

SYNTHESIS OF TRITIUM AND CARBON-14 LABELLED LINOLEIC ACID ESTERS OF 3-PHENYLAMINO-1,2-PROPANEDIOL, COMPOUNDS POTENTIALLY INVOLVED IN THE ETIOLOGY OF THE TOXIC OIL SYNDROME

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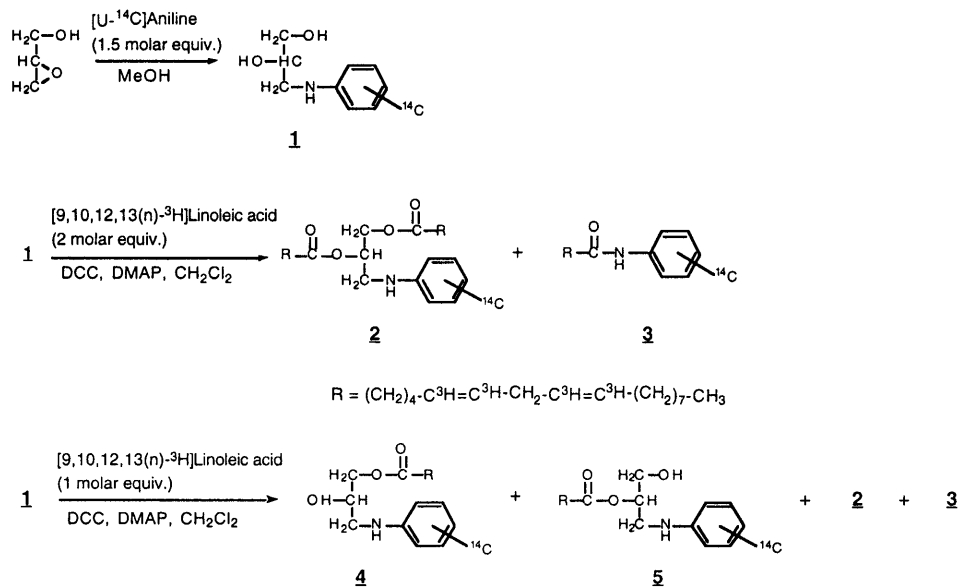
Summary. A simple, one-pot procedure for the preparation of ^3H and ^{14}C labelled mono and diesters of fatty acids with 3-phenylamino-1,2-propanediol, compounds shown to have a strong association with Toxic Oil Syndrome, is reported. Ring opening of glycidol with carbon-14 aniline in methanol led to the regioselective insertion of the phenylamino moiety at C-3. This intermediate was allowed to react with the appropriate molar equivalents of tritiated linoleic acid in the presence of DCC and DMAP to give the desired compounds in satisfactory overall yields.

Key words: Spanish Toxic Oil Syndrome. Synthesis. Tritium. ^{14}C Carbon. 3-Phenylamino-1,2-propanediol esters.

Since the Toxic Oil Syndrome (TOS) occurred in 1981, researchers around the world have pursued the etiologic agent responsible for the disease that affected 20,000 people and caused more than 800 deaths. Epidemiological investigations showed that this disaster was due to the ingestion of aniline-denatured rapeseed oil initially intended for industrial use, but diverted fraudulently for human consumption (1-3). The identification of fatty acid anilides in TOS oil samples led to the consideration of these compounds as the first markers of toxic oils (4,5). Consequently, the availability of synthetic standards of these anilides, both none and isotopically labelled, was required. Our laboratory was commissioned to fulfill this demand and the corresponding synthetic procedures to this aim were developed (6-8). Unfortunately, all efforts to

implicate these anilides and several other contaminants in the etiology of TOS have been unsuccessful. On the other hand, Vázquez Roncero *et al.* have reported the presence of 3-phenylamino-1,2-propanediol (**1**) and its mono and dioleyl esters in oils associated to TOS (9,10). Recent investigations performed by LC/MS/MS techniques showed the presence of these derivatives in aniline-denatured rapeseed oils from the refining company with the clearest link to TOS cases (11). These results, in conjunction with others reported elsewhere (see refs. in 11), suggest that 3-phenylamino-1,2-propanediol esters or their bioconversion metabolites are the most plausible causing agents of the toxic effects elicited by the contaminated oil. Once again, our laboratory was charged to supply the standards for performing analytical and toxicological studies on these derivatives. In this context, the preparation of the non labelled compounds was first accomplished (12). In the present communication, a versatile, simple one-pot method for the preparation of the title compounds is described.

