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NOTE

AN IMPROVED SYNTHESIS OF LABELLED FATTY ACID CARBOXAMIDES. *N*-PHENYL [9,10(n)-^{3}H] OLEAMIDE AND *N*-[ring-G-$^{13}C_6$] PHENYLOLEAMIDE AS STANDARDS FOR SPANISH TOXIC OIL SYNDROME STUDIES.

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Summary. A one-pot procedure for the high yield preparation of title compounds is reported. The key features involve the conversion of the fatty acid into the corresponding mixed anhydride by reaction with ethyl chloroformate, followed by reaction with aniline, using acetonitrile as solvent to ensure homogeneous reaction mixtures and keeping reaction times short to avoid the formation of undesirable side products.

Key words: *N*-Phenyl[9,10(n)-^{3}H]oleamide. *N*-[ring-G-$^{13}C_6$]Phenyloleamide. Synthesis. Amides. Spanish Toxic Oil Syndrome.

Despite efforts carried out in recent years to find out the causal toxicity mechanism of the epidemic disease known as Spanish Toxic Oil Syndrome (1-3), numerous questions remain still unresolved. Among them, the implication of anilides of fatty acids in the ethiopathogenesis of the disease has not been possible to confirm or rule out definitively. Therefore, interest on the availability of these compounds for biochemical and toxicological studies has demanded a continuous synthetic effort for furnishing these anilides, particularly as labelled standards.

In the present communication we report an improved method over our

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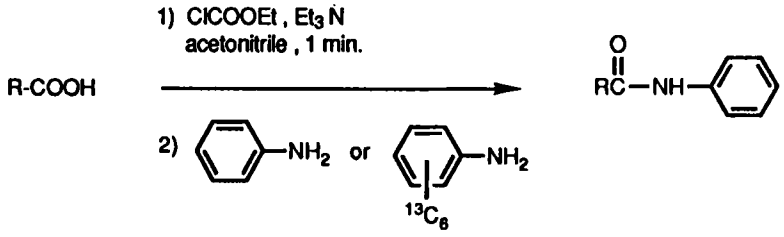
previous reported procedure (4,5), for the preparation of labelled fatty acid carboxamides (Scheme). This is based on a methodology widely used in peptide synthesis and can be carried out in a one-pot reaction with simple and rapid manipulation. The synthetic pathway involves the *in situ* formation of a mixed anhydride from the fatty acid and ethyl chloroformate in the presence of base, followed by the addition of the amine. The procedure has been optimized in two fundamental aspects. Firstly, use of acetonitrile as solvent maintains a homogeneous reaction through the whole process, thus facilitating the course of the involved reactions. Use of less polar solvents (toluene or ether) led to incomplete conversion of the fatty acid and to the concomitant formation of side products coming from the reaction of the chloroformate with the amine (particularly the corresponding carbamate). Secondly, the reaction time was kept to a minimum. We found that one minute was sufficient to achieve a total conversion of the starting fatty acid into the activated intermediate and thus avoiding further reaction of this intermediate with ethyl chloroformate to yield the corresponding ethyl ester.

As representative examples of the above procedure, we report the preparation of title compounds, which are standards for the above mentioned toxicology studies and represent cases where either the fatty acid or the aniline are the labelled starting substrates. Labelled oleamides were characterized by comparison with authentic standards available in our laboratory.

SCHEME



R = CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$- or CH_3-$(CH_2)_7$-CT=CT-$(CH_2)_7$-

EXPERIMENTAL

MATERIALS AND METHODS

Aniline (puriss. p.a.), oleic acid (puriss., 99%), triethylamine (puriss. p.a.) were from Fluka A.G. Ethyl chloroformate (99%) was from Janssen. Solvents were all of analytical grade and used as received. [ring-G-$^{13}C_6$]Aniline (99%) was from Cambridge Isotope Laboratories. [9,10(n)-^{3}H]Oleic acid (10 Ci/mmol) was from Amersham International.

Thin layer chromatography (TLC) analyses were performed on Merck precoated Silica gel 60K-254 plates (aluminum sheets, 0.2 mm thickness and glass plates 0.5 mm thickness). Spots were revealed by UV irradiation at 254 nm and with a RITA TLC radioscanner (Isomess, Germany). Radioactivity was determined with a LKB 1217 Rackbeta scintillation counter following the addition of 10 mL Optiphase Hi Safe II cocktail (LKB). Gas chromatography (GC) analyses were carried out with a Hewlett-Packard 5890 fitted with bonded phase capillary column (30 m, SPB-5, Supelco). Nuclear magnetic resonance (NMR) spectra were recorded in neutralized $CDCl_3$ solutions with a Varian Unity 300 spectrometer and chemical shifts are given in ppm downfield from tetramethylsilane. The gas chromatographic and mass spectrometric (GC-MS) determinations were carried out with Hewlett-Packard 5985 A spectrometer coupled to a 5890 Hewlett-Packard apparatus (25 m, OV-101 capillary column).

