SYNTHESIS OF 3-METHYL-4-PHENYL-3-BUTENAMIDE-1-^{14}C AND N,N-DIDEUTERO-3-METHYL-4-PHENYL-3-BUTENAMIDE.

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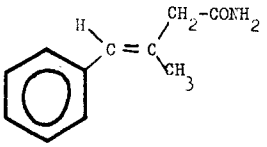
SUMMARY

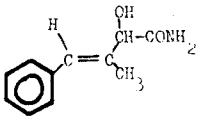
The syntheses of 3-methyl-4-phenyl-3-butenamide, an hypolipemic compound, labelled 1-^{14}C (VIII) and N,N-dideutero (XI) are described. The 2-methyl-3-phenyl-2-propenyl chloride (VI), reacting with cuprous cyanide-^{14}C, gave the 3-methyl-4-phenyl-3-butenonitrile-1-^{14}C (VII) which, treated with hydrochloric acid-water, gave the 3-methyl-4-phenyl-3- butenamide-1-^{14}C (VIII). The 3-methyl-4-phenyl-3-butenonitrile (X), treated with hydrochloric acid-D_2O, gave the N,N-dideutero-3-methyl-4-phenyl-3--butenamide (XI).

INTRODUCTION

The 3-methyl-4-phenyl-3-butenamide (I) is an hypolipemic compound[1-4], by reducing, at least in part, the biosynthesis of cholesterol and fatty acids[3]



The amide (I) is metabolized in rat and rabbit giving the following compounds[5]:



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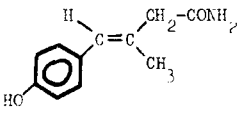

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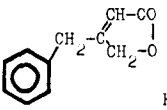

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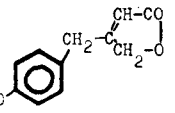

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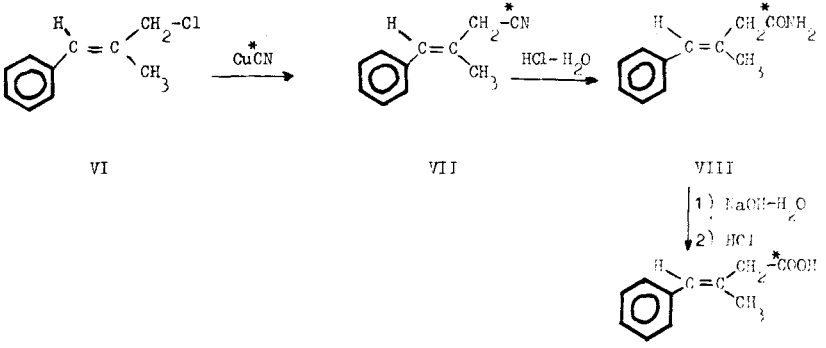
The mechanism of the biological lactonization of the amide (I) into the lactone (IV) has been suggested[6]. The stereochemistry of the compound (I) has been studied[7].

In order to confirm our previous results, regarding the metabolism of the amide (I), and in order to study more in detail such a metabolism(other minor metabolic products and mechanism of their formation), it was necessary to prepare the labelled compound I. The syntheses of 3-methyl-4-phenyl-3-butenamide-1-^{14}C(VIII) and N,N-dideutero-3-methyl-4-phenyl-3-butenamide (XI) are described in this report.

RESULTS

2-methyl-3-phenyl-2-propenyl chloride (VI), reacted with cuprous cyanide-^{14}C(obtained from sodium cyanide-^{14}C and cupric sulphate in the presence of sodium bisulphite), gave the 3-methyl-4-phenyl-3-butenonitrile-1-^{14}C (**VII**) ; the nitrile (VII),treated with hydrochloric acid and water, gave the 3-methyl-4-phenyl-3-butenamide-1-^{14}C (VIII).

The amide (VIII), hydrolized with sodium hydroxide, gave the 3-methyl-4-phenyl-3-butenoic acid-1-^{14}C (IX).

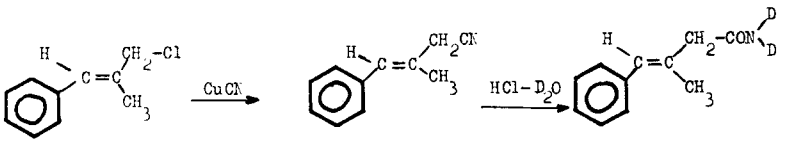


VI VII VIII

IX

* ^{14}C label

2-methyl-3-phenyl-2-propenyl chloride (VI), reacted with cuprous cyanide, gave the 3-methyl-4-phenyl-3-butenonitrile (X), which, treated with hydrochloric acid and D_2O, gave the N,N-dideutero-3-methyl-4-phenyl-3-butenamide (XI)



VI X XI

EXPERIMENTAL

Materials and methods. Melting and boiling points are uncorrected, infrared spectra were determined in nujol with Perkin-Elmer mod.157G, ultraviolet spectra were measured in methanol with Beckman DU G 2400.

