Table II. Control Experiments with $^{18}O_2$

origin of formic acid	composition, % $^2HCOOCH_2C_6H_5$, m/z 137	$^2HC^{18}OOCH_2C_6H_5$, m/z 139	$^2HC^{18}O^{18}OCH_2C_6H_5$, m/z 141
(1) $^2HC^{18}O^{18}ONa$[a]	17	46	37
(2) same as 1, incubated 60 min[b]	20	41	39
(3) same as 2	19	43	38
(4) from incubation of **4**, H = ^2H, in $^{18}O_2$, 20 min	35	65	
(5) same as 4, incubated 60 min	38	62	

[a] Synthetic sample used also in Experiments 2 and 3. GC-MS analyses were carried out as described.[17] [b] For conditions of incubation, see text (4 and 5 are averages of duplicate experiments).

that at least 50% of the molecules (**6a**) were labeled with ^{18}O at the 2β-hydroxy moiety. The silyl ether was cleaved with aqueous acetic acid and, after TLC (hexane–EtOAc 2:1), homogeneous [2β-^{18}O,19-^3H]-2β-hydroxy-10β-formylandrost-4-ene-3,17-dione (**6b**) was obtained.

Two sets of experiments were then carried out. In the first experiment, **6b** [100 μg (3×10^4 dpm of ^3H) in each of five flasks] was incubated in Tris-buffer (pH 7.4) with placental aromatase for 1 h at 37 °C, under nitrogen, as previously described.[4,15] At the termination of the reaction, the contents of the flasks were rapidly combined, the mixture was acidified and frozen in liquid nitrogen, and the formic acid was recovered by lyophilization.[4,15] The derived sodium formate was then converted to benzyl formate.[16,9] The second set of experiments was carried out exactly as above[4,15] but *without placental aromatase*, and the recovered formic acid was also converted to benzyl formate. Each of the two samples of benzyl formate contained tritium (1.35×10^5 dpm), indicating that ca. 90% of the substrate **6b** was aromatized. The GC-MS[17] of the two samples were recorded, and the results are summarized in Table I. The benzyl formates showed peaks at m/z 136 and 137, but none was present at m/z 138 indicating the absence of ^{18}O.

Usually, variable amounts of endogenous [^{16}O]formic acid were recovered from the placental aromatase preparations. It is therefore of importance that the benzyl formate derived from aromatization of [^{18}O]-**6b** in the *absence of placental aromatase* gave formic acid (analyzed as benzyl formate) which contained only ^{16}O. This benzyl formate could not be contaminated with a formate of endogenous origin and must have originated solely from C-19 of the [2β-^{18}OH]-**6b** substrate.

To determine whether ^{18}O was exchanged (lost) under the experimental conditions, $^2HC^{18}O^{18}ONa$ (80% ^{18}O enrichment) was prepared,[9] and an aliquot of the salt was converted to benzyl formate.[16,9] A second aliquot was incubated with human placental aromatase (1 h, at 37 °C, in the air), and the formic acid was recovered by lyophilization of the acidified mixture.[4,15] The GC-MS analyses of the two [$^{18}O_2$]benzyl formates indicated that *no* detectable loss of ^{18}O occurred (Table II, entries 1–3).

We have also incubated [19-^2H]-10β-formylandrost-4-ene-3,17-dione **4**, H = ^2H] in an atmosphere of $^{18}O_2$. Two sets of incubations (in duplicate) were carried out for 20 and 60 min with human placental aromatase[4,15] in an atmosphere of $^{18}O_2$ (98% excess). The recovered formic acids were analyzed (as benzyl formates) by GC-MS,[17] and all four samples showed ions at m/z 139 for $^2HC^{16}O^{18}OCH_2C_6H_5$ (Table II, entries 4 and 5). These results confirm the Akhtar et al.[9] observations that the "third" mole of oxygen is incorporated into the extruded formic acid.

To evaluate the operation of pathway C (Scheme II), 19-DT aldehyde **4** was incubated with placental aromatase in $^{18}OH_2$ (30% excess of ^{18}O) in the air. The rationale of the experiment was based on the premise that, if Fishman's hypothesis is correct, the $^{16}O_2$ should be utilized for C-2 hydroxylation to give **6b**, 2β-^{16}OH, which will then aromatize with the incorporation of ^{18}O from the water into the formic acid. The recovered formic acid (60% yield) contained only ^{16}O. These results when taken together with the results on the aromatization of [^{18}O]-**6b** exclude the operation of pathway C, Scheme II.