***N*-[ring-G-$^{13}C_6$]Phenyloleamide.** Triethylamine (16.5 µL, 120 µmol) and ethyl chloroformate (11.5 µL, 120 µmol) were added to a solution of oleic acid (33 mg, 120 µmol) in acetonitrile (1 mL) and the mixture was stirred for 1 minute at 20 °C. [ring-G-$^{13}C_6$] Aniline (9 µL, 100 µmol) was added and the homogeneous crude reaction mixture was stirred a further 10 minutes. Solvent and excess of reagents were evaporated under nitrogen and the residue was redissolved in a 1: 1 hexane:^{t}butyl methyl ether (10 mL) and washed with 1N HCl (2 x 3 mL), water (3 mL), brine (3 mL) and dried over $MgSO_4$. The residue obtained after solvent removal was purified by

preparative TLC eluting with a 4:1 hexane:ethyl acetate mixture to give 31 mg of the labelled oleanilide (86% yield; > 98% chemical purity by capillary GC, one spot by TLC). ^{1}H NMR: 0.94 (t, 3H, J = 7.5 Hz), 1.1-1.2 (22H), 1.65-1.8 (m, 2H, CH_2-CH_3), 1.95-2.05 (4H, allylic), 2.34 (t, 2H, J = 7.8 Hz, CH_2-CO), 5.15-5.20 (2H), 6.8-7.8 (6H, ArH, NH as broad complex absorptions); ^{13}C NMR: 14.115, 22.692, 25.629, 27.188, 27.243, 29.140, 29.267, 29.297, 29.326, 29.449, 29.553, 29.592, 29.723, 31.916, 37.861, 119.715 (dt, J_1 = 75 Hz, J_2 = 10 Hz, 2 *o* -Ar-C), 124.065 (dt, J_1 = 52 Hz, J_2 = 10 Hz, *p* -Ar-C), 129.000 (dt, J_1 = 60 Hz, J_2 = 10 Hz, 2*m* -Ar-C), 137.963 (dt, J_1 = 63 Hz, J_2 = 9 Hz, *ipso*-Ar-C), 171.54 (CONH) (olefin carbon atom absorptions masked under the aromatic absorptions); MS, m/z (%): 363 (M^+ in agreement with the presence of six ^{13}C atoms, 331, 141, 99).

***N*-Phenyl[9,10(n)-^{3}H]oleamide.** A solution of [9,10(n)-^{3}H]oleic acid (10 Ci/mmol, 5 mCi/mL, 0.057 mg, used as received, with a 96% radiochemical purity according to TLC/radioscanner analysis) in toluene (0.4 L) was evaporated under nitrogen in a 3 mL conic vial. The residue was redissolved in acetonitrile (100 µL) and diluted with a solution of oleic acid (1.071 mg) in 100 µL of the same solvent (total amount of oleic acid 4 µmol). Then, 1.7 µL (12 µmol) of triethylamine and 1.2 µL (12 µmol) of ethyl chloroformate were added to the crude reaction mixture. After standing for 1 minute at 20 °C, the homogeneous crude mixture was treated with 0.9 µL (12 µmol) of aniline and reaction was prolonged a further 10 minutes. Excess solvent and reagents were evaporated under nitrogen and the residue was dissolved in 0.5 mL of a 1:1 isooctane:^{t}butyl methyl ether solvent mixture and washed with 1N HCl (0.3 mL), water (0.3 mL) and dried over $MgSO_4$. A TLC (3:1 hexane:^{t}butyl methyl ether eluent mixture) / radioscanner analysis revealed that the spot corresponding to the oleanilide accounted for over 93% of the total radioactivity present. The residue obtained after filtration and solvent evaporation under nitrogen was redissolved in chloroform and spotted onto a silica TLC aluminum sheet and eluted with the above eluent mixture. The

olcanilide zone was visualized by UV light and the product was recovered from the silica by careful scrapping and extraction with chloroform (radioscanner monitoring). The combined eluates were evaporated and the residue, which contained the radiolabelled oleanilide, was stored in toluene solution at -20 °C.

The radiochemical yield, determinined by scintillation counting of aliquotes in different dilutions, was over 90%. The radiochemical purity was greater than 93% (TLC / radioscanner monitoring). There was no free oleic acid and most of the remaining radioactivity was due to the impurity already present in the tritiated oleic acid batch. Specific activity for the isolated oleanilide was 0.5 Ci/mmol. Finally, chemical purity was determined to be over 95% by TLC.

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