harvey Mudd College, Claremont, California 91711*

*Received November 29, 1989*

Bond distances can be determined with about 1% accuracy in orientationally disordered solids by use of a recently developed nutation NMR technique.[1] This method has provided important structural data in several systems,[2] and it seems particularly promising as a means of determining bond lengths and local geometries in reactive intermediates, substances which are difficult to prepare as X-ray quality single crystals.

Reactive carbocation salts represent a class of reactive intermediates that have been resistant to direct structural study. An enormous amount of structural and dynamic information about carbocations has been deduced from NMR studies, but there is very little *direct* information available concerning bonding distances and geometries of these reactive intermediates. This is not from lack of effort. Major programs have been mounted in several laboratories to do diffraction studies on reactive carbocation salts, but only a few structures have been solved successfully.[3a,4] The kinds of problems encountered are noted in some published reports.[3]

These diffraction studies are major achievements that have provided important data. However, a critical evaluation indicates that all had rather special features: strategically placed methyl group substituents,[3a,4a] donor heteroatoms,[4b,5] or extended π-systems.[4a,5] These special features tend to minimize crystal lattice disorder problems, problems which appear repeatedly in unsuccessful X-ray studies of carbocation salts.

While not without difficulties, nutation NMR appeared to be a more generally applicable method of gaining direct structural data about reactive intermediates. We have initiated a program that makes use of this technique to study some carbocations of particular interest. Nutation NMR involves the use of a pulse train that efficiently suppresses chemical shift information while retaining the dipolar coupling information.[1] After Fourier transformation, this dipolar coupling information, extracted from an amorphous or polycrystalline solid, is presented in the form of a Pake doublet.[6] Since the magnitude of a dipolar coupling constant is inversely proportional to the cube of the internuclear separation, distances between proximate atoms and localized geometries may be determined in a fairly direct manner.[1,2e] The application of this method to a carbon compound requires that the reactive intermediate be labeled with carbon-13 at *two* specific sites. Further, those sites in the labeled intermediate should be enriched to the 99% level in order to minimize the number of monolabeled molecules in the system.[7] Unlike diffraction methods, a "complete" structure determination can only be projected for the most simple or symmetrical carbocations.[2e] However, key features of the carbon framework can be determined by judicious synthetic design.

(1) Yannoni, C. S.; Kendrick, R. D. *J. Chem. Phys.* **1981**, *74*, 747. (b) Horne, D.; Kendrick, R. D.; Yannoni, C. S. *J. Magn. Reson.* **1983**, *52*, 299.

(2) (a) Yannoni, C. S.; Clarke, T. C. *Phys. Rev. Lett.* **1983**, *51*, 1191. (b) Clarke, T. C.; Yannoni, C. S.; Katz, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 7787. (c) Katz, T. J.; Hacker, S. M.; Kendrick, R. D.; Yannoni, C. S. *J. Am. Chem. Soc.* **1985**, *107*, 2182. (d) Yannoni, N.; Kahr, B.; Mislow, K. *J. Am. Chem. Soc.* **1988**, *110*, 6670. (e) Yannoni, C. S.; Kendrick, R.; Myhre, P. C.; Bebout, D. C.; Petersen, B. L. *J. Am. Chem. Soc.* **1989**, *111*, 6440.

(3) (a) Laube, T. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 560. (b) Dinnocenzo, J. P. Ph.D. Thesis, Cornell University, 1985.

(4) (a) Childs, R. F.; Faggiani, R.; Lock, C. J. L.; Mahendran, M. *J. Am. Chem. Soc.* **1986**, *108*, 3613–3617. (b) Montgomery, L. K.; Grendze, M. P.; Huffman, J. C. *J. Am. Chem. Soc.* **1987**, *109*, 4749–4750. (c) Laube, T. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 349. (d) Laube, T. *J. Am. Chem. Soc.* **1989**, *111*, 9224.

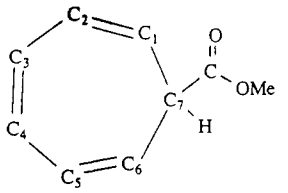
(5) For a summary of earlier X-ray diffraction studies on stabilized carbocations, see: Ku, A. T.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 1688.

(6) Pake, G. E. *J. Chem. Phys.* **1948**, *16*, 327.

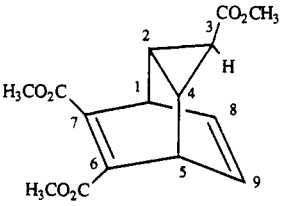
(7) The nutation sequence gathers the intensity of all monolabled species as line centered within the Pake doublet.