SCHEME



The procedure developed for the synthesis of diester **2** and monoesters **4** and **5** is shown in the Scheme. The key step was the regioselective opening of the glycidol oxirane ring with aniline in the presence of methanol as solvent and acid catalyst to render the amino moiety linked at C-3. Parallel studies conducted with non radioactive substrates have shown that the regioselectivity of

this reaction is over 96% (HPLC monitoring). An excess of carbon-14 aniline and prolonged reaction times (20-24 hour) at 50-60 °C were required for the reaction to proceed with satisfactory conversion yields. Then, the second step, *i.e.*, the esterification of the hydroxyl residues, was performed by using the appropriate molar equivalents of the tritiated fatty acid in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). When 2 molar equivalents of acid were used, good overall yields of diester **2** were isolated; in this case, the excess of aniline present in the reaction medium led to the formation of a small amount of the corresponding anilide **3**, which could be easily separated from **2** by preparative TLC. It is worth noting that these anilides are still used as markers for toxicological and immunological studies related to TOS. On the other hand, when 1 molar equivalent of linoleic acid was used, the two desired monoesters **4** and **5** were formed in an approx. 2:1 isomeric ratio, respectively, and in satisfactory overall yields. This reaction also afforded a small amount of diester **2** and anilide **3**. All four products were isolated by preparative TLC. In conclusion, we deem that the above procedure can be generalized to any fatty acid and it opens the possibility of combining the insertion of two different acid residues, which might be of interest in connection with the metabolism studies on these 3-phenylamino-1,2-propanediol derivatives.

Experimental Section

Materials

Aniline (Aldrich, 99%) and glycidol (Merck, 98%) were previously distilled and stored at -20°C. Linoleic acid (>99%) was from ICN. *N,N*-Dicyclohexylcarbodiimide and 4-dimethylaminopyridine were from Fluka. Solvents were all of analytical grade and used as received. [¹⁴C]Aniline hydrochloride (129 mCi/mmol) was from Amersham and [9,10,12,13(n)-³H]linoleic acid (40 Ci/mmol, 1 mCi/mL, ethanol solution) was from ARC.

Thin layer chromatography (TLC) analyses and purifications were performed on Merck Kieselgel 60 F₂₅₄ plates (aluminum sheets, 0.2 mm thickness, and glass plates, 0.5 mm thickness), and were revealed by UV irradiation at 254 nm and by immersion in anisaldehyde solution (2:2:100 v/v conc. H₂SO₄/*p*-methoxybenzaldehyde/ethanol), followed by heating at 120 °C. Radioactivity was determined with a LKB 1217 Rackbeta scintillation counter following the addition of 10 mL Optiphase Hi Safe II cocktail. Appropriate windows were adjusted for achieving a maximum

discrimination between ^3H and ^{14}C . All evaporations of solvents and excess of volatile reagents were conducted under nitrogen atmosphere in a well ventilated hood provided with the adequate filter systems.

Synthesis of 3-[[U- ^{14}C]phenyl]amino-1,2-propanediyl di[9,10,12,13(n)- ^3H]linoleate (2)

A solution of [U- ^{14}C]aniline hydrochloride (160 μCi in methanol) was evaporated to dryness and the residue was redissolved in *tert*-buthyl methyl ether (1 mL). This solution was washed with 0.1 N NaOH (200 μL). The organic phase was separated and evaporated to render free [U- ^{14}C]aniline. To this residue, aniline (14 mg, 0.15 mmol), glycidol (7.4 mg, 0.10 mmol) and methanol (230 μL) were added and the mixture was stirred overnight at 50 $^{\circ}\text{C}$. Then the temperature was increased to 60 $^{\circ}\text{C}$ and the stirring was prolonged for 4 hours. The reaction course was followed by TLC using a parallel reaction developed under identical conditions, but with no radioactive aniline. The residue obtained from the evaporation of solvent was redissolved in dichloromethane (200 μL) and reevaporated, and this operation was repeated to ensure that all methanol was eliminated. By this procedure, a residue containing aniline and the expected 3-[[U- ^{14}C]phenyl]amino-1,2-propanediol (**1**), as major products, was obtained.

