PREPARATION AND INVESTIGATION OF [^{14}C]PADIMATE-O AND [^{14}C]N-NITROSO-N-NOR-PADIMATE-O

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SUMMARY

Carbonation of the aryl lithium obtained from p-N,N(dimethylamino)bromobenzene with carbon-14 labeled carbon dioxide, followed by esterification gave [CO_2-^{14}C]p-N,N(dimethylamino)benzoate 2-ethylhexyl ester (Padimate-O). This material underwent slow decomposition to [^{14}C]N-nor-Padimate-O and [^{14}C]N-formyl-N-nor-Padimate-O. Nitrosation of [^{14}C]Padimate-O with sodium nitrite afforded [^{14}C]N-nitroso-N-nor-Padimate-O.

Key Words: Padimate-O, N-nitroso-N-nor-Padimate-O, N-nor-Padimate-O, N-formyl-N-nor-Padimate-O, carbon-14

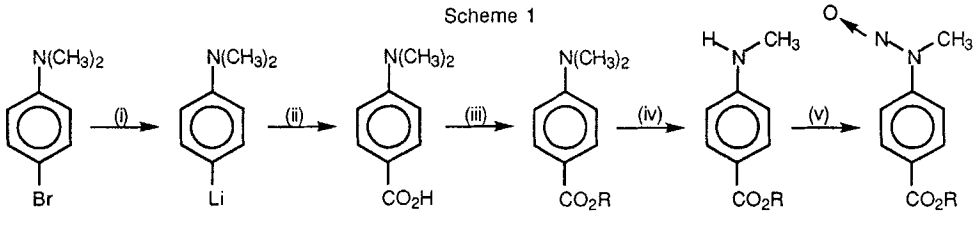
INTRODUCTION

Padimate-O, the 2-ethylhexyl ester of p-dimethylaminobenzoic acid (**1a**), is a common component of sunscreen preparations. To determine the skin penetration of Padimate-O and of its potential oxidation product, N-nitroso-N-nor-Padimate-O (**2a**), radiochemically labeled samples were required.

RESULTS AND DISCUSSION

One of the least expensive sources of carbon-14 is carbon dioxide generated from barium carbonate. Since carbonation of aromatic organometallic agents is a well-documented process, the carboxyl group of Padimate-O was selected as the preferred site for isotopic labeling with carbon-14. The synthetic route (Scheme 1) involved carbonation of the organolithium reagent **4** with carbon-14

Scheme 1



3 **4** **5** **1** **6** **2**

(i) n-BuLi
(ii) $BaCO_3$, H_2SO_4
(iii) 2-Ethylhexanol
(iv) 1. α-Chloroethyl chloroformate
2. MeOH, reflux
(v) HCO_2H, $NaNO_2$

a. R = $CH_3(CH_2)_3CHCH_2$-
CH_3CH_2

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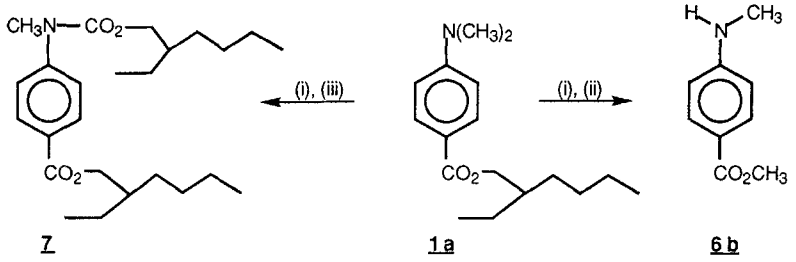
labeled carbon dioxide (from barium carbonate) to obtain the benzoic acid **5** followed by esterification to give carbon-14 labeled Padimate-O (**1a**). N-demethylation of **1a**, followed by nitrosation, was expected to afford carbon-14 labeled N-nitroso-N-nor-Padimate-O (**2a**).

