Synthesis of [Carbonyl-^{14}C] Labeled Carbonate and Carbamate Esters

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SUMMARY

A two-step synthesis of three carbonate and four carbamate esters labeled at the carbonyl with carbon-14 is described. The method utilizes the readily available [^{14}C]-phosgene which is first converted to an isolable [^{14}C]-labeled alkyl or aryl chloroformate and subsequently reacted with the appropriate alcohol or amine to give the corresponding ester.

KEYWORDS: Carbon-14, carbamate esters, carbonate esters, phosgene, choloroformates.

INTRODUCTION

Carbonate and carbamate esters are frequently utilized in the design and synthesis of prodrugs of alcohols (1). These esters are usually slowly hydrolyzed in phosphate buffer at pH 7.4, but exhibit fast hydrolytic rates in alkaline pH (2). However, the hydrolysis of such compounds is highly accelerated in the presence of esterolytic and/or proteolytic enzymes (3). In this connection, Shah and Connors (4) have presented evidence, from in vitro kinetic experiments, suggesting that the enzymatic hydrolysis of carbonate esters involves the intermediate formation of an enzyme carbonate (acyl enzyme) the deacylation of which is rate determining. Indeed,

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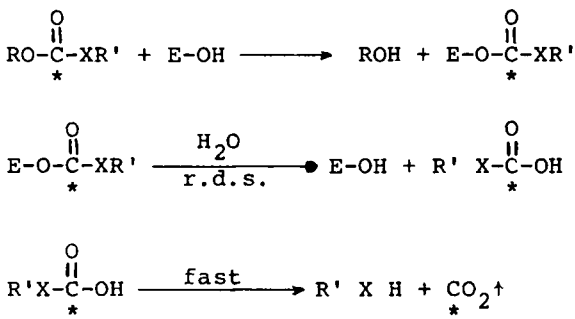
the same principle applies in the inhibition of acetylcholine esterase by the carbamate esters neostigmine and physostigmine (5). In either case, the deacylation step leads to the recovery of the enzyme and production of carbon dioxide (scheme 1).



X = O, carbonate ester

X = NH, carbamate ester

E-OH, serine-dependent esterase

Scheme 1

As seen from the above scheme, a carbonate or carbamate ester labeled at its carbonyl function would produce labeled carbon dioxide, the monitoring of which could be used as an index of the reaction progress.

In the course of synthesizing carbonate/carbamate esters with potential as active-site directed inhibitors of porcine pancreatic (PP) elastase, need was felt that these esters be synthesized labeled at their carbonyl function with carbon-14. The present communication describes procedures for the conversion of the commercially available [^{14}C]-phosgene to [^{14}C]carbonate or [^{14}C]carbamate esters via an isolable [^{14}C]chloroformate intermediate.

Experimental

Melting points were determined on a Thomas-Hoover Uni-Melt

apparatus and are uncorrected. ^{1}H NMR spectra were obtained using a Varian EM-360 (60 MHz) spectrometer; IR spectra were recorded on a Perkin-Elmer 567 spectrophotometer. Micro-analyses were performed by Atlantic Microlab, Inc., Atlanta, Georgia.

Carbon-14 labeled phosgene was purchased from New England Nuclear, Boston, MA., and was provided in an ampule as a chloroform solution (1 mCi/2 ml $CHCl_3$). [^{14}C]-phosgene (1 mCi) was dissolved in a 12.5% solution of carrier phosgene in toluene (48 ml) to give a stock solution containing 20 μCi/ml/1.2 mmole phosgene. Note: All reactions were carried out in a well-ventilated hood.

Synthesis of [Carbonyl-^{14}C] Ethyl Chloroformate

To one milliliter of [^{14}C]-phosgene stock solution (1-2 mmole, 20 μCi) was added 8.5 ml (10.8 mmol) of non-radioactive phosgene (12.5% in toluene). The resulting solution was then added, dropwise, to ice-cooled absolute ethanol (0.58 ml, 10 mmole). The reaction mixture was stirred in an ice bath for four hours, and at room temperature (25°C) for the following two hours. Hydrogen chloride gas and excess phosgene were removed under vacuum and trapped in 10% potassium hydroxide solution (150 ml).

The resulting reaction mixture was used in the synthesis of the carbonate and carbamate esters without further purification. However, for identification purposes, the reaction mixture was distilled under reduced pressure to produce pure [^{14}C]-ethyl chloroformate (0.54g, 50%); radiochemical yield, 6 μCi (30%); bp 93°C, Lit. 95°C (6).

General Procedure for the Synthesis of [Carbonyl-^{14}C] Ethyl Carbonate Esters (2,8,3) (Table 1).