0022-3263/90/1955-3417$02.50/0 

**Table I. NMR Characterization of Methyl Cycloheptatriene-7-carboxylate and Trimethyl Tricyclo[3.2.2.0$^{2,4}$]-6,8-nonadiene-3,6,7-tricarboxylate**



| | | | coupling constants, Hz | | | | | |
|---|---|---|---|---|---|---|---|---|
| no. | $\delta^C$ | | C2 | C3 | C4 | C5 | C6 | C7 |
| 1 | 116.8 | C1 | 68.0 | −2.5 | 0.7 | 0.1 | −3.1 | 38.3 |
| 2 | 125.6 | C2 | | 54.0 | −1.3 | 2.6 | 0.1 | 5.1 |
| 3 | 130.9 | C3 | | | 57.4 | −1.3 | 0.7 | 1.8 |
| 4 | 130.9 | C4 | | | | 54.0 | −2.5 | 1.8 |
| 5 | 125.6 | C5 | | | | | 68.0 | 5.1 |
| 6 | 116.8 | C6 | | | | | | 38.3 |
| 7 | 43.8 | | | | | | | |



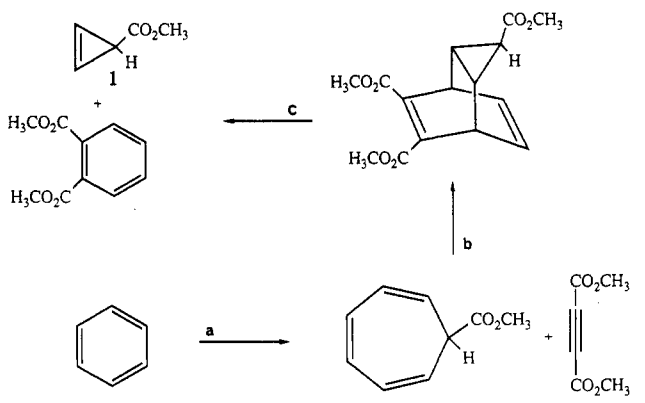
| | | | coupling constants, Hz | | | | |
|---|---|---|---|---|---|---|---|
| no. | $\delta^C$ | | C2 | C4 | C5 | C9 | C8 |
| 1 | 40.1 | C1 | 35.3 | −2.7 | 11.4 | −2.4 | 38.5 |
| 2 | 26.8 | C2 | | 19.7 | −2.7 | 3.0 | −3.1 |
| 4 | 26.8 | C4 | | | 35.4 | −3.1 | 3.0 |
| 5 | 40.1 | C5 | | | | 38.5 | −2.4 |
| 8 | 130.8 | C9 | | | | | 69.8 |
| 9 | 130.8 | | | ($^1J_{C2C3}$ = 12.8 Hz) | | | |

The synthetic effort required to prepare appropriately labeled reactive intermediates meeting the constraints noted can be minimized by identifying paths to doubly labeled precursors that serve as synthetic branch points. Such precursors can then be transformed to a number reactive intermediates for structural study. One such precursor is methyl cyclopropene-3-carboxylate (1), a compound that can be easily converted to the cyclopropenyl cation, the cyclopropylcarbinyl cation, and a number of other reactive intermediates. We record here details of our synthetic procedures for the preparation of two double label isomers of 1. We also report spectral data that serve to characterize this versatile precursor.

## Results and Discussion

The general pathway to 1 used in these syntheses (Scheme I) was identified by Doering.[8] The cycloaddition of dimethyl acetylenedicarboxylate with cycloheptatriene derivatives was explored by Alder,[9] and the stereochemistry of the cycloaddition was investigated in greater detail by Goldstein.[10] Subsequently, others have used this methodology to prepare unlabeled samples of 1.[11,12] However, experimental details concerning the retro-

**Scheme I**[a]



[a] (a) $N_2CHCO_2CH_3/Rh_2(O_2CCF_3)_4$; (b) $CCl_4$, 98 °C; (c) 410 ± 20 °C, $10^{-2}$ Torr.

Diels–Alder reaction, a key step in the pathway, are very sketchy, and some reports indicate a number of uncharacterized byproducts.[11a] Further, there is very little published data that aids characterization of methyl cyclopropene-3-carboxylate. Nevertheless, the continued development of this approach appeared attractive, particularly since use of commercially available glycine-*1,2*-$^{13}C_2$ or benzene-*u*-$^{13}C_6$ as starting materials would yield methyl cyclopropene-3-carboxylate-*3,carboxyl*-$^{13}C_2$ or methyl cyclopropene-3-carboxylate-*1,2*-$^{13}C_2$.