Our results show that the oxygen atom of the [^{18}O]-2β-hydroxyl of **6b** was *not incorporated* into the extruded formic acid derived from C-19. It may therefore be concluded that the mechanism proposed by Fishman et al.[10–13] via enzymatic formation of 2β-hydroxy-10β-formylandrost-4-ene-3,17-dione (**6b**) and its non-enzymatic aromatization is *not an obligatory pathway* of estrogen biosynthesis by placental aromatase.

(14) The synthesis was carried out by treating [19-^3H]-6β-bromo-19-acetoxyandrost-4-ene-3,17-dione with $^{18}O_2$-labeled potassium acetate in glacial ^{18}O-labeled acetic acid to give [2β-$^{18}O_2$,19-^3H]-2β,19-diacetoxyandrost-4-ene-3,17-dione. Saponification followed by selective silylation of the resulting 2β,19-diol furnished 19-hydroxy-2β-(*tert*-butyldimethylsilyl) ether which on mild oxidation gave the required **6a**. The proton NMR of **6a** showed signals at δ 9.78 (1 H, s, 19-CHO), 5.85 (1 H, s, C_4–H), 3.98 (1 H, br s, 2α-H), and 0.82 (3 H, s, C_{18}–H) in accordance with published data.[10] The MS of [^{16}O]-2β-silyl ether **6a** showed ions at m/z 402 ($M^+ - 28$) and 373 ($M^+ - C_4H_9$), while the MS of [^{18}O]-2β-silyl ether **6a** showed ions at m/z 402 (49%), 404 (51%), 373 (45%), and 375 (55%).

(15) Caspi, E.; Arunachalam, T.; Nelson, P. A., manuscript in preparation.

(16) Corina, D. L. *Anal. Biochem.* **1977**, *80*, 639.

(17) A Varian Model 3700 GC instrument equipped with a glass capillary column (25 m) coated with OV-101 was used. Injection port temperature 270 °C; column temperature 70–280 °C; temperature gradient 3 °C/min. The GC was linked to a Varian-MAT Model 312 mass spectrometer via a direct inlet. Spectra were recorded at 70 eV.

Reaction of Ketene Alkylsilyl Acetals with Bromoform–Diethylzinc. An Unprecedented Cyclopropanation Reaction

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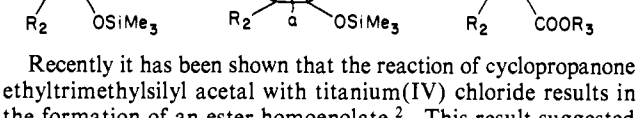
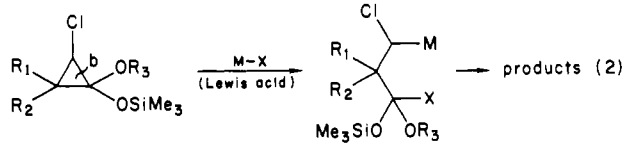
In conjunction with our program dealing with the reactivity of ketene alkylsilyl acetals and carbenes, we have reported that the reaction of those species with chlorocarbenes provides a convenient route to α,β-ethylenic esters via a-bond cleavage of an intermediate cyclopropane (eq 1[1]).



(1)

Recently it has been shown that the reaction of cyclopropanone ethyltrimethylsilyl acetal with titanium(IV) chloride results in the formation of an ester homoenolate.[2] This result suggested that chlorocyclopropanone acetals could lead to a carbenoid species by cyclopropane b-bond ring cleavage (eq 2) if the reaction was carried out in the presence of a Lewis acid.



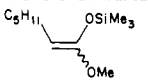
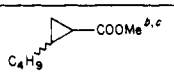
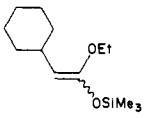
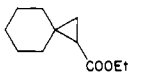
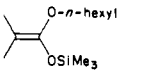
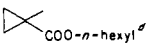
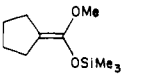
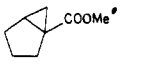
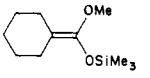
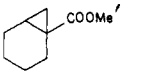
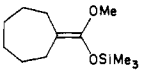
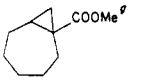
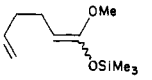
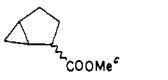
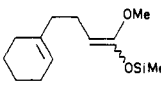
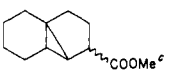
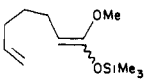
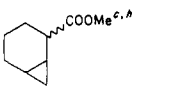
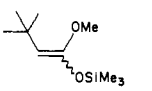
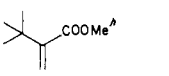
(2)

(1) Slougui, N.; Rousseau, G.; Conia, J. M. *Synthesis* **1982**, 58. Slougui, N.; Rousseau, G. *Synth. Commun.* **1982**, *12*, 401.