Nuclear magnetic resonance spectra were recorded on a Perkin-Elmer R-10(60 Mc) spectrometer with deuterated pyridine as solvent and tetramethylsilane as internal reference: chemical shifts are reported in delta values, parts per milion(multiplicity, J, number of protons and attribution are indicated in parentheses).

Paper chromatography was performed on propylene glycol-methanol(1:3) impregnated Whatman N.1 paper, descending technique,using as eluent benzene-water-methanol-2:2:1 V/V(top phase), 100 µeg of compound in 10 µcl of methanol, and examining the chromatogram with ultraviolet light.

Thin layer chromatography was performed on silica gel PF 254 of Merck, thickness 0.25 mm, ascending technique, using eluent, acetonitrile-water 95:5 V/V, 20 µeg of compound in 10 µcl of methanol, time of elution 30 min. and examining the chromatoplate with ultraviolet light (λ max 254 mµ).

Mass spectra were recorded on a LKB 9000 gas chromatograph-mass spectrometer at 70 eV, using a glass column 4 mm i.d. packed with chromosorb W-2.5% E.G.S. at the following temperatures: flash heater 250° , column 220° , molecular separator 250° , ion source 290° .

Gas-liquid chromatography was performed on Perkin-Elmer mod. 990 gas-chromatograph, using a glass column packed with SE 30 1% on chromosorb G, pressure of air 2 atm., pressure of H_2 2 atm., pressure of N_2 2 atm., detector flame ionization, column 120°, detector 250°, injector 250°.

3-methyl-4-phenyl-3-butenonitrile-1-^{14}C (VII)

To a solution of 18.85 g of $CuSO_4$ $5H_2O$ (75.4 mmole) in 60 ml of water, it was added 9.8 ml of $NaHSO_3$ 40% W/V in water (37.7 mmole) and then, immediately, 2.84 g of NaCN (58 mmole) and 975 μCi of Amersham Na^{14}CN dissolved in 30 ml of water.

After having cooled 2 hours at +5°, the Cu^{14}CN was filtered, washed with distilled water (4 x 10 ml), dried at 40° under vacuo in the presence of P_2O_5.

4.8 g of Cu^{14}CN, specific activity 0.183 μCi/mg, were obtained.

Chemical yield 93%, radiochemical yield 90%.

To a solution of 13.45 g of 2-methyl-3-phenyl-2-propenyl chloride (VI)[7] (81 mmole) in 25 ml of chloroform, it was added 4.79 g of Cu^{14}CN (54 mmole, specific activity 0.183 μCi/mg). The mixture was stirred and refluxed for 7 hours. After 20 hours at 20°, 10 g of Na_2CO_3 dissolved in 70 ml of water, were added; after having stirred 2 hours at 20°, the solid product was filtered and washed 2 x 20 ml of chloroform.

From the limpid filtrate, the chloroformic phase was separated, washed with 10 ml of water to a neutral reaction, dried on Na_2SO_4 and evaporated in vacuo to dryness.

The residue was distilled at 10 mm of residue pressure collecting the product at 136-140°: 6.5 g of 3-methyl-4-phenyl-3-butenonitrile-1-^{14}C (VII) were obtained, λ_{max}^{MeOH} 242 mμ (ε 13,600), I.R. bands at 2220, 1720, 1610, 1500, 750, 705 cm^{-1}, retention time in gas-liquid chromatography 10 min.

The product was G.L.C. pure, and had SA 0.095 μCi/mg.

Chemical yield (based on used Cu^{14}CN) 77%, radiochemical yield 70%.

3-methyl-4-phenyl-3-butenamide-1-^{14}C (VIII)

To 20 ml of HCl 35% in water, 6.3 g of 3-methyl-4-phenyl-3-butenonitrile-1-^{14}C

(VII)(40 mmole, specific activity 0.095 μCi/mg) were added.
With stirring, HCl gas was passed into solution for 5 hours at 0° + 5°.
The solution was neutralized with NaOH 30% in water at 0°, the solid product was filtered and washed 2 x 10 ml of benzene, dried in vacuo at 40° in the presence of P_2O_5. It was obtained 5.8 g of raw 3-methyl-4-phenyl-3-butenamide-1-^{14}C with mp 129-131°, λ_{max}^{MeOH} 204 mμ(ε 20,800) and 244 mμ (ε 14,800), I.R. at 3400, 3200, 1660, 1180, 750 and 705 cm^{-1}, only one spot in the paper chromatography(Rf 0.6).