The use of hexalabeled benzene does introduce a complication. The expense of this material dictates that it be a limiting reagent. However, efficient ring enlargement procedures require that benzene be used in excess. A simple technique for conducting this ring enlargement step

(8) Doering, W. von E.; Laber, G.; Vonderwahl, R.; Chamberlin, N. F.; Williams, R. B. *J. Am. Chem. Soc.* **1956**, *78*, 5448.
(9) Alder K.; Jungen, H.; Rust, K. *Ann. Chem.* **1957**, *602*, 94–117.
(10) Goldstein, M. J.; Gervitz, A. H. *Tetrahedron Lett.* **1965**, 4417.
(11) (a) Farnum, D. G.; Mehta. G.; Silberman, R. G. *J. Am. Chem. Soc.* **1967**, *89*, 5048–5049. (b) McMullen, G.; Sedelmeir, G.; Hildebrand, R.; Fritz, H.; Prinzbach, H. *Tetrahedron Lett.* **1979**, 3847–3850. (c) Maier, G.; Hoppe, M.; Reisenauer, H. P.; Kruger, C. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 437.
(12) For an analogous application, see: Staley, S. W.; Norden, T. D.; Su, C-F.; Rall, M.; Harmony, M. D. *J. Am. Chem. Soc.*, **1987**, *109*, 2880.

**Table II. NMR Characterization of Pyrolysis Byproducts[a]**

| | $H-C^4\equiv C^3-CH_2^2-CO_2CH_3^1$ | | $H_2C^4=C^3=CH^2-CO_2CH_3^1$ | |
|---|---|---|---|---|
| no. | $\delta^C$ | $^1J_{CC}$, Hz | $\delta^C$ | $^1J_{CC}$, Hz |
| 1 | 168.5 | 61.0 | 166.5 | 80.2 |
| 2 | 26.0[b] | | 88.2[d] | |
| 3 | 76.2 | 176 | 216.1 | 98.5 |
| 4 | 71.7[c] | | 78.3 | |

[a] Most data were obtained from label isomers of 1 that gave significant amounts (>10%) byproduct. [b] $^1J_{CH}$ = 133 Hz. [c] $^1J_{CH}$ = 176 Hz. [d] $^1J_{CH}$ = 175 Hz.

repetitively was designed and is outlined in the Experimental Section.

Application of the reaction chemistry noted in Scheme I gave appropriately labeled samples of methyl cycloheptatriene-7-carboxylate and trimethyl tricyclo-[3.2.2.$0^{2,4}$]-6,8-nonadiene-3,6,7-tricarboxylate. The proton decoupled $^{13}C$ NMR spectra of the labeled samples could be best fit by the sets of carbon–carbon couplings summarized in Table I.

The retro-Diels–Alder reaction proved to be the most difficult step to optimize. A small vacuum pyrolysis apparatus consisting of a 12-mm quartz tube packed with small pieces of quartz tubing was used. Trial runs were made at temperatures ranging from 275 °C to 550 °C and at a range of pressures. With our apparatus, the optimum conditions appeared to be 390–430 °C at $10^{-2}$ Torr. These conditions minimized the byproducts which formed at higher temperatures and gave acceptable conversions in one pass. Temperature control in our apparatus was not as precise as one would like. However, we were able to reproducably obtain conversions in the 70–85% range with product purity greater that 90% under the conditions noted. In several runs product purity exceeded 97 mol %. It was critical for us to find a set of conditions that mimized volatile byproducts, since we were unable purify contaminated samples of 1 by gas chromatography or liquid chromatography. Samples of 1 were readily separated from less volatile dimethyl phthalate and unconverted reactant by bulb-to-bulb vacuum distillation.

NMR spectra indicated that the major byproducts were methyl 3-butynoate and methyl 2,3-butadienoate. The ratio of these products appeared to vary from about 2:1 when the extent of byproduct formation was low to about 5:1 at higher reactor temperatures, when byproducts represented about 25% of the product mixture. Preparations of label isomers of 1 indicate that the byproducts are formed without rearrangement of the carbon skeleton. Thus, $^{13}C$ NMR spectra of contaminated preparations of 1-*1,2*-$^{13}C_2$ showed the major impurity as an AB quartet ($^1J_{CC}$ = 176 Hz) assignable to C3 and C4 of methyl 3-butynoate. The minor impurity in the spectrum showed as two doublets in the carbon spectrum at 78.3 and 216.1 ppm ($^1J_{CC}$ = 98.5 Hz) assignable to C4 and C3 of methyl 2,3-butadienoate. Complementary data for C2 and C1 of these byproducts were obtained by examination of the spectra of preparations of 1-*3,carboxyl*-$^{13}C_2$. Spectral data are summarized in Table II.

