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SYNTHESIS OF ^{14}C LABELLED ACRYLIC DERIVATIVES: DIETHYL [3-^{14}C] METHYLIDENEMALONATE AND ISOBUTYL [3-^{14}C] CYANOACRYLATE

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SUMMARY

*Isobutyl [3-^{14}C] cyanoacrylate and diethyl [3-^{14}C] methylidenemalonate were synthesized by the intermediate of their protective Diels-Alder adduct with anthracene. These adducts (**2a-b**) were obtained in a one-pot procedure by Knoevenagel condensation of [^{14}C] paraformaldehyde with isobutyl cyanoacetate and diethyl malonate respectively in the presence of a basic catalyst and anthracene. The adducts are stable crystalline compounds easily purified by recrystallization. The olefinic target coumpounds (**1a-b**) were obtained in high chemical and radiochemical purity (>99%) by thermolysis at 220 °C in mineral oil in the presence of maleic anhydride.*

Key words: Carbon-14, diethyl [3-^{14}C]methylidenemalonate, isobutyl [3-^{14}C]cyanoacrylate, Diels-Alder, Knoevenagel, 2-methylpropyl 2-cyano-[3-^{14}C]propenoate.

INTRODUCTION

Alkyl cyanoacrylate (**1b**) and dialkyl methylidenemalonate (**1a**) are two highly reactive derivatives of acrylic acid which are useful synthetic intermediates in Michael, Diels-Alder, cyclopropanation, and epoxidation reactions (1) as well as in polymer synthesis (2).

1a-b undergo rapid anionic polymerization in the presence of traces of water and can be polymerized in aqueous medium to yield discrete polymeric particles in the nanometer size range termed "nanoparticles" which are under investigation as submicroscopic colloidal drug carriers (3-5)

To evaluate the biodistribution and the biodegradability of the poly(ethyl methylidenemalonate)

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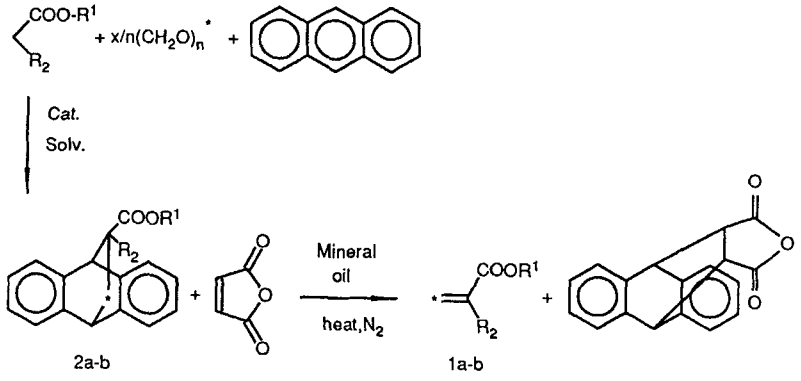
nanoparticles, we were in need for ^{14}C-labelled **1a**. The purpose of this paper is to describe the synthesis of **1a** and the extension of this approach to the synthesis of **1b**.

RESULTS AND DISCUSSION

The most frequently used method for synthesizing **1a-b** involves Knoevenagel condensation of paraformaldehyde with dialkyl malonate and alkyl cyanoacetate respectively in the presence of various basic catalysts (6). This method gives access to the polymer of **1a-b** which after thermal depolymerization yields the corresponding monomeric unit. Although this method was described in the literature for the synthesis of ^{14}C labelled **1b** (7), it proved to be somewhat cumbersome for this purpose.

We have recently developed a synthesis of **1a** via the intermediate of a protective Diels-Alder adduct (**2a**) of **1a** with anthracene (8,9) (scheme 1). **2a** was obtained in a one-pot procedure by a Knoevenagel condensation of paraformaldehyde with dialkyl malonate in the presence of anthracene. **2a** is a stable crystalline compound easily purified by recrystallization and which regenerates the monomeric unit (**1a**) by thermolysis in high boiling point mineral oil at rather low temperature (200 to 220°C). A similar approach proposed by Ray and Doran in the patent literature for the synthesis of unlabeled **1b** (10) differs from ours in that the thermolytic step was performed by refluxing **2b** for long period of time in an aromatic solvent.



	R_1	R_2
1a,2a	C_2H_5	$CO_2C_2H_5$
1b,2b	$CH_2CH(CH_3)_2$	CN

Scheme 1

This method was adapted for the synthesis of [3-^{14}C] **1a-b** with [^{14}C] paraformaldehyde as labelled starting material in order to obtain the backbone-labelled polymer after nanoparticles preparation. The preparation of **2a-b** was performed in sealed tube. Table 1 indicates the catalyst, solvent and formaldehyde molar ratio (x) employed for the synthesis of **2a-b**.

	x	Catalyst	Solvent
2a	2	Cu(OAc)2	AcOH/2-butanone
2b	1	piperidine	benzene

Table 1

The thermolytic step was performed in redistilled mineral oil (bp>190 °C, 0.2 Torr) in the presence of maleic anhydride to trap the anthracene produced, which otherwise proved to be troublesome in the distillation of **1a-b**. Although the thermolysis of **2a** did not require any anionic polymerization inhibitor, the retro-Diels-Alder reaction of **2b** did require the presence of SO_2 in order to stabilize the more reactive **1b**.

