Application of a Novel Double-Labeling Procedure to Measurement of Carbonyl Oxygen Isotope Effects on the Alkaline Hydrolysis and Hydrazinolysis of Methyl Benzoate

Sir:

Heavy-atom kinetic isotope effects[1,2] are ordinarily measured by competitive experiments in which the isotopic composition of a mixture of isotopically labeled and unlabeled substrates (or products) is measured as a function of extent of reaction.[3] The isotope ratio mass spectrometer used for such measurements can only be used with volatile substances such as CO, CO_2, N_2, and CH_3Cl, and chemical conversion of the substrate or product is often required prior to analysis. Sometimes the atom of interest is buried deep within the molecule and such conversion is not convenient.

In this paper we illustrate a novel method which may overcome many problems inherent in the competitive method. This technique uses a substrate which is labeled in two positions, the first accessible to analysis by isotope ratio mass spectrometry but sufficiently isolated from the reacting bond(s) that no isotope effect is observed, and the second the site of interest in the kinetic isotope effect experiment. This doubly labeled substrate is mixed with unlabeled substrate and the isotopic composition of the accessible position is monitored as a function of extent of reaction. Since the accessible position has no isotope effect of its own, the variation in isotopic composition at this position reflects the kinetic isotope effect at the position of interest.

By this technique we have measured carbonyl oxygen kinetic isotope effects on the alkaline hydrolysis and hydrazinolysis of methyl benzoate. [*methyl*-13C]Methyl [*carbonyl*-18O]-benzoate, 90% enriched at both positions,[4] was mixed with natural abundance methyl benzoate in the ratio of 1:4. This substrate was subjected to hydrolysis or hydrazinolysis and the 13C/12C isotope ratio of the product methanol was measured at ~30% reaction and at 100% reaction.[5] Because of the nature of the double-labeling method, the apparent carbon isotope effect obtained by this measurement is actually the product of the methyl carbon isotope effect and the carbonyl oxygen isotope effect. The former isotope effect is nearly unity;[8] so our measurements provide the carbonyl oxygen isotope effect directly. The data are summarized in Table I. The isotope effects given in Table I include small corrections for (a) 5% enrichment of 18O in the ether oxygen; (b) secondary isotope effects at the methyl carbon;[8] (c) the presence of singly labeled methyl benzoate. No correction for oxygen exchange between solvent and the carbonyl oxygen is necessary.[9]

In these experiments care was taken to eliminate all sources of natural abundance carbon compounds which could cochromatograph with methanol. A small amount of such carbon would cause a large error in the analysis of enriched samples.[10] The correctness of our results is indicated by several tests. (1) The isotope effect was constant from experiment to experiment, within the precision of the analytical procedure. (2) The isotope ratios of all 100% samples were the same and the isotope ratios of the low conversion samples were constant for hydrolysis and for hydrazinolysis. (3) No methanol could be detected if substrate or nucleophile was omitted from the reaction mixture. (4) Methanol of known isotopic composition could be carried through our entire procedure with no significant change in isotopic composition.

The accepted mechanism for the reaction of esters with nucleophiles involves a tetrahedral intermediate.[11,12] The large oxygen isotope effects observed for both the carbonyl oxygen and the ether oxygen[8] in the hydrazinolysis of methyl benzoate are consistent with the occurrence of such an intermediate. Qualitatively, these isotope effects indicate that both car-

Table I. Carbonyl Oxygen Isotope Effects for Reactions of Methyl Benzoate in Water at 25 °C

	Isotope ratios[b] $\times 10^4$		
% reaction[a]	Low conversion	100% conversion	k^{16}/k^{18} [c]
Alkaline Hydrolysis			
30.0	3164	3174	1.0043
28.9	3164	3169	1.0019
30.0	3150	3161	1.0048
30.7	3164	3179	1.0065
20.0	3156	3163	1.0028
35.0	3168	3184	1.0071
		Mean	1.0046
			±0.0020
Hydrazinolysis at pH 7.9, 0.3 M Free Hydrazine			
33.7	3127	3179	1.0179
32.5	3126	3175	1.0163
36.3	3132	3186	1.0185
33.1	3124	3179	1.0197
31.6	3125	3182	1.0195
		Mean	1.0184
			±0.0014

[a] Determined spectrophotometrically at 282 nm for alkaline hydrolysis and at 258 nm for hydrazinolysis. [b] Decade settings for *m/e* 45/44, corrected to tank standard = 3150. [c] The isotope effects are corrected for percent reaction, for the presence of singly labeled ester, for a methyl $k^{12}/k^{13} = 1.0022$, and for the presence of 1% [*methyl*-13C]-methyl [*ether*-18O]benzoate.

bon–oxygen bonds are substantially weakened in the transition state.

The alkaline hydrolysis of methyl benzoate is more difficult to understand in terms of a two-step mechanism. The small magnitudes of both oxygen isotope effects[8] indicate that the carbon–oxygen bonds are not extensively broken in the transition state. Available data do not allow us to convincingly eliminate the possibility that the alkaline hydrolysis occurs by way of a direct displacement without the intervention of a tetrahedral intermediate.

