SYNTHESIS OF [$^{13}C_2$]-AMITRIPTYLINE, NORTRIPTYLINE AND DESMETHYLNORTRIPTYLINE

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SUMMARY

The syntheses of the antidepressant drug, amitriptyline, and its metabolites nortriptyline and desmethylnortriptyline, doubly labelled with carbon-13 have been accomplished in thirteen stages. $Ba^{13}CO_3$ was used for the carbonation of o-tolylmagnesium bromide, which subsequently provided a ^{13}C-label distributed between positions 5, 10 and 11 in the tricyclic ring. A second carbon-13 atom was introduced at the terminal side-chain carbon, adjacent to nitrogen, using $Na^{13}CN$.

Key words: Carbon-13, Carbonation, Amitriptyline

INTRODUCTION

Stable isotopes are now frequently used complementary to radioisotopes as labels for drugs in metabolism and pharmacokinetic studies[1]. Stable isotope labelled compounds can be used as internal standards for the

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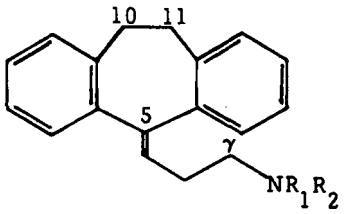
development of sensitive and specific methods for the measurement of drugs and metabolites in biological fluids. In these methods a mass spectrometer is used as a gas chromatographic detector to enable separate measurement of the drug and internal standard.

In connection with research into the application of stable isotopes in drug metabolism, we have recently synthesised carbon-13 labelled analogues of the antidepressive agent, amitriptyline (I), and its metabolites nortriptyline (II) and desmethylnortriptyline (III).



I ($R_1 = R_2 = CH_3$)

II ($R_1 = H$, $R_2 = CH_3$)

III ($R_1 = R_2 = H$)

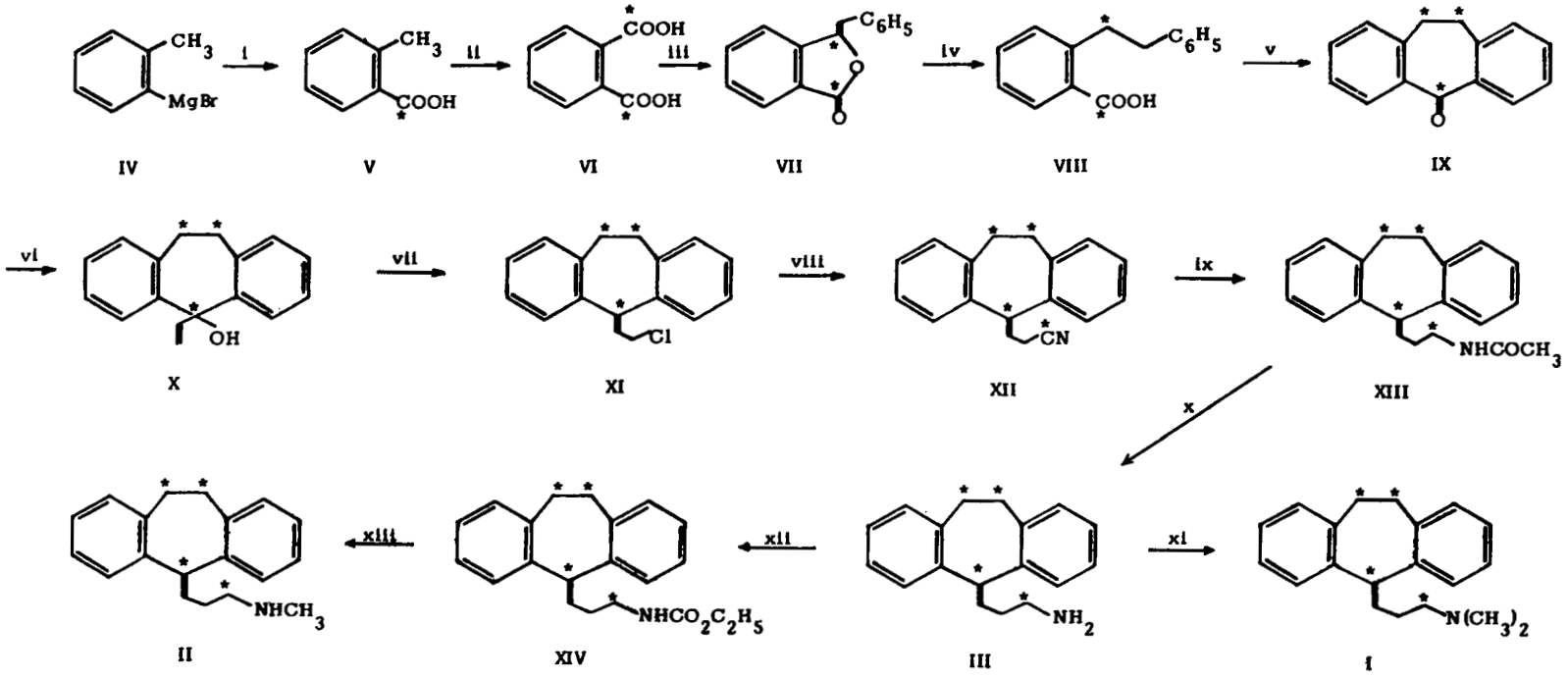
In order to provide labelled analogues suitable for use in studies using mass spectrometry[2] and ^{13}C-NMR spectroscopy[3] the three compounds were prepared doubly-labelled with carbon-13. One of the labels was located on the terminal (γ) carbon of the side-chain, and the other - due to the

equivalence of the 5 and 10, and 10 and 11 positions of the tricyclic moiety at certain stages of its synthesis - was distributed between positions 5, 10 and 11 in the ratio 50:25:25% (see Scheme).

