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Synthesis of ^{14}C-labeled Copolymers for Drug Delivery Studies*

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SUMMARY

^{14}C-labeled polyanhydride copolymers [(20:80/1,3-bis(p-carboxyphenoxy)-propane (CPP):sebacic acid (SA)] (**7** and **13**) were synthesized according to schemes I and II: ^{14}C-labeled sebacic acid (2,9-$^{14}C_2$) (**5**) and ^{14}C-labeled CPP 1,3-bis(p-carboxyphenoxy)-propane (labeled at C-1, C-3 of the propyl group) (**11**) were transformed to the corresponding mixed anhydrides (**6** and **12**) as prepolymers respectively by reaction with acetic anhydride. The labeled mixed anhydride prepolymers (**6** and **12**) were condensed with unlabeled counter-prepolymers (12' and 6') to give the labeled polyanhydride copolymers (**7** and **13**). The labeled copolymers (**7** and **13**) were identified and characterized by gel permeation chromatography (GPC).

Key words: Suberic acid-$^{14}C_2$; Sebacic acid (SA-$^{14}C_2$); CPP-$^{14}C_2$; SA prepolymer, CPP prepolymer and 20:80/CPP:SA copolymers.

INTRODUCTION

Poly((p-carboxyphenoxy)propane:sebacic acid) (CPP:SA) has been used as a biodegradable carrier for the delivery of 1,3-bis[2-chloroethyl]-n-nitrosourea (BCNU) to the brain for the treatment of brain cancer. This delivery system provides several advantages. First, it is possible, with this implant, to achieve high local concentrations of drug to areas and organs, such as the brain that are difficult to access. In addition, as the polymer degrades, it releases drug over an extended duration of time, and finally vanishes. Since the anticancer compound delivered by this particular formulation, BCNU, is toxic when circulating levels are sufficient to pass the blood brain barrier, delivery directly into the brain minimizes systemic exposure, therefore reducing the risk factors. This polymeric implant is now in Phase III clinical trials in humans.

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In order to determine the metabolic fate and elimination of this polymer ^{14}C-radiolabeled polymer that contained ^{14}C-SA and ^{14}C-CPP were used. The incorporation of ^{14}C into the polymer allowed a relatively simple way to track the course of the polymer biodegradation over time. The sebacic acid was labeled near, but not on the carboxyl. The CPP was labeled on the phenol ether linkage to determine the accessibility of this portion of the polymer to metabolic degradation.

DISCUSSION

It was thought desirable to prepare labeled SA in which the label was not on the carbonyl moiety where it could theoretically be lost by early decarboxylation. Accordingly, we devised Scheme 1 which introduced the ^{14}C-label at positions α to the carboxyl groups. Carbonation of the Grignard reagent [$BrMg(CH_2)_6MgBr$] in THF gave suberic acid $^{14}C_2$-carboxyl (**2**) in quantitative yield. The dicarboxylic acid was converted to the desired sebacic acid (2,9-$^{14}C_2$) utilizing well established reactions as outlined in Scheme I. The pure sebacic acid-$^{14}C_2$ (**5**), recrystallized from acetone, was treated with acetic anhydride to give ^{14}C-SA prepolymer (**6**). The condensation of **6** with unlabeled CPP prepolymer (12') was carried out in the polymerization apparatus (Figure 1) to give [20:80/CPP-^{14}C-SA polyanhydride copolymers with average molecular weight of 40,000 according to GPC.