The product was crystallized with 50 ml of acetone and gave 4.75 g of pure 3-methyl-4-phenyl-3-butenamide-1-^{14}C (VIII), mp 132-133°, λ_{max}^{MeOH} 204 mμ(ε 21,350) and 245 mμ (ε 15,500), I.R. bands at 3400, 3200, 1660, 1180, 750, 705 cm^{-1}, in nuclear magnetic resonance spectrum bands at 2.07(doublet, J = 1.5 cps, 3H, CH_3-C=CH-), 3.35(singlet, 2H, $-CH_2$-CO-), 6.60 (broad singlet, 1H, -C=CH-), 7.32 (broad singlet, 5H, aromatic protons), 8.0 (very broad singlet, 2H, $-CONH_2$);mass spectrum: m/e 175 (molecular ion), 132, 131, 117, 116, 115, 91,59.

The product had only one spot in paper chromatogtaphy(Rf 0.6) and in thin layer chromatography (Rf 0.8), had SA 0.071 μCi/mg.

A sample of the product was crystallized twice more from acetone and gave SA 0.0705 μCi/mg.

100 mg of product (VIII) with SA 0.071 μCi were diluted with 1000 mg of pure, non radioactive (I), crystallized from acetone and gave 803 mg of the product (VIII) with SA 0.00695 μCi/mg.
Chemical yield 68%, radiochemical yield 57%.

3-methyl-4-phenyl-3-butenoic acid 1-^{14}C (IX)

500 mg of 3-methyl-4-phenyl-3-butenamide-1-^{14}C (VIII)(SA 0.071 μCi/mg) were refluxed 7 hours with 2 g of NaOH in 20 ml of water and 20 ml of methanol. After standing overnight at room temperature, the methanol was evaporated in vacuo.

The solution was diluted with 100 ml of water, extracted 3 x 100 ml of ethyl ether(in order to remove the neutral product), and the water phase was treated at 0° with HCl 35% in water to pH 2. The solid product was filtered, washed with distilled water, dried in vacuo.

The product was diluted with 2 g of pure,non radioactive 3-methyl-4-phenyl-3--butenoic acid and crystallized with 50 ml of petroleum ether: 2.1 g of 3-methyl-

-4-phenyl-3-butenoic acid 1-^{14}C(IX) were obtained, mp 111-113°, λ_{max}^{MeOH} 247-248 mμ (ε 15,400), I.R. bands at 1700, 1250, 750, 710 cm^{-1}, only one spot in paper chromatography (Rf 0.1) and in thin layer chromatography (Rf 0.45), SA 0.0132 μCi/mg.

A sample of the product was crystallized twice more from petroleum ether and gave SA 0.0128 μCi/mg.

100 mg of product (IX) with SA 0.0132 μCi/mg mixed with 100 mg of pure, non radioactive 3-methyl-4-phenyl-3-butenoic acid were together crystallized from petroleum ether. 160 mg of product (IX) with SA 0.0063 μCi/mg were obtained.

Radiochemical yield 78%.

N,N-dideutero-3-methyl-4-phenyl-3-butenamide (XI)

To a solution of 300 g of 2-methyl-3-phenyl-2-propenyl chloride (VI)[7] (1.81 mole) in 1 l of $CHCl_3$, it was added 246 g of CuCN (2.77 mole) and the mixture was refluxed 6 hours. After cooling at 20°, 1 l of Na_2CO_3 10% in water was added and the solid product was removed by filtration. From the limpid filtrate, the chloroformic phase was separated, washed with water, the solvent was evaporated and the residue was distilled at 10 mm of residue pressure collecting the product at 136-139°: 200 g of 3-methyl-4-phenyl-3-butenonitrile (X) were obtained, λ_{max}^{MeOH} 242 mμ (ε 13,650), I.R. bands at 2220, 1720, 1610, 1500, 750 and 705 cm^{-1}, retention time in gas-liquid chromatography 10 min.

To 36 g of 3-methyl-4-phenyl-3-butenonitrile (X) (230 mmole) 4 ml of D_2O (99.7% D_2O Fluka, 218 mmole) were added and at 0° HCl gas was passed into the solution for 4 hours.

To the mixture ,100 ml of benzene were added, and then $NaHCO_3$ 10% in water at 5° to pH 7. Immediately the solid product was filtered, washed with 3 x 10 ml of benzene, dried in vacuo at 40°. The product (29 g)was crystallized from methanol and 20.7 g (117 mmole) of N,N-dideutero-3-methyl-4-phenyl-3-butenamide (XI) were obtained, mp 135-7°, λ_{max}^{MeOH} 204 mμ (ε 21,000) and 244 mμ (ε 15,100), I.R. bands at 3400, 3200, 1660, 1180, 750, 705 cm^{-1}, only one spot in paper chromatography(Rf 0.6), and in thin layer chromatography (Rf 0.8), mass spectrum: m/e 177(molecular ion), in nuclear magnetic resonance spectrum, no band of CONH at 8.0 δ .

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