Before undertaking the synthesis of carbon-14 labeled 2-ethylhexyl 4-(N,N-dimethylamino)-benzoate (Padimate-O, **1a**) and 2-ethylhexyl 4-(N-nitroso-N-methylamino)benzoate (N-nitroso-N-nor-Padimate-O, **2a**), the proposed synthetic pathway (Scheme 1) was investigated. Treatment of commercial p-bromo-N,N-dimethylaniline (**3**) with n-butyl lithium (1) followed by exposure to carbon dioxide (generated from barium carbonate) afforded p-(N,N-dimethylamino)benzoic acid (**5**) in 48% yield. Refluxing of this product with 2-ethylhexanol in the presence of borontrifluoride etherate (2) for 6 hours gave an oil. *Purification by Kugelrohr distillation provided the ester* **1a** *in 78% yield.*

In order to prepare the N-nor compound **6a** it was planned to N-demethylate Padimate-O (**1a**) using α-chloroethyl chloroformate (3,4). Although this procedure had given good results, in our hands, even with compounds containing ester groups, treatment of **1a** with α-chloroethyl chloroformate followed by reflux in methanol (Scheme 2), afforded, instead of the desired product **6a**, the analogous methyl ester **6b**. Since this product was presumably formed by *trans* esterification with the solvent methanol, the reaction was repeated using 2-ethylhexanol as solvent. This time the product isolated was the 2-ethylhexyl carbamate of N-nor-Padimate-O (**7**) which *resisted all attempts* to cleave it to the desired product **6a**.

Scheme 2



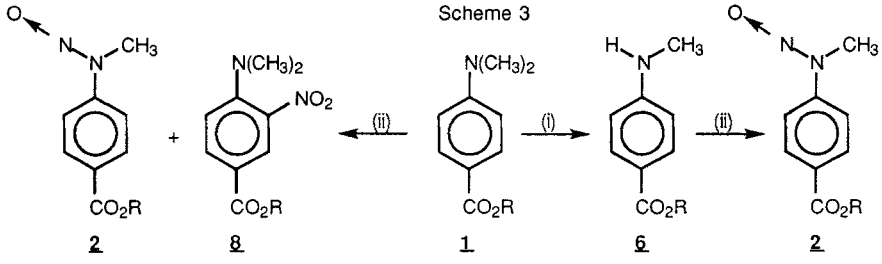
7 **1a** **6b**

(i) α-Chloroethyl chloroformate (ii) Methanol, reflux (iii) 2-Ethylhexanol, reflux

In view of these disappointing results demethylation with diethyl azodicarboxylate (DEAD) (5) was investigated (Scheme 3). Treatment of 2-ethylhexyl 4-(N,N-dimethylamino)benzoate (**1a**, Scheme 3) with diethyl azidodicarboxylate afforded the demethylated product, 2-ethylhexyl 4-(N-methylamino)benzoate (**6a**), in 87% yield, after flash chromatography. Nitrosation with one equivalent of nitrous acid gave the target compound, 2-ethylhexyl 4-(N-nitroso-N-methylamino)benzoate (**2a**), in 24% yield, after flash chromatography. Although this product (**2a**) matched an authentic sample, we were dissatisfied with the low overall yield (21%). Since the product of demethylation of 2-ethylhexyl 4-(N,N-dimethylamino)benzoate (**1a**) exhibited some extraneous ^1H NMR signals, we were concerned that the apparent high yield of **6a** (87%) was, in fact, inaccurate. We therefore prepared **6a** from commercially available 4-(N-methylamino)benzoic acid (**6c**) and nitrosated it; the product **2a** was obtained in 64%, demonstrating that, indeed, there was a problem with the intermediate

6a produced by N-demethylation of **1a**. During the handling of the secondary amino ester **6a** we also discovered that it was fairly labile, undergoing hydrolysis and *trans*-esterification quite easily. We therefore decided to explore the possibility of circumventing this intermediate entirely.