In the preparation of ^{14}C-CPP (Scheme II), the key intermediate labeled 1,3-dibromo propane (**10**), was obtained from reaction sequence starting with CH_3CO_2Et. Carbonation of [$LiCH_2CO_2Et$] in THF at -78°C gave the mono ethyl ester of malonate ^{14}C-carboxyl which, without isolation, was treated with excess CH_3CHN_2 to give the corresponding diethyl malonate (**8**). The labeled malonate was reduced with lithium aluminum hydride in ether to the diol (**9**) which was converted to the 1,3-dibromopropane 1,3-$^{14}C_2$ (**10**). The bromide was treated with 2 equivalents potassium salt of methyl *p*-hydroxybenzoate to give the diester of CPP which was hydrolyzed to give CPP-$^{14}C_2$ (**11**). Pure CPP-$^{14}C_2$ after recrystallization EtOAc was obtained as pale yellow solid which was identified in GLC with comparison of an authentic sample.

Following the same polymerization procedure as described to prepared **7**, CPP-$^{14}C_2$, diluted with unlabeled CPP was converted to ^{14}C-CPP prepolymer and subsequently condensed with unlabeled SA prepolymer to give [20:80/^{14}C-CPP-SA polyanhydride copolymer.

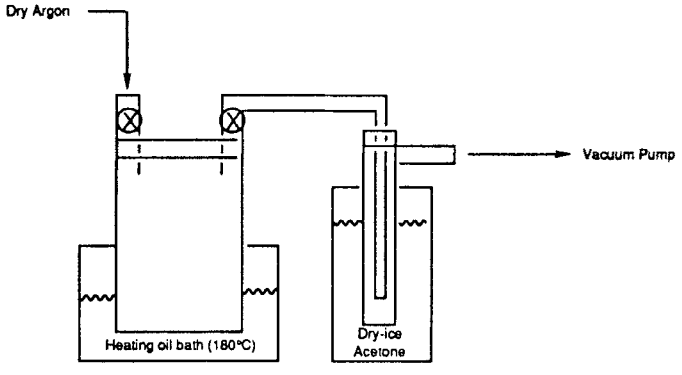
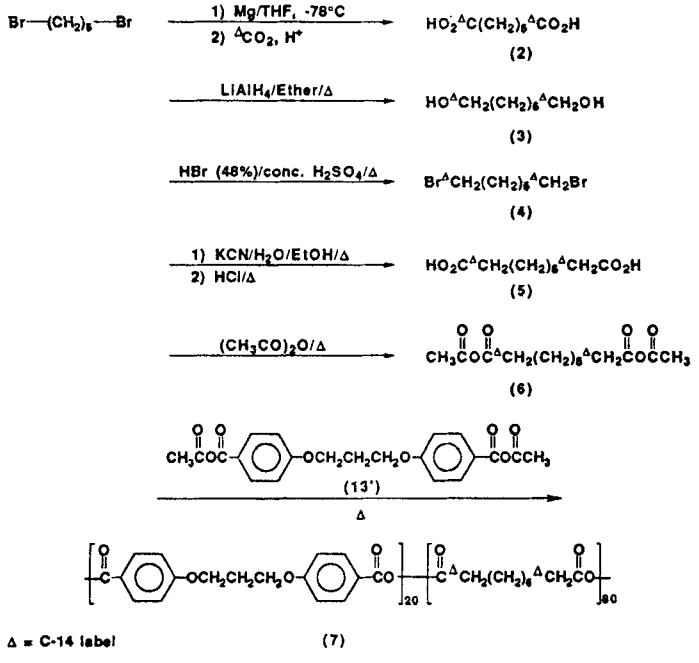
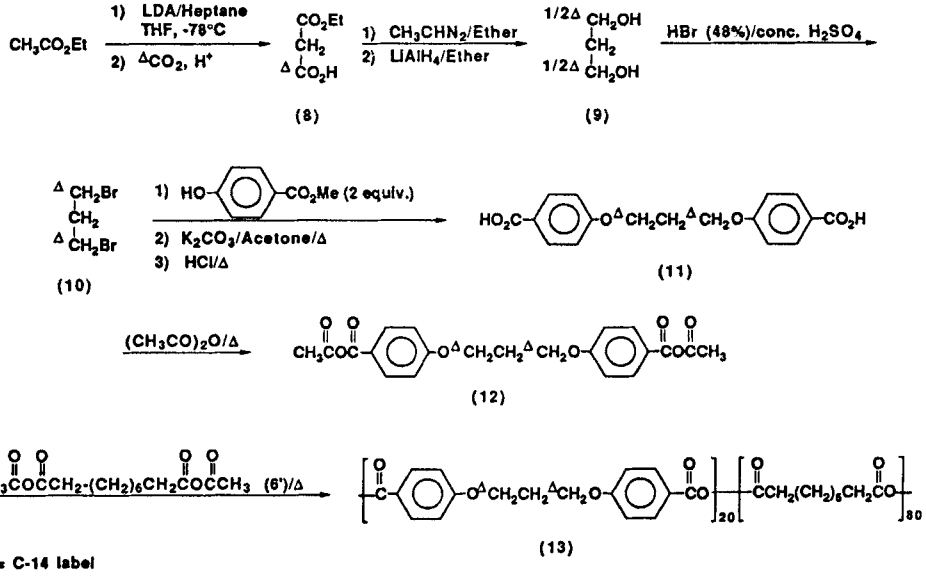