Solution-state NMR spectra of 1 and its label isomers have a certain aesthetic appeal. The molecules are quite simple. With narrow sweep widths and a little patience one can acquire proton-coupled $^{13}C$ NMR spectra that reveal one-, two-, three-, and four-bond proton–carbon couplings clearly. The AA′MXX′ character of the vinyl proton and the vinyl carbon spectra of 1-*1,2*-$^{13}C_2$ is shown in part in Figure 1. The $^1H$ and $^{13}C$ wings of this pattern appear identical owing to nearly equal values of $J_{AM}$ and $J_{MX}$. Analysis of the spectral patterns provides $^1J_{CC}$ and

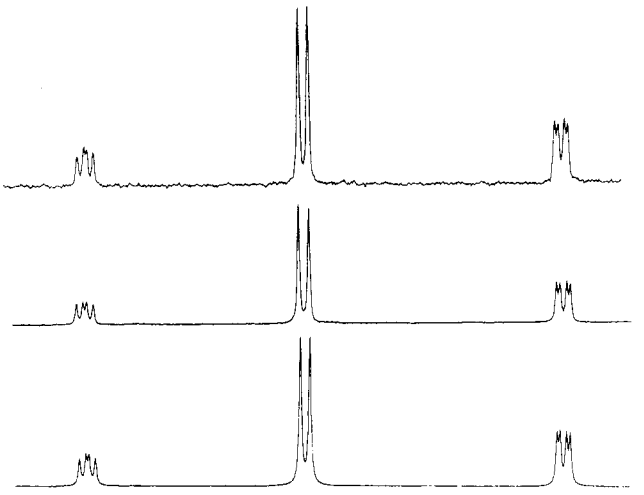
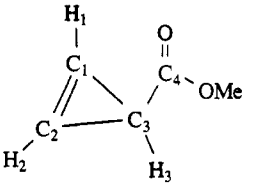


**Figure 1.** $^1H$ and $^{13}C$ NMR spectra of 1-*1,2*-$^{13}C_2$: upper trace, the downfield wing of half of the AA′MXX′ pattern in the $^{13}C$ region; middle trace, downfield wing of the other half of the spectrum in the $^1H$ region; lower trace, calculated spectrum.

**Table III. NMR Characterization of Methyl Cyclopropene-3-carboxylate**




| no. | $\delta^C$ | $\delta^H$ |
|---|---|---|
| 1 | 104.0 | 6.93 |
| 2 | 104.0 | 6.93 |
| 3 | 17.3 | 2.16 |
| 4 | 177.6 | 3.63 |

Coupling Constants, Hz

| | H2 | H3 |
|---|---|---|
| H1 | 0.66 | 1.45 |
| H2 | | 1.45 |

| | C2 | C3 | C4 |
|---|---|---|---|
| C1 | 67.3 | 7.6 | |
| C2 | | 7.6 | |
| C3 | | | 77 |

| | C1 | C2 | C3 | C4 |
|---|---|---|---|---|
| H1 | 239.5 | 7.7 | 1.8 | 1.4 |
| H2 | 7.7 | 239.5 | 1.8 | 1.4 |
| H3 | 1.4 | 1.4 | 178.5 | 9.25 |

$^3J_{HH}$ for the vinyl carbons and vinyl protons. The other spectral patterns are first-order. The proton–proton, proton–carbon, and carbon–carbon coupling constant data for 1 are summarized in Table III.

The carbomethoxy group must perturb the magnitudes of some of the scalar couplings. We note, however, that with the use of the relationships connecting the one-bond carbon–carbon and proton–carbon coupling constants to carbon atom hybridizations,

$$^1J_{CC} = 550(s_{Ci})(s_{Cj})$$

$$(s_C) = {}^1J_{CH}/500$$

together with the usual boundary conditions, one finds the C–H bonds at C1 and C2 are approximately sp hybrids ($sp^{1.1}$), and the C1, C2 double bond is nearly $sp^2$ ($sp^{1.9}$). This leaves approximately $sp^5$ ($sp^{4.8}$) orbitals on C1 and C2 to bond with C3. Extention of this analysis to C3 with

the assumption that the carboxyl carbon is $sp^2$ yields a predicted $^1J_{C1(2)C3}$ of 11 Hz. This may be compared with the observed value of 7.6 Hz. This simple estimate does not account for the possible additive effects of a positively signed one-bond coupling and a negatively signed two-bond coupling between atoms in question.[13]

Preliminary nutation NMR studies of 1-*1,2*-$^{13}C_2$ shows a Pake doublet with a peak separation that indicates a C1–C2 bond length of 1.31 (2) Å. Microwave studies indicated the C1–C2 bond length to be 1.296 Å in cyclopropene,[14] 1.292 Å in 3-cyanocyclopropene,[12] and 1.321 Å in 3,3-difluorocyclopropene.[15] Preliminary nutation studies of 1-*1,carboxyl*-$^{13}C_2$ indicate that the exocyclic single bond length is 1.51 (2) Å.

We conclude that 1 can be made in acceptable yield and purity by the procedure outlined. Careful control of pyrolysis conditions minimizes the formation of byproducts. Byproducts can be rationalized by the pathways discussed in earlier studies by Bergman[16] and others.[17] Methyl cyclopropene-3-carboxylate proves to be relatively reactive at room temperature. NMR studies on an undiluted sample indicate a half-life of the order of 1–2 h at room temperature. However, 1 may be conveniently stored and handled as a dilute solution in organic solvents.