(2) Nakamura, E.; Kuwajima, I. *J. Am. Chem. Soc.* **1983**, *105*, 651.

0002-7863/84/1506-7283$01.50/0

Table I. Cyclopropane Ester Synthesis from Ketene Acetals

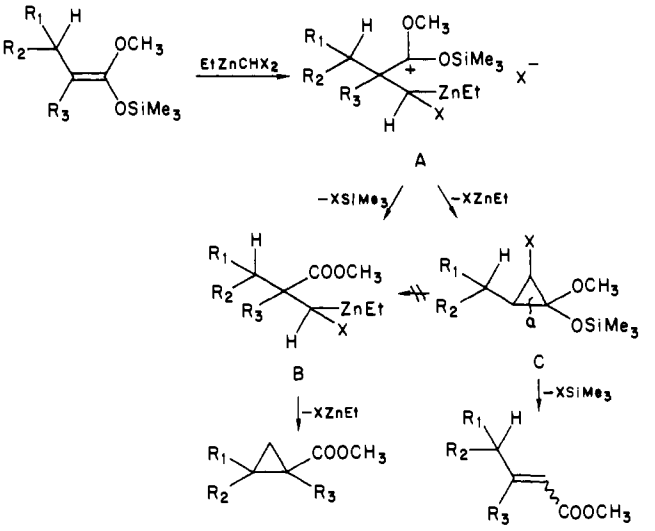
entry	ketene acetal	cyclopropane ester[a]	yield, %
a			80
b			82
c			72
d			55
e			63
f			58
g			80
h			78
i			10
j			40

[a] All reactions were performed at 20 °C. 5 mmol of ketene acetal, 2.5 mL of pentane, and 3.75 mL of 1 M Et_2Zn in pentane (1.5 equiv) were mixed under argon; 0.655 mL of $CH Br_3$ (1.5 equiv) was added dropwise (30 min); then, oxygen bubbling for 1 min[5] initiated the reaction. After 2 h at room temperature and a standard workup, the products were isolated via liquid chromatography. [b] The reaction conducted with the $E:Z = 89:11$ or the $E:Z = <2:98$ ketene acetal mixtures gave similar results. [c] The product stereochemistry was not determined. [d] *n*-Hexyl 3-methyl-2-butenoate (18%) was also formed. [e] Methylcyclopentylidene acetate (30%) was formed. [f] Methyl cyclohexylideneacetate (3) (27%) was also formed. [g] Methyl cycloheptylideneacetate (27%) was also formed. [h] Nonidentified products also formed.

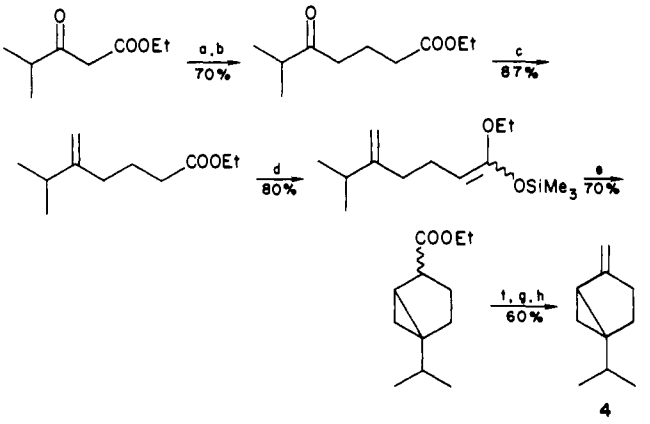
We report here a novel synthesis of cyclopropanecarboxylic esters from the reaction of ketenealkylsilyl acetals[3] with bromoform–diethylzinc.[4]