Figure 1. Co-polymerization apparatus.



Scheme I. 20:80 CPP-SA polyanhydride copolymer (C-14 labeled at SA)



Scheme II. 20:80 CPP-SA polyanhydride copolymer (C-14 labeled at CPP)

EXPERIMENTAL

Radioassays were carried out in 10 mL of Scintisol cocktail (Isolab Inc.) with internal standards and counted with a Beckman LS-250 liquid scintillation system. TLC analyses and radioautography were conducted on 20 cm TLC plates of Merck silica gel GF 254 using benzene/dioxane/acetic acid (90:15:4) for developing solvent and bromocresol purple as visualizing reagent. Analyses by HPLC were obtained from a Waters 6000A, solvent delivery system, Model 450 variable wavelength detector and Waters Ultrastyragel (Linear) gel permeation column. GLC analyses were obtained from a Hewlett-Packard 5710A gas chromatograph on a 10% DC 200 carbowax column.

Suberic Acid-(^{14}C-carboxyl) (2)

Carbonation of the Grignard reagent prepared from Mg turnings (226 mg, 5.7 mmol) and 1,6-dibromohexane (1.39 g, 5.7 mmole) in 20 mL of dry THF with $^{14}CO_2$ generated from $Ba^{14}CO_3$ (227 mCi, 4 mmole) with 15 mL of conc. H_2SO_4 gave the crude di-acid (**2**) (137 mCi) after continuous extraction with ether (2 × 200 mL). Recrystallization from acetone gave suberic acid ($^{14}C_2$-carboxyl) (107 mCi, 224 mg, 107 mCi/mmole) which was confirmed by comparison of unlabeled sebacic acid in the TLC system (Rf = 0.35).

1,8-Octanediol-1,8-$^{14}C_2$ (3)

The diacid (**2**) (71 mCi, 116 mg, 0.66 mmole) in anhydrous ether (26 mL) was refluxed with 20 mL of $LiAlH_4$ solution, (1.0 M solution in ether, Aldrich) for 18 hr. The reaction was quenched by cautious addition of water (10 mL), then was extracted with ether (3 × 30 mL). The combined ether extracts were dried ($MgSO_4$) and concentrated to give the crude diol (**3**) (60 mCi, 81.8 mg) as a white solid. The diol was characterized by comparison with unlabeled **3** in the TLC (Rf = 0.21) and, corresponding radioautography, then was used in the bromination step without further purification.

1,8-Dibromooctane-1,8-$^{14}C_2$ (4)[1,2]

Labeled diol (**3**) (60 mCi, 81.8 mg) was mixed with HBr (48%, 20 mL) and conc. H_2SO_4 (1.7 g). The resulting yellow mixture was refluxed for 24 hr then cooled and extracted with ether. Concentration of the ether extracts (3 × 20 mL) gave a reddish residue which was purified by column chromatography (4 g silica gel in CH_2Cl_2). The dibromo compound (46 mCi, 224 mg, R_f = 0.8) was used in the next step without further purification.