Determination of heavy-atom isotope effects by the double-label procedure described here makes available a new class of studies of heavy-atom isotope effects at positions which could formerly be studied only by the use of radioactive labels. This method might be of use, for example, in measuring carbonyl carbon isotope effects in the reaction of esters. This method can also be adapted to the high-precision measurement of secondary deuterium isotope effects.[13]

References and Notes

(1) A. MacColl, *Annu. Rep. Chem. Soc.*, **71A**, 77 (1974).
(2) M. H. O'Leary in "Isotope Effects on Enzyme-Catalyzed Reactions", W. W. Cleland, M. H. O'Leary, and D. B. Northrop, Ed., University Park Press, Baltimore, Md., 1976, p 233.
(3) J. Bigeleisen and M. Wolfsberg, *Adv. Chem. Phys.*, **1**, 15 (1958).
(4) H. Eilingsfeld, M. Seefelder, and H. Weidinger, *Chem. Ber.*, **96**, 2671 (1963).
(5) For the alkaline hydrolysis experiment two portions of freshly prepared 4 mM methyl benzoate in water were equilibrated at 25 °C, after which KOH was added. At the appropriate time one reaction was stopped by neutralization with H_2SO_4 and the unreacted substrate was removed by twice extracting with purified Norit. Reaction of the second sample was allowed to proceed beyond 10 half-lives, after which the sample was treated in an identical manner. methanol was isolated by fractional distillation, followed by gas chromatography, and was then converted to CO_2 by pyrolysis at 1300 °C in the presence of a twofold excess of water in the apparatus of Borowitz et al.[6] Isotope effects were calculated as previously described,[7] with the additional corrections described in the text. Hydrazinolysis was conducted similarly at pH 7.9 in the presence of 0.3 M free hydrazine.
(6) J. L. Borowitz, A. Raviv, P. Rona, D. Sedeh, D. Samuel, and F. S. Klein, *J. Labelled Compd.*, **4**, 259 (1965).
(7) M. H. O'Leary, D. T. Richards, and D. W. Hendrickson, *J. Am. Chem. Soc.*, **92**, 4435 (1970).
(8) In experiments to be reported in detail later we have measured ether oxygen and methyl carbon isotope effects on the alkaline hydrolysis ($k^{16}/k^{18} = 1.0062 \pm 0.0006$; $k^{12}/k^{13} = 1.0006 \pm 0.0005$), and hydrazinolysis at pH

7.9 (k^{16}/k^{18} = 1.0403 ± 0.0034; k^{12}/k^{13} = 1.0020 ± 0.0003) of methyl benzoate in aqueous solution at 25 °C.

(9) S. A. Shain and J. F. Kirsch, *J. Am. Chem. Soc.*, **90**, 5848 (1968).
(10) Four precautions served to eliminate most of this background. (1) All water used in these experiments was doubly distilled; the last distillation was from alkaline permanganate in an all-glass fractional distillation apparatus. (2) All glassware was cleaned in alkaline permanganate and rinsed with the above water. (3) Norit was purified by heating to 1000 °C in a quartz tube under high vacuum. (4) Large sample sizes (greater than 120 μmol) were generated to reduce the effect of any possible contamination.
(11) S. L. Johnson, *Adv. Phys. Org. Chem.*, **5**, 237 (1967).
(12) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).
(13) This research was supported by Grants PCM 75-15315 and PCM 77-00812 from the National Science Foundation.

Marion H. O'Leary,* John F. Marlier

*Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706*

Received November 7, 1977

# A New and Efficient Total Synthesis of (±)-Longifolene

Sir:

Despite the well-documented synthetic power of the bimolecular photoaddition–retroaldol reaction sequence (de Mayo reaction), depicted in Scheme I,[1] the potential of intramolecular variants starting from 1,3 diketones or their enol derivatives A, bearing olefinic substituents R^1, R^2, or R^3, has been neglected so far.[2] We now report the first example of one of these intramolecular alternatives which constitutes the key reaction leading to a ready synthesis of (±)-longifolene (**11**) (Scheme II).[3] The intricate carbon network of this sesquiterpene has served as a challenging test case for synthetic methodology[4] and planning[5] throughout the past 15 years.

