NOTE

SYNTHESIS OF N-[ring-G-^{3}H] PHENYLLINOLEAMIDE AND N-PHENYL[1-^{14}C]LINOLEAMIDE AS LABELLED STANDARDS FOR SPANISH TOXIC OIL SYNDROME STUDIES

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The anilides of fatty acids, particularly those of oleic and linoleic, have recently been subject of interest for their presumed implication in the mechanism of toxicity of the epidemic disease known as Spanish Toxic Oil Syndrome (1-4). Although still a matter of controversy, detection of these compounds, collectively referred as "oleoanilides", in significant amounts in aniline denatured rapeseed oil batches which were later exposed to different fraudulent refining and mixing treatments to make them suitable for human consumption, has led to the establishment of a reasonable link between these oleoanilides and the ethiopathogenesis of the disease (2). Consequently, interest on the availability of these compounds for biochemical and toxicological studies has demanded the development of procedures for the preparation of both non radioactive and isotopically labelled standards of high purity requirements. A previous study carried out in these laboratories for the case of non labelled anilides showed that the procedure involving the reaction of the corresponding acid chloride with aniline in the presence of a tertiary amine should be the one to be used to fulfill the demanded purity requirements (5). The present report describes

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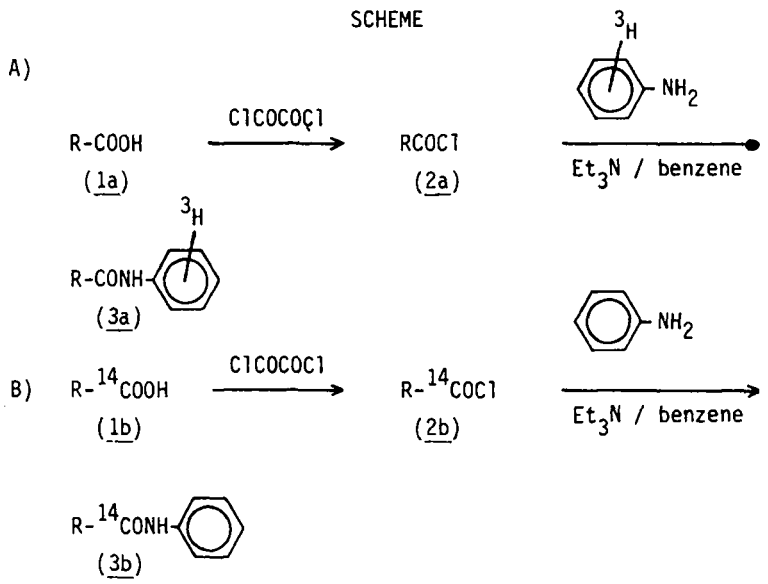
the application of the above procedure to the synthesis of two isotopically labeled anilides of linoleic acid, namely, N-[ring-G-^{3}H]phenyllinoleamide (3a) and N-phenyl[1-^{14}C]linoleamide (3b) (see Scheme)*

* Concerning the preparation of isotopically labeled anilides, Mancha et al. have recently reported the synthesis of N-[ring-G-^{3}H] Phenyl [1-^{14}C] oleamide (6).

SCHEME



R = CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-$(CH_2)_7$-

EXPERIMENTAL

Aniline (puriss. p.a.), linoleic acid (puriss., 99%) and oxalyl chloride were from Merck. Triethylamine was from Fluka A.G.. Solvents were all of analytical grade and were used as received. [ring-G-^{3}H] Aniline (25 mCi, 163 mCi/mmol) was from Amersham International plc and [1-^{14}C] linoleic acid (1mCi, 59 mCi/mmol, as specified by the supplier) was from New England Nuclear. Infrared spectra were recorded in a Perkin Elmer 399B spectrometer.

Thin layer chromatography (TLC) analyses were performed on Merck precoated Silica Gel 60K-254 plates (aluminum sheets, layer thickness 0.2 mm for analytical controls and 0.25 mm thick glass plates for preparative separations). Spots were revealed by UV irradiation at 254 nm.