Sebacic Acid (SA-$^{14}C_2$) (5)

To the crude dibromo compound (**4**) in a warm solution (2 mL of H_2O and 8 mL of ethanol) was added KCN (153 mg, 2.34 mmole) in one portion and the resulting solution was refluxed for 8 hr. The resulting dinitrile without isolation was subjected to hydrolysis with conc. HCl (3 mL). After refluxing for 14 hr, the mixture was extracted with ether (200 mL) in a continuous extractor for 24 hr. The labeled crude sebacic acid (**5**) was diluted with unlabeled sebacic acid (200 mg) and was recrystallized from acetone 3 times successively. Labeled sebacic acid (^{14}C-2) (**5**) (11.29 mCi, 145 mg, 15.7 mCi/mmole) obtained as a white solid, was confirmed by comparison with an authentic sample of sebacic acid by TLC (Rf = 0.38). It had a radiochemical purity of 95%.

^{14}C-labeled Sebacic Acid Prepolymer (6)

Labeled sebacic acid (5) (9.8 mCi, 112 mg) was mixed with cold sebacic acid (1.89 g), then was added to 11 mL of acetic anhydride. After 2 hr of reflux the excess acetic anhydride was removed under reduced pressure and the resulting residue was further dried in high vacuum overnight. Without further purification, the viscous residue (7.35 mCi, 2.01 g) was used in the polymerization reaction.

20:80 CPP-SA Polyanhydride Copolymer (^{14}C-labeled at SA) (7)

Crude sebacic acid prepolymer (6) (7.35 mCi, 2.01 g, 7.02 mmole, 8 equiv.) was mixed with unlabeled CPP-prepolymer (12') (702 mg, 1.75 mmole, 2 equiv.) in the reaction kettle (see Figure 1) equipped with an argon inlet and a stirrer. The reaction kettle was immersed in on an oil bath at 180°C. After the prepolymers were melted and well stirred, a high vacuum was applied ($<10^{-2}$ mmHg). The melt polycondensation by-product—acetic anhydride—was trapped in a dry ice/acetone trap. During the polymerization, a strong argon sweep was performed for 30 seconds every 3-5 minutes. At the end of the reaction (~20 min), the kettle was removed from the oil bath and the viscous polymer was allowed to cool to room temperature under argon. The crude polymer (7) was purified by dissolving in 25 mL of dichloromethane and precipitation using 50 mL of dry petroleum ether (b.p. 35-36°). A white fiber-like, precipitate was swirled in anhydrous ether (50 mL) for 2 hrs. The ether was removed and the labeled polyanhydride (7) (3.59 mCi, 1.8 g, 1.99 μCi/mg) was obtained as a white solid.

The characterization of the pure copolymer (7) was checked out by GPC and had radiochemical purity of 97.9%.

Diethyl Malonate (^{14}C-carboxyl) (8)[3]

5 mmole equivalent $LiCH_2CO_2Et$ in THF, prepared from 2.0 M lithium diisopropylamine in THF (2.5 mL, 5.0 mmole, Aldrich) and CH_3CO_2Et (0.5 mL, 5.1 mmole) at -78°C was introduced by a syringe to a carbonation flask which contained $^{14}CO_2$ (285 mCi) generated from $Ba^{14}CO_3$ (1.028 g, 56 mCi/mmole) and conc. H_2SO_4 (20 mL). After 30 min of stirring at -78°C, the reaction was quenched by addition of saturated NH_4Cl solution (10 mL), followed by 6N H_2SO_4 (60 mL). The reaction mixture was extracted with ether (200 mL) in a continuous extractor. The crude product, mono ethyl ester of malonic acid, in ether (50 mL), after drying over anhydrous $MgSO_4$, was treated with freshly prepared ethereal CH_3CHN_2[4] solution (150 mL).