The results are summarized in Table I. When monosubstituted ketene acetals are used, cyclopropanecarboxylic esters are formed by a novel C–H insertion (entries a and b). When disubstituted ketene acetals are used, α,β-ethylenic ester byproducts were also formed (entries c–f) (eq 1). Interestingly, γ or δ unsaturated ketene acetals resulted in an intramolecular cyclization (entries g–i). This reaction provides a convenient method for the preparation of the bicyclo[3.1.0]hexane system and can be advantageously compared to the copper-catalyzed intramolecular cyclization of unsaturated α-diazo ketones.[6] As a consequence of entropy problems, the scope of this intramolecular cyclopropanation is somewhat limited (entry i). Finally, when the ketene acetal substituent has no allylic hydrogen or unsaturation, a completely different reaction sequence occurred (entry j).[7]

Scheme I. Postulated Mechanism of the Bromoform–Diethylzinc–Ketene Acetal Reaction

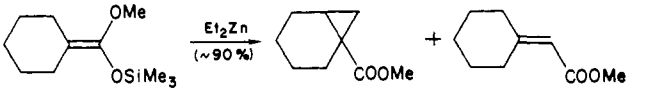


Scheme II.[a] Preparation of (±)-Sabinene



[a] (a) NaH, THF, $BrCH_2CH_2COOEt$; (b) Me_2SO, NaCl;[10] (c) $Ph_3P{=}CH_2$, potassium *tert*-amylate benzene;[11] (d) LDA, THF, $ClSiMe_3$;[3] (e) $CHBr_3$, Et_2Zn, pentane; (f) $LiAlH_4$ ether, room temperature; (g) TsCl then LiBr acetone; (h) KO-*t*-Bu, Me_2SO.

We have also studied the behavior of the ketene acetal **1** toward Et_2Zn–bromoform and analogous reagents under different reaction conditions (eq 3). By use of diethyl ether with bromoform,



	2	3
$HCBr_3$, ether	28	72
$HCBr_3$, benzene	75	25
$HCBr_3$, pentane	70	30
$HCBr_2Cl$, pentane	68	32
$HCBrCl_2$, pentane	73	27

(3)

methylcyclohexylidene acetate **3** was the main product. The yield of this material decreased dramatically when pentane was used. In this solvent, the same products were formed when bromodichloro-, chlorodibromomethane, or bromoform was used. Since the haloform does not effect the ester ratio, direct formation of halocyclopropanone acetal C can be excluded. Formation of

(3) Ainsworth, C.; Chen, F.; Kuo, Y. N. *J. Organomet. Chem.* **1972**, *46*, 59.

(4) Miyano, S.; Matsumoto, Y.; Hashimoto, H. *J. Chem. Soc., Chem. Commun.* **1975**, 364. See also: Nishimura, J.; Furukawa, J. *Ibid.* **1971**, 1375.

(5) Miyano, S.; Hashimoto, H. *J. Chem. Soc., Chem. Commun.* **1971**, 1418.

(6) Burke, S. D.; Grieco, P. A. *Org. React. (N. Y.)* **1979**, *26*, 361.

(7) Ketone enoxysilanes did not lead to such a reaction. Blanco, L., unpublished results.

intermediate A can be explained by two different pathways[7] (see Scheme I).

The predominant formation of the α,β-ethylenic ester when ether is used can be explained by assuming that the metal atom in intermediate A is better solvated than in pentane. This solvatation results in a weakening of the the carbon–zinc bond, and halocyclopropanone acetal C is formed by internal electrophilic substitution. In pentane (or benzene), species A loses $XSiMe_3$ giving rise to carbenoid B, which undergoes insertion into a carbon–hydrogen bond. This same intermediate can undergo intramolecular cyclopropanation if a CC double bond is available. Carbenoid formation seems to be without precedent. It is well-known that carbenes and carbenoids insert into carbon–hydrogen bond.[8] The exclusive insertion into the β-position is probably due to the presence of the ester functionality.

The usefulness of this reaction was illustrated by a new eight-step (±) synthesis of sabinene (**4**) (Scheme II).

(8) Kirmse, W. "Carbene Chemistry", 2nd ed.; Academic Press: New York, 1971; pp 209–266.
(9) Preceding synthesis: Fanta, W. I.; Erman, W. F. *J. Org. Chem.* **1968**, *33*, 1656. Mori, K.; Ohki, M.; Matsui, M. *Tetrahedron* **1970**, *26*, 2821. Vig, O. P.; Bhatia, M. S.; Gupta, K. C.; Matta, K. L. *J. Indian Chem. Soc.* **1969**, *46*, 991. Alexandre, C.; Rouessac, F. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2241.
(10) Krapcho, A. P.; Lovey, A. J. *Tetrahedron Lett.* **1973**, 957.
(11) Conia, J. M.; Limasset, J.-C. *Bull. Soc. Chim. Fr.* **1967**, 1936.