To realize our objective, the acid chloride **2**[6,7] (bp 66 °C (28 Torr)) was treated with 1-morpholino-1-cyclopentene under the usual conditions[8] to give the 1,3 diketone **3**[7] (bp 63 °C (0.05 Torr), 82%), which on O-acetylation (1.5 equiv of acetyl chloride, pyridine, 0 °C, 4 h) furnished a crude enol acetate[7] (IR (CCl$_4$) 1770, 1718 (m), and 1640 cm^{-1}; NMR (CDCl$_3$) δ 2.22 (s, 3 H); UV (hexane) 243 nm (log ε 4.05); 80%) to which structure **4a** has been assigned.[9] Irradiation of the crude acetate **4a** (mercury high-pressure lamp, cyclohexane, Pyrex filter, 15–30 °C) afforded regioselectively in high yield (78%) the adducts **5a**[7,11] (1:3 mixture of stereoisomers). Hydrolytic cleavage of the acetoxy group, however, required heating of **5a** with 4% KOH in dioxane/water, 1:1, at 100 °C for 20 min to give directly the aldol **12**[7,11] (72%), presumably via the transient retroaldol intermediate **6**. As a way of avoiding the undesired secondary reaction **6 → 12**,[12] **3** was first converted to its benzyloxycarbonyl derivative **4b**[7,9] (2.7 equiv of benzyloxy chloroformate/pyridine, 5 °C, 8 h, 88%) which under the above-mentioned irradiation conditions furnished the adducts **5b**[7] (2:3 mixture of stereoisomers, 83%). Hydrogenolysis of **5b** (3 atmH$_2$, Pd/C (10%), HOAc, 25 °C 18 h) resulted in clean retroaldol cleavage giving the 1,5 diketone **6**[7,11] (mp 63–64 °C, 83%).

Having achieved a simple and efficient entry to the skeleton of longifolene (**11**), **6** was functionalized by initially converting the less hindered 8-carbonyl to a geminal dimethyl group[13] as follows. Regioselective Wittig methylenation of **6** to **7**[7,11] (5.8 equiv of methyltriphenylphosphonium bromide, 5.0 equiv of sodium *tert*-amylate, toluene,[14] 25 °C, 1.5 h, 88%), followed by a modified Simmons–Smith cyclopropanation of **7** to **8**[7,11] (6 equiv of zinc/silver couple,[15] 3 equiv of diiodomethane, refluxing ether, 60 h, 78%) and selective cyclopropane hydrogenolysis[16] (2 to 3 atmH$_2$, PtO$_2$, HOAc, 25 °C 18 h, 96%) gave the ketone **9**[7,11] which reveals superimposable ^{1}H NMR and IR spectra on those of (±)-**9**, obtained by another route.[4c] The previously described α-methylation of **9**[4c] furnished **10**

Scheme I
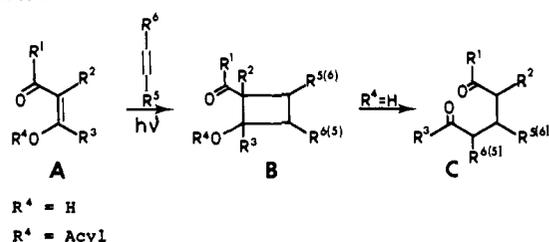
Scheme II
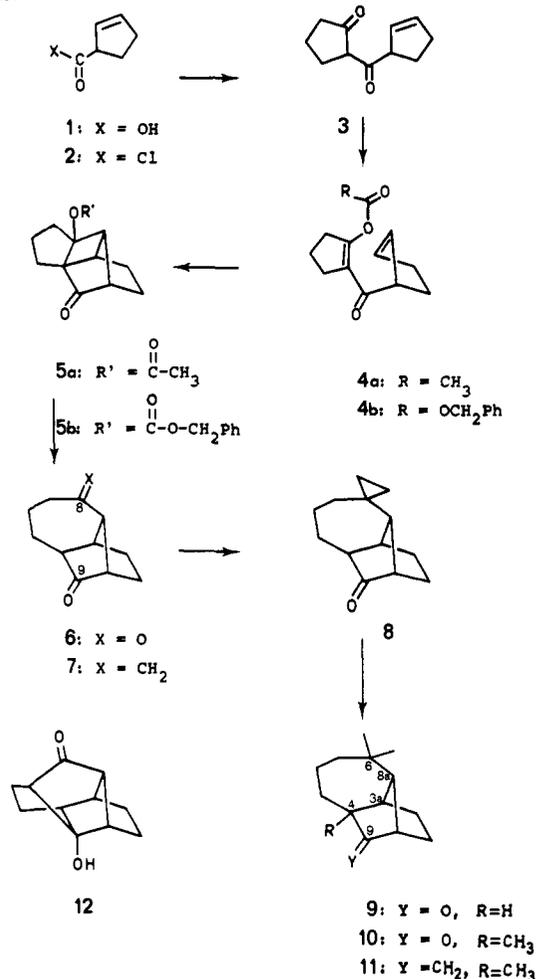
(94%), which was identified by comparison with a sample of naturally derived longicamphenylone (**10**) using GC (coinjection), ^{1}H NMR, ^{13}C NMR, IR, and mass spectral evidence.[17] Finally, using known reaction conditions,[4] **10** was converted into (±)-longifolene (**11**) (80%), the identity of which followed from similar comparison with the natural product.

In summary, this approach affords (±)-longifolene (**11**) in a nonoptimized overall yield of 25% from the acid chloride **2**.[18] The key step **4b → 5b** involves the formation of two C–C bonds, one of which (C(8)–C(8a)) has not been regarded as a particular strategic one;[5] furthermore, the asymmetric center of **4b** induces correctly the other three chiral centers of longifolene. The enantioselective synthesis of (±)-longifolene, as well as further exploration of intramolecular de Mayo reactions, are presently under study in our laboratory.