Future reports will provide accounts of the use of the label isomers of 1 as precursors to appropriately labeled samples of reactive intermediates of particular interest for nutation NMR studies.

## Experimental Section

**General.** Melting points were recorded on a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded in $DCCl_3$ (unless noted otherwise) on an IBM/Bruker AF 200 NMR spectrometer. Spectral simulations and iterative fits of observed and calculated spectra were obtained with the use of the program PANIC available in the IBM/Bruker software package. Chromatographic separations were accomplished with the use of a Harrison Research Model 7924T chromatotron equipped with glass disks coated with Merck silica gel PF-240 containing calcium sulfate hemihydrate. The syntheses of both labeled and unlabeled samples of glycine methyl ester hydrochloride, methyl diazoacetate, methyl cycloheptatriene-7-carboxylate, and trimethyl tricyclo[3.2.2.0$^{2,4}$]-6,8-nonadiene-3,6,7-tricarboxylate were adapted from literature procedures.[18-20] The modifications appropriate for synthesis of the labeled materials are recorded in this Experimental Section.

**Glycine-*1,2*-$^{13}C_2$ Methyl Ester Hydrochloride.** A suspension of glycine-*1,2*-$^{13}C_2$ (1.02 g, 13.6 mmol, Aldrich 99.5 atom %) and methanol (10 mL) was saturated with hydrogen chloride gas, and the stirred mixture was allowed to react for 24 h under argon at room temperature. Evaporation of the solvents under vacuum gave the colorless, crystalline ester (1.65 g, 98%). $^1$H NMR ($D_2O$): $\delta$ 3.65 (d, $^3J_{CH}$ = 5 Hz, 3 H), 3.71 (dd, $^1J_{CH}$ = 145 Hz, $^2J_{CH}$ = 6 Hz, 2 H). $^{13}$C NMR ($D_2O$): $\delta$ 43.7 (d, $^1J_{CC}$ = 62.6 Hz), 171.4 (d).

**Methyl Diazoacetate-*1,2*-$^{13}C_2$.** A 25-mL pear-shaped flask equipped with a mechanical stirrer, thermometer, and septum port was charged with glycine-*1,2*-$^{13}C_2$ methyl ester hydrochloride (1.64 g, 13.1 mmol), and 3 mL of water. The flask was protected from incident light by an aluminum foil wrapping. Methylene chloride (6.8 mL) and aqueous sodium nitrite (2.8 mL, 5.0 M) were added to the flask, and the stirred contents were cooled to −10 °C. An aqueous solution of sulfuric acid (1.1 mL, 5 wt %) was added via syringe at a rate that maintained the temperature below −8 °C. The mixture was allowed to stir for 20 min, and the layers were separated in a cold separatory funnel. The methylene chloride layer was run into a cold 30-mL flask containing sodium bicarbonate solution, and the aqueous phase was counterextracted with four 1-mL portions of methylene chloride. The combined methylene chloride layers were shaken with aqueous sodium bicarbonate until neutral and dried over sodium sulfate. Evaporation of the filtered methylene chloride solution under vacuum at room temperature gave 1.14 g (87.4%) of methyl diazoacetate. $^1$H NMR: $\delta$ 3.75 (d, $^3J_{CH}$ = 4 Hz, 3 H), 4.77 (dd, $^1J_{CH}$ = 205 Hz, $^2J_{CH}$ = 3 Hz, 2 H). $^{13}$C NMR: $\delta$ 45.9 (d, $^1J_{CC}$ = 97.0 Hz), 167.1 (d).

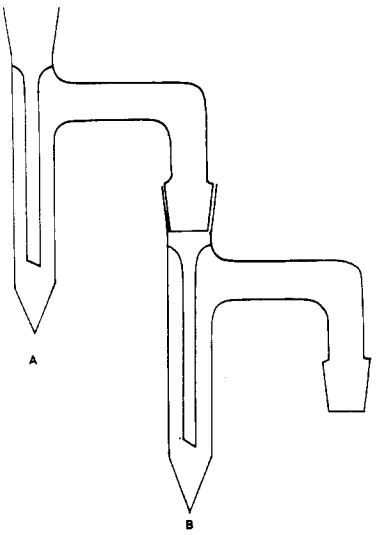



**Figure 2.** Reactor/trap assembly used in ring enlargements of hexalabeled benzene. During reaction, trap A contains benzene and rhodium catalyst. Methyl diazoacetate is added through a septum to the stirred solution. Entrained vapor is collected in trap B. The positions of the traps are reversed during isolation, and the unconverted benzene is bulb-to-bulb distilled from B to A under reduced pressure.