Scheme 3



2 **8** **1** **6** **2**

(i) Diethyl azodicarboxylate
(ii) $NaNO_2$, H^+

a. R = $CH_3(CH_2)_3CHCH_2$- (CH_3CH_2)
b. R = CH_3
c. R = H

Direct nitrosation to **2b** is known to occur (Scheme 3) when methyl 4-(N,N-dimethylamino)-benzoate (**1b**) is treated with sodium nitrite in aqueous hydrochloric acid, although the major reaction product is methyl 3-nitro-4-(N,N,-dimethylamino)benzoate (**8b**) (6). Since we knew the 2-ethylhexyl ester moiety to be stable in formic acid, but were concerned about its potential lability in aqueous acid, 2-ethylhexyl 4-(N,N-dimethylamino)benzoate (**1a**) was treated with sodium nitrite in formic acid. As expected (6), this produced the desired product, 2-ethylhexyl 4-(N-nitroso-N-methylamino)-benzoate (**2a**), (20% yield) along with an aromatic nitro compound (50% yield), presumably **8a**. To explore the possible retardation of the nitration reaction, several trial reactions were carried out at -20°C, utilizing organic diluents. The results of these trial reactions, which were monitored by HPLC (Table), suggested that reaction in ethyl acetate at -20°C might afford a somewhat improved yield of **2a**. It was also noted that after the yield of **2a** reached 25% (ca. 85% consumption of the starting material) the amount of nitrosation product **2a** no longer increased. Carrying out this reaction on the scale on which the radiosynthesis would be performed led to a 23% yield of **2a**. However, stopping the reaction when the amount of **2a** reached 25%, isolating the starting material **1a** and re-nitrosating, increased the yield of purified **2a** to 31%.

Table

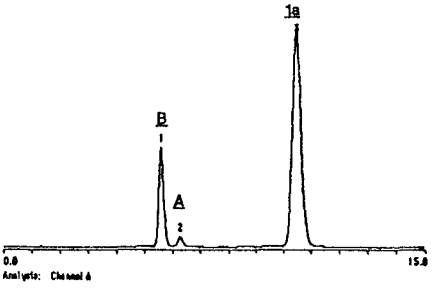
Effect of Diluents on the Reaction of 2-Ethylhexyl 4-(N,N-Dimethylamino)benzoate (**1a**) with Sodium Nitrite and Formic Acid at -20°C

	Product Composition (%)		
Diluent	**7a**	**8a**	**1a**
EtOAc	27	35	35
THF	8	73	19
CH_2Cl_2	4	13	88
CH_3CN	4	71	22
$(CH_3)_2CO$	8	39	51

Use of carbon-14 labeled barium carbonate in the procedure developed for the synthesis of **1a** afforded carbon-14 labeled p-(N,N-dimethylamino)benzoic acid (**5**) in 50% yield. Dilution with an equal amount of authentic, unlabeled p-(N,N-dimethylamino)benzoic acid followed by recrystallization afforded 13.4 mCi of pure p-(N,N-dimethylamino)benzoic acid (**5**) of specific activity 20.9 mCi/mmol in 20% radiochemical yield. Esterification of 12.1 mCi following the procedure described above, followed by extensive chromatographic purifications, gave 6.4 mCi of carbon-14 labeled Padimate-O (**1a**) (53% yield) of >98% purity. The balance of **1a** (5.7 mCi) was subjected to direct nitrosation by treatment with sodium nitrite in formic acid at 0°C. After purification, 1.77 mCi (31% yield) of **2a** was obtained.

Storage of Padimate-O (**1a**), either as a solid or in methanolic solution, for several weeks, indicated that decomposition was taking place. Analysis by both HPLC and TLC indicated the presence of two impurities, **A** and **B**, both more polar than Padimate-O (**1a**) (Figure). Isolation of these

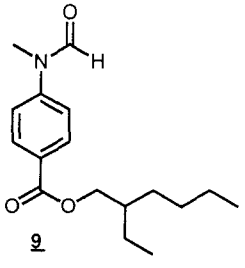
Figure
HPLC[1] of Decomposed Padimate-O (**1a**)



Time	Area (μV-sec)	Area %
5.576	2010849	19.598
6.251	202486	1.973
10.436	8046967	78.428
Total Area	10260302	99.999

[1] Waters RCM Resolve radial pak C18, 10 μm, 8 x 100 mm, 90% CH_3OH-H_2O, 1.2 mL/min, 254

compounds and spectral analysis showed the less polar of the two impurities, impurity **A**, to be 2-ethylhexyl p-N-methylaminobenzoate (**6a**). The second impurity (**B**) was identified as 2-ethylhexyl p-(N-formyl-N-methylamino)benzoate (**9**). HPLC analysis indicated that both **6a** and **9** underwent further decomposition.