[^{14}C]-Ethyl chloroformate in toluene (10 ml, 0.54g, 5 mmol, 6 μCi) was added dropwise to a solution of the appropriate alcohol

or phenol (8 mmol) dissolved in pyridine (0.54 g, 12 mmol). The reaction mixture was heated on a steam bath for 10 minutes and left overnight at room temperature. To this mixture, distilled water (3 ml) was added to dissolve all the pyridinium chloride formed. The organic layer was then separated, successively washed with 5% HCl, 5% NaOH and water, dried over anhydrous magnesium sulfate, filtered and the toluene evaporated under reduced pressure. The resulting [^{14}C] carbonate esters were purified by distillation, and were identified by TLC (silica gel) in two different eluting solvent systems against a non-radioactive sample prepared in a similar manner. The non-radioactive esters exhibited boiling points comparable to reported literature values. Rf values, boiling points, chemical and radiochemical yields are shown in Table 1.

General Procedure for the Synthesis of [Carbonyl-^{14}C] Ethyl Carbamate Esters (4,5,6) (Table 1)

The appropriate amine (10 mmol) was added dropwise to [^{14}C]-ethyl chloroformate in toluene (5 ml, 0.27 g, 2.5 mmol, 3 μCi) and the reaction mixture was stirred for 5 minutes in an ice bath. To this solution, an additional amount of [^{14}C] ethyl chloroformate (0.27 g, 2.5 mmol, 3 μCi) and 1 M NaOH solution (5 ml) were added simultaneously, and stirring was continued for another 10 minutes. The organic layer was separated, washed successively with 5% HCl and water, dried over anhydrous magnesium sulfate, filtered and the toluene evaporated under reduced pressure. The liquid carbamate ester 4 was purified by distillation under vacuum whereas the solid carbamate esters 5 and 6 were purified by recrystallization from petroleum ether (30-60°C) and ethanol-water respectively. The [^{14}C]-esters thus obtained were identified by TLC in two different elution solvent systems against non-radioactive samples prepared under identical reaction conditions. The identity of the non-radioactive carbamates was established by

comparing their melting points (or boiling points) to literature values (Table 1). Chemical and radiochemical yields as well as Rf values are reported in Table 1.

Synthesis of [Carbonyl-^{14}C]Phenyl Chloroformate

Two milliliters (40 μCi, 2.4 mmol) of the stock preparation of [^{14}C]phosgene were diluted with 13.8 ml (17.4 mmol) of non-radioactive phosgene (12.5% in toluene). To this solution, phenol (1.88 g, 20 mmol) was added and the resulting solution was placed in a 50 ml 2-neck flask equipped with a dropping funnel. Triethylamine (2.02 g, 20 mmol) was added dropwise with continuous stirring at 0°C for 30 minutes. The triethylammonium hydrochloride formed during the reaction was filtered off and the filtrate washed with 5% HCl and water, dried over anhydrous magnesium sulfate, filtered and the toluene removed under reduced pressure. The residue was purified by distillation under reduced pressure to give 1.88 g (60%) of [^{14}C]-phenyl chloroformate (radiochemical yield, 20%, specific activity 0.025 μCi/mmole, bp 187-188°C, lit. bp_{20}, 80°C (7).

Synthesis of [Carbonyl-^{14}C]n-Butyl Phenyl Carbamate (7).

[^{14}C]Phenyl chloroformate (1.88 g, 12 mmol, 8 μCi) prepared as described above, was dissolved in dry benzene (10 ml) and the resulting mixture was added dropwise to a solution of n-butyl amine (0.96 g, 13.2 mmol) and pyridine (1.14 g, 14.4 mmol) in dry benzene (10 ml). After stirring for 10 minutes at room temperature, the reaction mixture was slowly poured into 5% HCl (30 ml). The benzene layer was separated, successively washed with 5% HCl, 5% aqueous $NaHCO_3$, dried over anhydrous magnesium sulfate, filtered and the benzene evaporated under reduced pressure. The residue was washed twice with cold petroleum ether, to remove unreacted starting materials, and recrystallized from ethanol-water to give 0.87 g (40%) of [^{14}C]n-butyl phenyl carbamate having a specific activity of 0.014 μCi/mmole.

The product co-chromatographed (TLC, silica gel) with a non-radioactive sample prepared in an identical manner. Two elution solvents were used and the R_f values were found to be 0.63 and 0.31 in acetone/$CHCl_3$ (50:50) and hexane/$CHCl_3$ (70:30) respectively.

The identity of the non-radioactive sample of n-butyl phenyl carbamate was confirmed by elemental analysis. Anal. calcd. for $C_{11}H_{15}NO_2$: C, 68.40; H, 7.82; N, 7.25. Found: C, 68.34; H, 7.82; N, 7.06.

RESULTS AND DISCUSSION

[Carbonyl- ^{14}C]-labeled carbamate esters have been reported in the literature in relation to their metabolism (8) and reaction with serine proteases (9). In all the reported examples, the [^{14}C]-labeled ester was synthesized from the reaction of a [^{14}C]-labeled isocyanate with the appropriate alcohol, a method not applicable to the synthesis of [^{14}C]-carbonate esters. A report by Roeda and Westera (10) describes the synthesis of the symmetric [^{11}C]-diethyl carbonate from [^{11}C]-phosgene and sodium ethanoate. In studying the potential of carbonate and carbamate esters as active-site directed inhibitors of PP elastase a general method was needed for the synthesis of such esters labeled at the carbonyl with carbon-14.