High performance liquid chromatography (HPLC) analyses were carried out with a Kontron system provided with two Model 414 pumps, a programmer series 200 and a Uvikon 722 UV detector, and using a 30 cm x 0.4 cm i.d. column packed with μ-Bondapack C-18 (10 μm). The mobile phase was an acetonitrile: acetic acid buffer (pH 3.4) mixture operating with a linear gradient from 75% to 95% acetonitrile in 12 min at a constant flux of 1.5 ml/min. Eluted fractions were monitorized by UV detection at 198 and 280 nm. Radiochromatograms to estimate radiochemical purities of compounds synthesized were obtained from 200 μl aliquotes corresponding to HPLC fractions of 30 sec. Radioactivity was determined with a LKB 1217 Rackbeta scintillation counter following the addition of 8 ml Unisolve cocktail (Koch Light).

N-[ring-G-^{3}H] Phenyllinoleamide (3a).

A mixture of linoleic acid (1a, 1.40 g, 5 mmol) and excess of oxalyl chloride (2.7 ml, 30 mmol) was allowed to react in a dry atmosphere for 12 h at room temperature. The residue obtained after removal of unreacted oxalyl chloride under reduced pressure was distilled bulb-to-bulb to give 1.32 g (125-30°/0.2 Torr) of pure linoleyl chloride (2a, 88% yield). IR (CCl_4: 3000, 2960, 2850, 1800, 1460 and 1400 cm^{-1}.

To a 25 ml round-bottomed flask containing a mixture of linoleyl chloride (2a, 0.17 g, 0.57 mmol) and triethylamine (70 μl, 0.5 mmol) in benzene (3 ml), was added with external cooling a solution of tritiated aniline (25 mCi, 0.15 mmol) in the same solvent (1 ml) and the mixture was stirred in a dry atmosphere for 3 h at room temperature. After dilution with diethyl ether (15 ml), the organic solution was washed with 1N hydrochloric acid (1 x 3 ml), 1N sodium hydroxide solution (2 x 3 ml), brine (1 x 3 ml) and dried over magnesium sulphate. The residue obtained after solvent removal under N_2 was purified by preparative TLC, eluting with hexane: ethyl acetate/ 2:1 to give a colourless oil (43.4 mg, Rf 0.5) identified as tritiated linoleanilide (3a) by comparison with an authentic non radiactive standard previously prepared (5). HPLC analyses of an aliquot gave a 94% chemical

purity (which implied a 75% yield in the conversion). A radiochemical purity over 97% was calculated from radiochromatographic analysis carried out as above described (specific activity 154 mCi/mmol).

N-Phenyl [1-^{14}C] linoleamide (3b).

An excess of oxalyl chloride (2 ml) was added to a 25 ml round-bottomed flask containing [1-^{14}C] linoleic acid (1b, 0.5 mCi, 0.008 mmol, obtained from evaporation to dryness, under N_2, of the ethanolic solution commercially supplied), and the mixture was stirred in a dry atmosphere for 15 h at room temperature. Excess of oxalyl chloride was carefully removed under N_2 and the residue, which contained the crude labelled linoleyl chloride (2b), was dissolved in benzene (2 ml). A solution of triethylamine (13 µl, .009 mmol) in benzene (1 ml) and a solution of aniline (30 µl, 0.3 mmol) in the same solvent (1 ml) were added subsequently and the mixture was stirred for 3 h at room temperature. Working-up and purification of the crude reaction mixture were carried out as described above for preparation of (3a) except that washings with sodium hydroxide solution were excluded. In this case preparative TLC afforded a purified fraction of the labelled linoleanilide (3b) (1.5 mg, overall chemical yield from the corresponding acid 1b: 53%). Chemical and radiochemical purity controls of (3b) performed as described for (3a) gave 98% and 99%, respectively. (Specific activity was 62 mCi/mmol, as measured under our counting conditions).

Preliminary data obtained on the stability of these compounds on storage in diluted acetonitrile solution at -40° during three months, have revealed a 6% of decomposition for tritiated derivative (3a), whereas lower values have been achieved in the case of ^{14}C-labelled anilide (3b) (2%). In both cases, HPLC radiochromatograms showed the presence of radioactive eluates which correspond to compounds of increased polarity probably arising from the oxidation of the fatty acid moiety.

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