In a separate vial, 125 μL of a solution of [9,10,12,13(n)- ^3H]linoleic in ethanol was evaporated. The residue (approx. 125 μCi) was diluted with a solution of linoleic acid (56 mg, 0.20 mmol) in dichloromethane (200 μL) and this solution was added to the crude reaction mixture of diol **1** obtained above. Then a solution of DCC (45 mg, 0.22 mmol), DMAP (1.2 mg, 0.01 mmol) in dichloromethane (400 μL) was also added to the reaction vial and the mixture was stirred at room temperature for 20 hours. As above, a parallel reaction without the radioactive reagents was used to control the reaction course. The crude reaction mixture was filtered, washed thoroughly with dichloromethane and evaporated. The residue was redissolved in chloroform and spotted onto two silica 0.5 mm thickness glass plates. Three elutions were performed using 10:1, 7:1 and 5:1 hexane:ethyl acetate mixtures, respectively. The fractions obtained were visualized by irradiation with 254 nm UV light and the major products were recovered by careful scrapping and extraction with chloroform. The combined eluates were evaporated to dryness to render 23 mg of pure diester **2** (33% overall yield). The specific activities obtained for this diester were of 0.9 mCi/mmol for ^{14}C (30 μCi), and of 1.2 mCi/mmol for ^3H (40 μCi). A second product isolated was [U- ^{14}C]N-phenyl[9,10,12,13(n)- ^3H]linoleamide (**3**) (yellow oil, 17 mg, 24% yield from linoleic acid; 0.54

mCi/mmol, 25.6 μ Ci, for ^{14}C , and 0.56 mCi/mmol, 27 μ Ci, for ^3H). For the chemical description of diester 2 and anilide 3, see refs. 12 and 6, respectively.

Synthesis of the [9,10,12,13(n)- ^3H]linoleic acid monoesters of 3-[[U- ^{14}C]phenyl]amino-1,2-propanediol (4 and 5)

These compounds were obtained by using the same procedure described above with slight modifications. Briefly, free [U- ^{14}C]Aniline (approx. 300 μ Ci) was diluted with aniline (14 mg, 0.15 mmol), and treated with glycidol (7.4 mg, 0.10 mmol) in methanol solution (400 μ L). The mixture was stirred overnight at 50 $^{\circ}\text{C}$ and for 4 hours at 60 $^{\circ}\text{C}$. The reaction course was monitored as indicated above and the crude reaction mixture was treated also as detailed above to render a residue containing the excess of aniline and the expected 3-[[U- ^{14}C]phenyl]amino-1,2-propanediol 1 as major products.

The esterification step was performed by using linoleic acid (125 μ Ci of the labelled sample which was diluted with 28 mg, 0.10 mmol, of the non radioactive substrate), DCC (23 mg, 0.11 mmol), DMAP (1.2 mg, 0.01 mmol) and dichloromethane (400 μ L). The mixture was stirred at room temperature for 15 hours. Work-up and purification of the crude reaction mixture by preparative TLC (two silica 0.5 mm thickness glass plates, 2 elutions by employing 4:1 and 3:1 hexane:ethyl acetate mixtures, respectively), gave as pure compounds: 15 mg of the linoleic acid ester at C-1 4 (12) (white solid, 35% yield from glycidol), and 7 mg of the ester at C-2 5 (12) (yellow oil, 16% yield from glycidol). In addition, 4 mg of the corresponding diester 2 (6% yield from glycidol), and 9 mg of anilide 3 (25% yield from linoleic acid), were obtained as side products. The specific activities of these compounds were as follows:

Cmpd.	^{14}C		^3H	
	(mCi/mmol)	Total μ Ci	(mCi/mmol)	Total μ Ci
<u>4</u>	0.57	20.1	1.0	34.9
<u>5</u>	0.47	7.6	0.85	13.6
<u>2</u>	0.78	4.5	1.62	9.4
<u>3</u>	0.47	12.0	0.86	21.6

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