The resulting yellow solution was stirred for 2 hr at 0°C. The dried ether solution (200 mL) (Na_2SO_4) was evaporated to give the labeled diethyl malonate (8) (172 mCi, 675 mg, 40.9 mCi/mmole), confirmed in GLC by comparison with authentic diethyl malonate.

1,3-Propanediol-1,3-$^{14}C_2$ (9)[5]

Labeled diethylmalonate (172 mCi, 1.09 g, 6.25 mmole, diluted by mixing of 325 mg cold diethylmalonate) in ether (20 mL) was refluxed with $LiAlH_4$ (685 mg, 18 mmole) for 16 hr under argon. After quenching by cautious addition of 8N HCl (15 mL), the resulting mixture was extracted with ether (160 mL) in a continuous extractor. The crude diol (40 mCi, 180 mg) was identified by comparison of an authentic sample in GLC and was used in the bromination step without further purification.

1,3-Dibromopropane-1,3-$^{14}C_2$ (10)

A mixture of the crude diol (40 mCi, 180 mg), HBr (48%, 1.4 mL) and conc. H_2SO_4 (0.8 mL) was heated under reflux for 4 hr. The resulting dark brown mixture was carefully neutralized with 5% $NaHCO_3$ solution (2 × 25 mL) and extracted with ether (3 × 30 mL). The combined phases were washed with water (20 mL), dried (K_2CO_3), and concentrated under a slow stream of argon. The dibromide (22.7 mCi, 239 mg 19.17 mCi/mmole) was analyzed by GLC then was used in the next step without further purification.

CPP-$^{14}C_2$(1,3-bis(p-carboxy-phenoxy)propane-1,3-$^{14}C_2$ (11))

A mixture of the labeled dibromide (22.7 mCi, 239 mg, 1.18 mmole), acetone (20 mL), methyl-4-hydroxybenzoate (358 mg, 2.35 mmole) and K_2CO_3 (324 mg, 2.34 mmole) was refluxed for 24 hr under argon. The reaction was filtered, then the filtrate was concentrated and dissolved in dichloromethane (40 mL), washed with 5% $NaHCO_3$ solution (20 mL) and finally with 5% NaOH (20 mL). The crude product was recrystallized from EtOAc (15 mL) to give a pale yellow solid (10 mCi, 154 mg) which was identified by GLC comparison with unlabeled material. The labeled diester, without isolation, in 20 mL THF was hydrolyzed to the diacid (**11**) by refluxing for 20 hr with 3N HCl (40 mL). As the solvent was removed under reduced pressure, a pale yellow solid precipitated and was collected, washed with water (2 × 15 mL), finally with ether (2 × 20 mL). The pure CPP (**11**) (8.5 mCi, 118 mCi, 2.6 mCi/mmole) was identified in GLC by comparison with an authentic sample.

^{14}C-labeled CPP Prepolymer (12)

Labeled CPP (8.5 mCi, 118 mg) was refluxed with acetic anhydride (5 mL) for 30 min. The excess acetic anhydride was distilled off and the resulting viscous residue was further dried under high vacuum. The dried white residue (6.8 mCi, 119 mg) was used in the copolymerization step without further purification.

20:80 ^{14}C-CPP/SA Polyanhydride Copolymer (C-14-labeled at CPP) (13)

The labeled CPP-prepolymer (6.8 mCi, 119 mg, 0.29 mmole, 2 equiv.) was mixed with unlabeled SA prepolymer (**6'**) (338 mg, 1.18 mole, 8 equiv.) in the polymerization kettle. The same procedure (see preparation of **7**) was performed to give the copolymer (**13**) (4.93 mCi, 402 mg, 12.26 μCi/mg) as confirmed by GPC and has radiochemical purity of 96%.

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