**Methyl Cycloheptatriene-7-carboxylate-*7,carboxyl*-$^{13}C_2$.** Dropwise addition (micro syringe) of methyl diazoacetate-*1,2*-$^{13}C_2$ (0.42 g, 4.1 mmol) to a mixture of benzene (9 mL, 0.1 mol) and rhodium trifluoroacetate (23 mg) followed by a 2-day reaction period at room temperature under argon afforded 0.55 g (88.5%) of crude, doubly labeled cycloheptatriene derivative. $^1$H NMR: $\delta$ 2.55 (dm, $^1J_{CH}$ = 134 Hz, 1 H), 3.79 (d, $^3J_{CH}$ = 4 Hz, 3 H), 5.43 (m, 2 H), 6.25 (m, 2 *H*), 6.85 (m, 2 H). $^{13}$C NMR: $\delta$ 43.8 (d, $^1J_{CC}$ = 62 Hz), 173.4 (d).

**Methyl Cycloheptatriene-7-carboxylate-*1,2,3,4,5,6*-$^{13}C_6$.** This ring enlargement is normally conducted with a large excess of aromatic.[20] Since hexalabeled benzene is the limiting reagent, a method for partial conversion and subsequent recycling of the unconverted benzene was designed and tested with unlabeled benzene. The reaction apparatus consisted of two traps fabricated from 15-mL centrifuge tubes equipped with standard taper 14/20 joints as shown in Figure 2. When used in the manner shown, the unconverted benzene could be collected in the collection trap, and in the next cycle this trap became the reaction trap. The product obtained from each cycle was carefully transferred from the reaction trap with the use of methylene-$d_2$ chloride.

The reactor/trap assembly was charged with benzene-*1,2,3,4,5,6*-$^{13}C$ (1.005 g, 12.0 mmol, Cambridge Isotope Laboratories, 99 atom %) and rhodium trifluoroacetate (15 mg). Methyl diazoacetate (0.25 g, 2.5 mmol) was added dropwise (microsyringe) to the ice-cooled, magnetically stirred mixture over a 90-min period. After the addition, an additional 3–5 mg of rhodium catalyst was added, and the reaction was allowed to proceed for

(13) Hansen, P. E. *Annu. Rep. NMR Spectrosc.* **1981**, *11A*, 65.
(14) Stigliani, W. M.; Laurie, V. W.; Li, J. C. *J. Chem. Phys.* **1975**, *62*, 1890.
(15) Ramaprasad, K. R.; Laurie, V. W.; Craig, N. C. *J. Chem. Phys.* **1976**, *64*, 4832.
(16) York, E. J.; Dittmar, W.; Stevenson, J. R.; Bergman, R. J. *J. Am. Chem. Soc.* **1973**, *95*, 5680.
(17) (a) Pincock, J. A.; Boyd, R. J. *Can. J. Chem.* **1977**, *55*, 2482. (b) Davis, J. H.; Goddard, W. A.; Bergman, R. J. *J. Am. Chem. Soc.* **1976**, *98*, 4015. (c) Yoshimine, M.; Pacansky, J.; Honjou, N. *J. Am. Chem. Soc.* **1989**, *111*, 2785 and references therein.
(18) Borders, C. L., Jr.; Blech, D. M.; McElvany, K. D. *J. Chem. Ed.* **1984**, *61*, 814–815.
(19) Searle, N. E. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 424.
(20) Anciaux, A. J.; Demonceau, A.; Noels, A. F.; Hubert, A. J.; Warin, R.; Teyssie, P. *J. Org. Chem.* **1981**, *46*, 873–876.

an additional 90 min. The reactor/trap assembly was then arranged in the evaporation configuration and attached to a vacuum manifold. After three freeze-thaw cycles, the volatiles were bulb-to-bulb distilled into the collection trap with magnetic stirring of the product material in the reaction trap. The residue in the reaction trap was carefully removed, and the procedure was repeated with proportionately less methyl diazoacetate. At the end of five such cycles, a total of 1.83 g of material containing the desired product was in hand. This material was subjected to rotary evaporation with trapping of the volatiles. The trapped volatiles were pooled with the unconverted hexalabled benzene for recovery. The crude product was filtered through a 1 × 8 cm column of Bio-Sil A, 100-200 mesh, with 1:1 pentane-methylene chloride elution. Evaporation gave 1.229 g of material. This material was subjected to distillation in a Kugelrohr oven (100-115 °C at 2 Torr) to yield two fractions, 0.668 g and 0.309 g. NMR indicated that the first fraction contained 90% and the second fraction 25% of the desired methyl cycloheptatriene-7-carboxylate-*1,2,3,4,5,6*-$^{13}C$, 0.68 g (72% yield based on recovered benzene).[21] A second preparation conducted with 2.13 g of hexalabeled benzene and 1.50 g (15.0 mmol) of methyl diazoacetate in six cycles afforded 1.34 g (8.6 mmol) of labeled methyl cycloheptatriene-7-carboxylate (70% based on recovered benzene). $^1H$ NMR: $\delta$ 2.56 (m, 1 H), 3.79 (s, 3 H) 5.45 (dm, 2 H), 6.26 (dm, 2 H), 6.85 (dm, 2 H). $^{13}C$ NMR: $\delta$ 116.9 (d), 125.6 (m), 130.6 (m).