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Padimate-O (**1a**) samples which contained up to 10% of these impurities were readily purified using a Sep Pak Cartridge to return 84% of >99% pure material.

EXPERIMENTAL

NMR spectra were recorded on a Bruker WM-250 or AM-500 spectrometer using tetramethylsilane as internal standard. Mass spectrometric determinations were carried out on a VGT ZAB-E ultrahigh resolution mass spectrometer in the EI$^+$ mode. TLC-radioscan analyses were performed using E. Merck Silica gel 60F-254 plates on a Berthold model LB Linear Analyzer. HPLC analyses were carried out on Rainin HPXL dual pump system with a Rheodyne injector and a IN/US System, Inc., β-RAM Flow-Through Monitor. IR spectra were recorded on Shimadzu IR-460 spectrophotometer. Samples were counted using Ultima Gold as scintillant on a Packard Tri-carb 4000 liquid scintillation spectrometer.

[^{14}C-CO_2H]p-(N,N-Dimethylamino)benzoic Acid (5). A mixture of p-bromo-N,N-dimethylaminoaniline (3) (216 mg, 1.1 mmol), n-BuLi (0.6 mL, 1.0 mmol) and anhydrous Et_2O (8 mL), in flame-dried glassware, was refluxed under N_2 for 45 min. The mixture was allowed to cool, then attached to a high vacuum manifold. Carbon-14 labeled carbon dioxide was generated by the slow addition of [^{14}C]$BaCO_3$ (235 mg, 67.9 mCi, 1.2 mmol) to H_2SO_4 (5 mL) and vacuum transferred to the reaction flask. To complete the transfer the reaction mixture was cooled by liquid N_2. The reaction mixture was then allowed to warm to 0°C and 5 mL H_2O was added. The layers were separated and the aq. layer was acidified with 5% HCl. The precipitated solid was filtered, washed with H_2O and dried under high vacuum for 16 h yielding a purple solid (88 mg, 50%). This material was diluted with authentic p-(N,N-dimethylamino)benzoic acid (93 mg) and the resulting mixture was recrystallized from EtOH. Gray needles were recovered (108 mg, 13.8 mCi); specific activity 20.85 mCi/mmol. The m.p. (235-240°C) was in general agreement with that observed for an authentic sample (241-243°C) and the ^{1}H NMR spectrum was identical to that of an authentic sample.

2-Ethylhexyl [^{14}C-CO_2H]p-(N,N-dimethylamino)benzoate (1a). A mixture of [^{14}C-CO_2H]p-(N,N-dimethylamino)benzoic acid (5) (96 mg, 12.1 mCi, 0.58 mmol), 2-ethylhexanol (1.5 mL, 9.8 mmol), and $BF_3 \cdot Et_2O$ (0.2 mL, 1.6 mmol) was refluxed for 7 h under argon. TLC (SiO_2; 2% MeOH-$CHCl_3$) indicated that the reaction was complete. The mixture was filtered through Celite into a cold solution of 5% aq. Na_2CO_3 (9.6 mL), extracted with $CHCl_3$ (2 x 5 mL), dried (K_2CO_3), and concentrated to an oil (9.6 mL) which was purified by Kugelrohr distillation (60-65°C, 0.3 mm Hg). Radio-TLC (SiO_2, $CHCl_3$) and radio-HPLC (Waters Resolve 5 μm SiO2, 3.9 x 150 mm, 6% EtOAc/hexane, 1 mL/min, 254 nm) showed the product to be 97% pure. The oil was passed through a Sep-Pak (600 mg SiO_2) eluting with 10% EtOAc/hexane. Combination of the appropriate fractions afforded 1a (6.4 mCi, 53%) shown by radio-TLC (10% EtOAc/Hexane) and HPLC (as above) to be of >98% purity.