Purity (>99%) and chemical stability of **1a-b** were found to be very good. Specific activity, though not very high as can be found in the experimental section, was quite satisfactory for the *in vitro* experiments.

EXPERIMENTAL SECTION

Melting points and boiling points are uncorrected. Melting points were measured on a Reichert micro hot stage. Infrared data were obtained on a Perkin-Elmer Model 457 spectrophotometer using KBr disks. GLC was performed on a Hewlett-Packard Model 5710A gas chromatograph (column: OV17 3%; detector: FID; carrier gas: nitrogen; temperature: 100 °C for 2 min, 10 °C/min up to 300 °C). TLC was performed on silica gel/UV 254 plates with CH_2Cl_2 as eluent and scanned with a Berthold LB 27-21 thin layer scanner. Radioactivity was measured with an Intertechnique SL30 liquid scintillation counter.

[12-^{14}C]-11-bis(ethoxycarbonyl)-9,10-dihydroanthracene (2a)

A 30 ml borosilicate tube was charged with 4.25 g (0.025 mol) of anthracene, 1.5 g (0.05 mol) of paraformaldehyde, 500 µCi (18.5 MBq) of [^{14}C] paraformaldehyde (881 µCi/mg (32.6 MBq/mg), Amersham International, UK), 4 g (0.025 mol) of diethyl malonate, 0.75 g of copper (II) acetate, 9 ml of 2-butanone and 3 ml of acetic acid. The tube was sealed, enclosed in a metal safety vessel and heated in an oil bath at 95 °C for 2 h and then up to 130 °C for 3 h. The tube was then cooled to room temperature and frozen in liquid nitrogen before opening. The reaction mixture was poured under magnetic stirring into 400 ml of distilled water. After 30 min, the insoluble adduct (2a) was isolated by reverse filtration and the washing procedure was repeated twice. 2a was then solubilized in 90 ml of hot ethanol/water mixture (90/10; v/v). The solution was filtered while hot to remove the remaining anthracene. The filtrate was cooled overnight at -20 °C and the crystalline 2a was filtered and vacuum dried (4 g, 45%, mp:130-131 °C).

^{14}C- labelled 2a was identical by TLC with an authentic cold sample (9). The only contaminant revealed by UV (254 nm) was anthracene (5% estimated). The radiochemical purity of 2a (86%) was estimated by radiochromatogram scanning (figure 1). The residual radioactivity remained at the starting point.

IR data for 2a were consistent which those found for the authentic compound(9): 2980(w), 1730(s), 1460(m), 1260(m), 1235(m), 1220(m), 775(m), 760(m), 580(m) cm^{-1}.

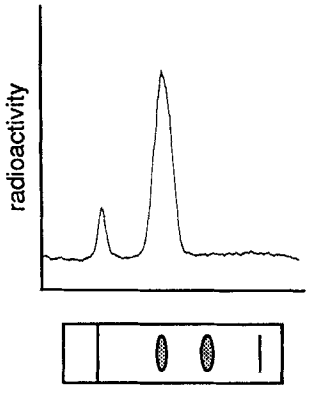


Figure 1

Radio TLC scanning of 2a.The non radioactive spot represents anthracene.

[12-^{14}C]-11-isobutoxycarbonyl-11-cyano-9,10-endoethano-9,10-dihydroanthracene(2b)

A 30 ml borosilicate tube was charged with 3.56 g (0.02 mol) of anthracene, 0.6 g (0.02 mol) of paraformaldehyde, 500 µCi (18.5 MBq) of [^{14}C] paraformaldehyde (210 µCi/mg (7.8 MBq/mg),

Amersham International, UK), 2.82 g (0.02 mol of isobutyl cyanoacrylate, 20 ml of benzene and 100 mg of piperidine. The tube was sealed, enclosed in a metal safety vessel and heated in an oil bath at 115 °C for 20 h. The tube was then cooled to room temperature and frozen in liquid nitrogen before opening. The reaction mixture was vacuum filtered to remove the unreacted anthracene. The filtrate was diluted to 50 ml with benzene and treated with 25 ml of concentrated phosphorous acid to remove the piperidine and thus avoid polymerization problem in the thermolytic step. The resulting emulsion was centrifugated at 2500 rpm for 15 min. The organic layer was separated and evaporated under reduced pressure to a yellowish oil which crystallized on standing. The product was recrystallized from 5 ml of methanol and vacuum dried to afford 2.3 g of **2b** (38 %;mp:97-98 °C). The labelled **2b** coeluted (silica gel/CH_2Cl_2) with the authentic unlabeled compound. As for **2a** the only contaminant revealed by UV was anthracene. As can be seen in figure 2, the radiochemical purity of **2b** was higher than 95%.

IR data for **2b**:2960(b), 2150(w), 1750(s), 1470(m), 1460(m), 1250(m), 1230(m), 1195(m), 1055(m), 770(m), 760(m), 740(m), 730(m), 640(m), 590(m), 570(m) cm^{-1}.