**Trimethyl Tricyclo[3.2.2.0$^{2,4}$]-6,8-nonadiene-3,6,7-tricarboxylate-*3,carboxyl*-$^{13}C_2$.** A 5-mm NMR tube was charged with a carbon tetrachloride (0.611 g) solution of distilled methyl cycloheptatriene-7-carboxylate-7,*carboxyl*-$^{13}C_2$ (0.669 g, 4.45 mmol) and freshly distilled dimethyl acetylenedicarboxylate (1.11 g 7.83 mmol).[22] The tube was cooled in dry ice and sealed with a torch, and an initial NMR spectrum was recorded. The tube was then inserted into a $^3/_8$ in. stainless steel tube closed on one end, and this assembly was heated at 98 °C in a vertical Kugelrohr oven for 24 h. A spectrum recorded at this time indicated complete conversion to products. The contents were transferred to a 10-mL flask, and the volatiles were removed by rotary evaporation followed by Kugelrohr distillation at 110-115 °C at 2 Torr to remove the excess dimethyl acetylenedicarboxylate. The amber residue (1.28 g, 99%) solidified, mp 60-65 °C. Recrystallization from ether-pentane gave a sample with mp 75-77 °C (lit. mp 76 °C, 74-75 °C, 48-50 °C).[8-10] $^1H$ NMR: $\delta$ 1.76 (m, $^1J_{CH}$ = 171 Hz, 1 H), 2.06 (m, 2 H), 3.61 (d, $^3J_{CH}$ = 4 Hz), 3.78 (s, 6 H), 4.23 (m, 2 H), 6.15 (m, 2 H). $^{13}C$ NMR: $\delta$ 30.1 (d, $^1J_{CC}$ = 74 Hz), 171.1 (d).

**Trimethyl Tricyclo[3.2.2.0$^{2,4}$]-6,8-nonadiene-3,6,7-tricarboxylate-*1,2,4,5,8,9*-$^{13}C_6$.** The procedure described for the doubly labeled cycloheptatriene derivative was used. A mixture of carbon tetrachloride (0.426 g), hexalabeled methyl cycloheptatriene-7-carboxylate (0.68 g, 4.3 mmol), and freshly distilled dimethyl acetylenedicarboxylate (1.044 g, 7.35 mmol) afforded 1.04 g, 81%, of the Diels-Alder adduct, mp 74-75 °C, after recrystallization from pentane-ether. $^1H$ NMr: $\delta$ 1.77 (t, 1 H), 2.07 (dm, $^1J_{CH}$ = 177 Hz, 2 H), 3.62 (s, 3 H), 3.79 (s, 6 H), 4.23 (dm, $^1J_{CH}$ = 147 Hz), 6.15 (dm, $^1J_{CH}$ = 176 Hz). $^{13}C$ NMR: $\delta$ 26.8 (m), 40.1 (m), 130.8 (m).

**Methyl Cyclopropene-3-carboxylate-*3,carboxyl*-$^{13}C_2$.** A number of trial vacuum pyrolyses with unlabeled Diels-Alder adduct indicated that the cycloreversion reaction was best conducted in our apparatus at 410 ± 20 °C.[23] A packed quartz pyrolysis tube (300 × 15 mm) equipped with standard taper 14/20 joints and two traps fabricated from 15-mL centrifuge tubes were attached to a vacuum manifold. The pyrolysis tube was heated with two 100-mm combustion tube heaters, and the thermocouple monitor was mounted at the point of contact between the two heaters. Heating tape was used to heat the exposed portion of the pyrolysis tube and trap side arm to minimize condensation of pyrolysate. The system was thoroughly baked out under vacuum and brought to atmospheric pressure with dry helium. A 5-mL flask containing the doubly labeled Diels-Alder adduct (0.520 g, 1.73 mmol) topped with a small plug of oven-dried glass wool was attached to the pyrolysis tube, and the system was evacuated to $10^{-2}$ Torr. With the tube heaters at temperature (390-430 °C through regions of the pyrolysis tube) and the traps cooled with liquid nitrogen, the flask was heated to 275 °C with a small heating mantle. After a 1-h period all the material had passed through the pyrolysis column. When the system had cooled, the apparatus was brought to atmospheric pressure with helium. The pyrolysis tube was removed, and the inlet to the first trap was sealed with a small flask. The first trap was then warmed to room temperature, and the methyl cyclopropane-3-carboxylate was transferred to the liquid nitrogen cooled second trap by bulb-to-bulb distillation at $10^{-2}$ Torr. Magnetic stirring of the sample in the first trap facilitated this transfer. In this manner, 82 mg of methyl cyclopropene-3-carboxylate-*3,carboxyl*-$^{13}C_2$ (49%, 73.5% based on recovered starting material) was isolated. A second pyrolysis of 0.500 g of the labeled Diels-Alder adduct afforded 0.137 g (81.5%) in one pass. $^1H$ NMR ($CD_2Cl_2$): $\delta$ 2.17 (ddt, $^1J_{CH}$ = 178 Hz, $^2J_{CH}$ = 9.25 Hz, $^3J_{HH}$ = 1 Hz, 1 H), 3.63 (d, $^3J_{CH}$ = 4 Hz, 3 H), 7.08 (m, 2 H). $^{13}C$ NMR ($CD_2Cl_2$): $\delta$ 17.3 (ddtq, $^1J_{CC}$ = 77 Hz), 177.6 (ddqt).[24]