2-Ethylhexyl [^{14}C-CO_2]4-(N-Nitroso-N-methylamino)benzoate (2a). To a solution of 2-ethylhexyl [^{14}C]4-(N,N-dimethylamino)benzoate acid ([^{14}C]-1a) (76 mg, 0.27 mmol, 5.7 mCi) in formic acid (1 mL) at 0°C was added portionwise $NaNO_2$, while monitoring by HPLC (Waters

Resolve C_{18} Radial Pak, 10 μm, 8 x 100 mm, 90% MeOH-H_2O, 1.2 mL/min, 254 nm). When the area of the product peak [^{14}C]-**2a** was 25% of the sum of all peaks, addition of $NaNO_2$ was stopped (47 mg, 0.68 mmol) and the reaction was worked up by adding the mixture to a separatory funnel containing CH_2Cl_2 (10 mL). The layers were separated and the organic phase was washed with H_2O (3 x 10 mL). The combined aqueous phase was re-extracted with CH_2Cl_2 (15 mL). The combined organic extract was dried (Na_2SO_4), filtered and evaporated. The residual yellow oil was purified by flash chromatography (SiO_2, 230-400 mesh, 10% EtOAc-hexanes). The pure fractions (TLC-RAM, SiO_2, 20% EtOAc/hexane) were combined to give 15.8 mg (20%) of the desired product [^{14}C]-**2a**. Also isolated from the chromatography was unreacted starting material [^{14}C]-**1a**. The product obtained by nitrosation of the recovered starting material, following the same procedure, was combined with impure fractions from the first reaction and chromatographed as above. Combination of the pure fractions with the previously obtained material yielded 24.8 mg (31%, 1.77 mCi) of [^{14}C]-**2a** of >99% purity (TLC-RAM and HPLC UV and RAM).

Decomposition Products of Padimate-O (1a). Flash chromatography of a sample of Padimate-O (**1a**) (350 mg), which showed the presence of two impurities (Figure) when analyzed by HPLC (Waters RCM Resolve radial pak C18, 10 μm, 8 x 100 mm, 90% MeOH-H_2O, 1.2 mL/min, 254; Waters Resolve spherical Silica, 5 μm, 3.9 x 150 mm, 94% hexane-EtOAc 1.0 mL/min, 254 nm) yielded 9 mg each of pure **A** and **B** (2.6% each). Impurity **A** coeluted with authentic 2-ethylhexyl p-(N-methylamino)benzoate (**6a**) and had ^{1}H NMR, IR and MS identical to those of **6a**. The MS of impurity **B** had a molecular ion (M+1) of m/z 292, 14 amu higher than **1a**; the ^{1}H NMR spectrum had a methyl signal integrating for only three protons and exhibited a new signal at 8.55 ppm. The ^{13}C NMR was similar to that of **1a**, but had an additional signal at 165.9 ppm. The IR spectrum showed the absence an N-H stretch. We deduced that we had a formyl derivative of **6a**.

Purification of Small Samples of Decomposed Padimate-O (1a). A solution of 90% pure Padimate-O (**1a**) in hexane was loaded onto a Sep Pak Cartridge (Waters 51900) which had been saturated with 10% EtOAc in hexane. Elution with 10% EtOAc in hexane afforded >99% pure **1a** in 84% yield.

CONCLUSIONS

The sunscreen ingredient Padimate-O (**1a**) labeled with carbon-14 at a chemically and metabolically stable site was prepared in high purity (>99%) and in 16% yield, in two steps from p-(dimethylamino)bromobenzene. Both the labeled material and its unlabeled analog were found to undergo decomposition to afford N-nor-Padimate-O (**6a**) and N-formyl-N-nor-Padimate-O (**9**). However, **1a** could be readily repurified to >99% purity using Sep-Pak chromatography. The N-nitroso derivative of N-nor-Padimate-O (**2**) of >99% purity was prepared from Padimate-O (**1a**) in 31% yield.

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