Thus, the synthesis of [^{14}C]-labeled carbonate and carbamate esters 1-7 was achieved utilizing the readily available [^{14}C]-phosgene following the synthetic routes represented by equations 1 and 2.

$$Cl\text{-}\overset{O}{\overset{\|}{C}}\text{-}Cl + R'OH \xrightarrow{\text{toluene}} R'O\text{-}\overset{O}{\overset{\|}{C}}\text{-}Cl + HCl \qquad \text{(equation 1)}$$

$$R'O\text{-}\overset{O}{\overset{\|}{C}}\text{-}Cl + R\,X\,H \xrightarrow{\text{base}} R'O\text{-}\overset{O}{\overset{\|}{C}}\text{-}XR + \text{Base} \cdot HCl \qquad \text{(equation 2)}$$

1: X = O, R = n-Bu, R' = Et

2: X = O, R = $PhCH_2$, R' = Et

3: X = O, R = Ph, R' = Et

4: X = NH, R = n-Bu, R' = Et

5: X = NH, R = $PhCH_2$, R' = Et

6: X = NH, R = Ph, R' = Et

7: X = NH, R = n-Bu, R' = Ph

Compounds 1-6 could be prepared by a one-pot synthesis without isolation of the chloroformate. However, we have found it imperative to remove the excess phosgene since failure to do so, resulted in the formation of urea and a mixture of symmetric and asymmetric carbonates.

Recently, it was reported by Fujinama et. al. (11) that symmetric dialkyl carbonate esters can be prepared from the reaction of K_2CO_3 with alkyl halides in presence of an organostannyl catalyst. The reaction of dialkyl carbonates with PCl_5 to give the corresponding alkyl chloroformate has also been described (12). It was therefore hoped that a combination of the above two reactions would provide a method for the preparation of [^{14}C]-labeled alkyl chloroformates starting with $Na_2{}^{14}CO_3$ (equations 3 and 4).

$$Na_2{}^{14}CO_3 + 2\ RBr \xrightarrow{\text{organostannyl catalyst}} RO-\overset{\overset{O}{\|}}{\underset{14}{C}}-OR + 2\ NaBr$$

(Equation 3)

$$RO-\overset{\overset{O}{\|}}{\underset{14}{C}}-OR + PCl_5 \longrightarrow RO-\overset{\overset{O}{\|}}{\underset{14}{C}}-Cl + RCl + POCl_3$$

(equation 4)

Both of the above reactions were tried in our laboratories utilizing non-radioactive materials. Although ethyl chloroformate was obtained in good yield from diethyl carbonate (equation 4), all attempts to prepare dialkyl carbonates by the route shown in equation 3 failed.

In conclusion, a general method is described for the preparation of carbonate and carbamate esters labeled at the carbonyl

Table 1: Radiolabeled (C-14) Carbonate and Carbamate Esters (Et-$\overset{O}{\overset{\|}{C}}$-CXR) Synthesized in this Study.

Compound Number	X	R	b.p./m.p. °C	Chemical[a] Yield %	Radiochemical[b] Yield %	Rf Values A	Rf Values B
1	O	n-Bu	166-167[d]	36	16	0.50	0.49
2	O	$PhCH_2$	231-232[e]	42	20	0.65	0.63
3	O	Ph	226-228[f]	46	21	0.63	0.56
4	NH	n-Bu	201-202[g]	48	23	0.62	0.52
5	NH	$PhCH_2$	44 (s)[h]	40	19	0.60	0.42
6	NH	Ph	52-53(s)[i]	38	18	0.68	0.43

[a]Overall yield was calculated on the basis of the amount of ethanol used to prepare the ^{14}C-ethyl chloroformate.

[b]Calculated on the basis of ^{14}C-phosgene utilized.

[c]Column A represents elution by Acetone/$CHCl_3$(50:50) and Column B, hexane/$CHCl_3$ (30:70). Each compound showed a single spot in both systems.

[d]Lit. b.p. 167°C (Ref. 13)

[e]Lit. b.p.$_{20}$ 122-4°C (Ref 14)

[f]Lit. b.p. 222-230°C (Ref 15)

[g]Lit. b.p. 202-203°C (Ref 16)

[h]Lit. m.p. 44°C (Ref 17a)

[i]Lit. m.p. 53°C (Ref 17b)

with carbon-14. This method unlike previously reported procedures (9) is not limited by the availability of the [^{14}C]-labeled isocyanates. Furthermore since the [^{14}C]-labeled chloroformate intermediate is isolable, asymmetric carbonates can be easily prepared.

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