**Methyl Cyclopropene-3-carboxylate-*1,2*-$^{13}C_2$.** A sample of the hexalabled Diels Alder adduct (0.389 g, 1.30 mmol) was pyrolyzed in the manner previously described to yield 70 mg (72%, based on recovered starting material, purity >97%). $^1H$ NMR ($CCl_2D_2$): $\delta$ 2.17 (m, $^2J_{CH}$ = 1.4 Hz, $^3J_{HH}$ = 1.45, 1 H), 3.65 (s, 3 H), 6.93 (part of AA'MXX' pattern, $^1J_{CH}$ = 239.5 Hz, $^1J_{CC}$ = 67.3 Hz, $^2J_{CH}$ = 7.7 Hz, $^3J_{HH}$ = 1.45 Hz, $^3J_{HH}$ = 0.66 Hz, 2 H). $^{13}C$ NMR ($CCl_2D_2$): $\delta$ 104 (part of AA'MXX' pattern).

**Acknowledgment.** This research was supported by NSF Grant CHE-8519522. We thank Dr. C. S. Yannoni, IBM Almaden Research Center, for the nutation NMR studies reported here in preliminary form. We also thank Julia Freutel and Elizabeth Starr for their work on these syntheses. NSF instrumentation grants CHE-8551436 and CHE-8419266 and grants from the Dreyfus Foundation and Research Corporation provided funding for the 200-MHz NMr spectrometer used in this investigation.

(21) NMR indicated that the contaminants in the fractions were dimethyl maleate and dimethyl fumarate, by-products previously observed in these rhodium catalysed reactions; see reference 20.

(22) Attempts to use unpurified samples of methyl cycloheptatriene-7-carboxylate, which contained the rhodium catalyst, led to the formation of significant quantities of cyclotrimerized dimethyl acetylenedicarboxylate. While this product could be separated by chromatography, it proved to be more efficient to purify the cycloheptatriene derivative before conducting the cycloaddition.

(23) This temperature was determined by direct measurement of the reaction zone of the pyrolysis column under simulated conditions. The external thermocouple monitor used in pyrolysis was calibrated with respect to these measured temperatures.

(24) Proton coupled $^{13}C$ NMR of unlabeled samples of 1 showed: $\delta$ 17.3 (dtq, $^1J_{CH}$ = 178.5 Hz, $^2J_{CH}$ = 1.84 Hz, $^4J_{CH}$ = 0.4 Hz), 104.0 (ddd, $^1J_{CH}$ = 239.5 Hz, $^2J_{CH}$ = 7.7 Hz, $^2J_{CH}$ = 1.4 Hz), 177.6 (dqt, $^2J_{CH}$ = 9.25 Hz, $^3J_{CH}$ = 3.7 Hz, $^3J_{CH}$ = 1.4 Hz).

## Amphidinolide E, a Novel Antileukemic 19-Membered Macrolide from the Cultured Symbiotic Dinoflagellate *Amphidinium* sp.

Jun'ichi Kobayashi,*[1a] Masami Ishibashi,[1b] Tetsuya Murayama,[1c] Masako Takamatsu,[1c] Michiko Iwamura,[1b] Yasushi Ohizumi,[1c] and Takuma Sasaki[1d]

*Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, Faculty of Science, Toho University, Funabashi 274, Japan, Mitsubishi Kasei Institute of Life Sciences, Tokyo 194, Japan, and Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan*

*Received December 14, 1989*

Marine microorganisms are of considerable current interest as new promising sources of bioactive substances,[2] and recently several unique secondary metabolites have

(1) (a) Hokkaido University. (b) Toho University. (c) Mitsubishi Kasei Institute of Life Sciences. (d) Kanazawa University.

(2) Kobayashi, J. *J. Nat. Prod.* **1989**, *52*, 225-238.

0022-3263/90/1955-3421$02.50/0 