Enzymatic Synthesis of Hydrocarbon-Soluble Peptides with Reverse Micelles

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Received May 15, 1984

Reverse micelles have been investigated also because of their capability to host several types of molecules.[1,2] Also enzymes can be solubilized in reverse micelles (Figure 1) without loss of activity.[3] Reverse micelles formed by the anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT), swell by increasing the amount of water in the system (generally expressed by the parameter $w_0 = [H_2O]/[AOT]$), and at the same time the physical properties of the water of the water pool become closer to those of bulk water.[4] Reverse micelles can then be viewed as microreactors, whose dimensions can be easily changed and with a milieu whose physical properties (e.g., dielectric constant, microviscosity, acidity) can be continuously modulated and possibly tailored to the characteristics of the reaction taking place in the water pool. Another important aspect of reverse micelles is the difference between the polar core and the outside (essentially hydrocarbon). This difference in environment can be utilized advantageously for mass transport and separation in chemical reactions.

It is the aim of this communication to show for the first time the use of reverse micelles for the enzymatic synthesis of peptides

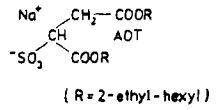

Figure 1. Schematic representation of the solubilization of enzymes in reverse micelles (cross section) and of the structure of the surfactant AOT used in our studies. Particularly at small w_0 values ($w_0 = [H_2O]/[AOT]$) the uptake of the protein will bring about a reequilibration of the material present in the micelles.[3i]

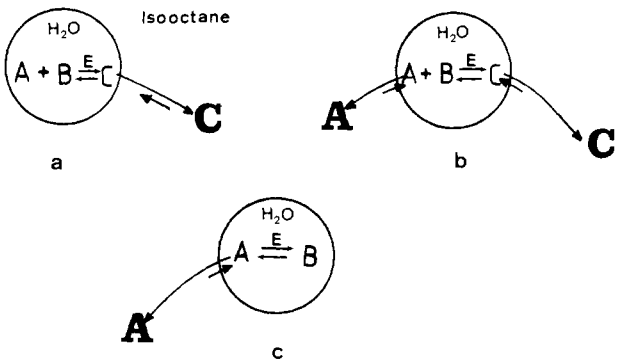

Figure 2. Three examples of compartimentalization of reactants in reverse micelles in enzymatic reactions. In the first case (a) the two reagents A and B are preferentially soluble in water and the product C in hydrocarbon. The second case (b) represents a different reaction, where one of the two reagents (A) is also soluble in hydrocarbon. Finally (c), the case of a quite different reaction is schematized, where an overwhelming hydrocarbon-soluble compound A yields, upon enzymatic cleavage, a water soluble product B which would remain entrapped in the water pools.

and to point out the potentialities of reverse micelles in enzymatic reactions involving lipophylic (and in particular water insoluble) reagents. It has been already shown that water-soluble enzymes solubilized in reverse micelles can accept highly lipophylic substrates.[3g,h] On the basis of this, and on the distribution of reagents as dictated by their relative solubilities, several possibilities can be envisaged, as shown in Figure 2. In the first and second case (Figure 2a, b) the product C, once formed in the water pool, will be expelled into the bulk hydrocarbon.

In this communication, we will illustrate this, using as an example the α-chymotrypsin-induced synthesis of a hydrocarbon-soluble (water insoluble) protected tripeptide. In the past, we have investigated the enzymatic synthesis of peptide bonds in aqueous solution,[5] and the extension to reverse micelles appeared an obvious thing to do, also in view of the small amount of water present in the hydrocarbon micellar system. The reaction chosen is

$$\underset{A}{\text{Z-Ala-Phe-OMe}} + \underset{B}{\text{H-Leu-NH}_2} \overset{E}{\rightleftharpoons} \underset{C}{\text{Z-Ala-Phe-Leu-NH}_2} + \text{MeOH} \quad (1)$$

catalyzed by α-chymotrypsin, where Z is the benzyloxycarbonyl protecting group. Both A and C are practically insoluble in water and soluble in isooctane, and therefore the situation can be depicted as in Figure 2b. Typically, the reaction conditions were room temperature, pH 10, 0.1 M borate buffer, and overall concentration[6] of A and B 1 mM and of the enzyme 5 μM. Enzyme

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