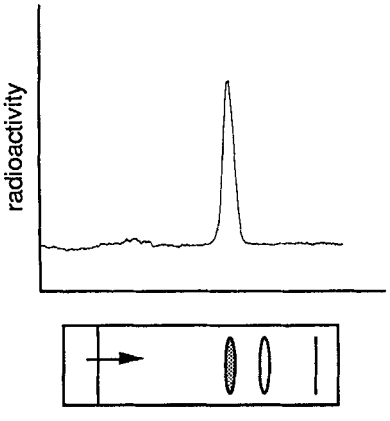


Figure 2

Radio TLC scanning of 2b. The non radioactive spot represents anthracene

Synthesis of the [3-^{14}C] monomeric units 1a-b

The procedure described below for **1a** was the same used for **1b** excepted that for the more reactive **1b** SO_2 (polymerization inhibitor) was bubbled into the reaction mixture before heating at 160°C for 45 min.The glassware was treated overnight with chromic acid cleaning mixture and flame-dried

under vacuum.A B-14 two-necked 30 ml round-bottomed flask fitted with a thermometer, a magnetic stirrer and a one-piece distilling apparatus (Vigreux column: 7x1cm) was charged under nitrogen with 1.5 g (4.3 mmol) of **1a**, 0.315 g (3.44 mmol) of powdered maleic anhydride and 15 ml of high boiling mineral oil (bp>190 °C, 0.2 mmHg). The mixture was heated while stirring at 220 °C for 45 min and allowed to cool to room temperature. While cooling, the maleic anhydride/anthracene adduct precipitated as a white crystalline material. Distillation of the reaction mixture under reduced pressure yielded **1a** (bp 60-61°C, 0.25 mmHg; 0.3 g, 40.5%). The chemical purity checked by GLC was estimated to be higher than 99%. The labelled **1a** was identical in retention time to the authentic unlabelled compound. The radiochemical purity (>99%) was evaluated by radio-TLC scanning (figure 3) of **1a** after derivatization with an excesss of bromine in CH_2Cl_2. Derivatization monitored by GLC was complete at room temperature in a few minutes. Specific activity for **1a**: 15.65 μCi/mmol(0.579 MBq/mmol). Overall radiochemical yield for **1a** :14.5 %.

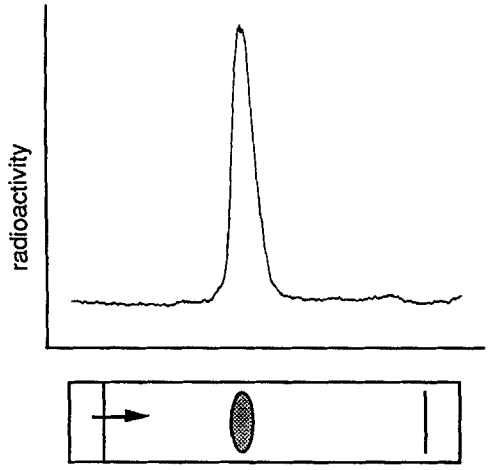


Figure 3

Radio TLC scanning of bromine derivatized 1a

The chemical purity of **1b**, measured by GLC, was higher than 99%. The derivarization, monitored by GLC, was complete in 15 min as performed for **1a**. Radio-TLC of the bromine derivatized **1b** (figure 4) did not show any contaminant. Specific activity for **1b**: 23.7 μCi/mmol (0.876 MBq/mmol). Overall radiochemical yield for **1b**: 18.6%.

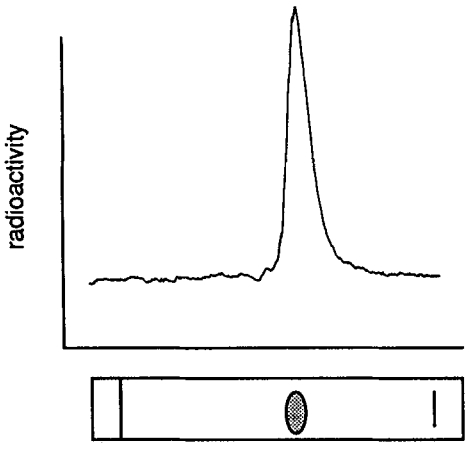


Figure 4

Radio TLC scanning of bromine derivatized ***1b***

CONCLUSION

Described in this paper is a simple and efficient synthesis of [3-^{14}C] labelled isobutyl cyanoacrylate and diethyl methylidenemalonate. Though very reactive, these compounds were obtained in high chemical and radiochemical purity without further purification procedure and should be very useful for either labelled polymer synthesis or as starting material for the synthesis of other labelled compounds. Moreover, this procedure could be extended to labelled cyanoacrylic and methylidenemalonic esters bearing other alcohol sidechains as was performed for the unlabelled methylidenemalonic esters (9) and to the production of higher specific activities. However, in view of the susceptibility of **1a-b** to radicalar polymerization, very high specific activities should not be envisaged if the material is to be stored for prolonged periods of time.

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