DISCUSSION

Dibenzosuberone-^{13}C (IX) was prepared from o-bromotoluene essentially by the method of Maul[4], with the exception that reduction of benzalphthalide (VII) to the phenethylbenzoic acid (VIII) was performed chemically (P/HI)[5], rather than catalytically (Pd/H_2). Conversion of this $^{13}C_1$ tricyclic ketone to the doubly labelled nitrile (XII) was accomplished by following the route described by Marshall et al.[6] using $Na^{13}CN$. Catalytic hydrogenation of (XII) in common organic solvents produced a dimeric amine, whereas the use of acetic anhydride prevented further reaction of the primary amine by trapping it as the N-acetate (XIII). Conventional alkaline hydrolysis then afforded the desired primary amine, desmethylnortriptyline (III), which could be smoothly converted to amitriptyline (I) by the Leuckart reaction, and to nortriptyline (II) via the carbamate (XIV)[7]. The three bases were purified as their hydrochlorides, whose identities and purities were checked by thin layer chromatography and mass spectrometry.

Scheme



(*denotes the positions of the ^{13}C label - see Introduction)

Reagents: i, $^{13}CO_2$; ii, $KMnO_4$; iii, $C_6H_5CH_2CO_2H$-NaOAc; iv, P-HI; v, $(H_3PO_4)n$; vi, CH_2=CHMgBr; vii, HCl; viii, $Na^{13}CN$; ix, H_2-Ac_2O; x, KOH; xi, HCO_2H-HCHO; xii, $ClCO_2C_2H_5$; xiii, $LiAlH_4$.

EXPERIMENTAL

Thin layer chromatograms were obtained on silica plates, melting points (uncorrected) on a Köfler hot-stage apparatus, and mass spectra using the direct insertion probe of a V.G. - Micromass 16F instrument operating in the electron impact mode.

o-Toluic acid-[carboxyl-^{13}C] (V)

In an evacuated manifold apparatus[8], barium ^{13}C-carbonate (90 atom %; 7.0 g; Prochem, London) was treated cautiously with excess concentrated sulphuric acid (75 ml) and the $^{13}CO_2$ liberated was trapped in a flask containing 106 mmoles of o-tolylmagnesium bromide (IV) in THF (72 ml), cooled in liquid nitrogen. When the evolution of gas was complete, the reaction mixture was allowed to warm up to room temperature. After stirring for 30 minutes, the resulting solution was poured onto dilute sulphuric acid and extracted three times with ether. The combined organic phases were then extracted with 2N sodium hydroxide solution and the ether-washed aqueous layers acidified. Extraction with ether provided the product as a colourless solid. Yield 4.8 g (99%).

Phthalic acid-[carboxyl-^{13}C] (VI)

A solution of the above acid (V, 9.9g) in 1 N sodium hydroxide solution (400 ml) was treated with potassium permanganate (35 g), then stirred and heated under reflux for 4 hours. After cooling, a saturated solution of sodium sulphite was added to discharge the purple colour and the precipitated manganese dioxide removed by filtration. The colourless filtrate was evaporated at 80°C in vacuo until precipitation commenced. This precipitate was redissolved by the judicious addition of water at 80°C and the solution poured onto concentrated

hydrochloric acid (150 ml). After standing overnight at 0°C, the product was filtered, washed and dried at 60°C in vacuo. Yield 9.4 g (78%), m.pt. 205°C, shown to be pure and identical to authentic phthalic acid by TLC (n-BuOH-HOAc-H_2O, 2:1:1; R_f 0.65).

^{13}C-Benzalphthalide (VII)

A mixture of phthalic acid-[carboxyl-^{13}C] (9.2 g), phenylacetic acid (8.35 g) and freshly-fused sodium acetate (0.2 g) was heated at 250°C for 5 hours. The solid formed on cooling was recrystallised from ethanol as yellow needles. Yield 5.0 g (41%), m.pt. 94-95°C, identical to authentic benzalphthalide by TLC (C_6H_6; R_f 0.50).

2-(α-^{13}C-β-Phenylethyl)benzoic acid-[carboxyl-^{13}C] (VIII)

^{13}C-Benzalphthalide (5 g), red phosphorus (3.2 g) and hydriodic acid (32 ml; 54% w/v) were heated under reflux overnight, cooled, poured onto ice-water and filtered. The precipitate and concentrated ammonia solution (30 ml) were refluxed for 30 minutes, cooled and filtered. The colourless solution was acidified with concentrated hydrochloric acid, cooled in ice and filtered. After washing, the product was dried at 50°C in vacuo. Yield 4.2 g (83%), m.pt. 130-132°C. TLC ($CHCl_3$-MeOH, 3:1; R_f 0.9) identical to authentic material.

[5,10(11)-^{13}C$_1$]-5H-Dibenzo[a,d]-10,11-dihydrocyclohepten-5-one (IX)

The above labelled acid (5.47 g)was added during 20 minutes to polyphosphoric acid (82% P_2O_5; 32 g) maintained at 170°C and stirred vigorously. After a further 3 hours at this temperature, the dark brown mixture was cooled, treated with ice-water and extracted several times with ether. The combined organic extracts were washed with 1%

(w/v) sodium hydroxide solution and water, dried ($MgSO_4$), evaporated to dryness and the residue distilled. Yield 1.7g (34%), b.p. 113-114°C/ 0.03 mm. TLC (C_6H_6, R_f 0.65) identical to authentic dibenzosuberone.

[5,10(11)-$^{13}C_1$]-5-Hydroxy-5-vinyl-5H-dibenzo[a,d]-10,11-dihydrocycloheptene (X)

A solution of vinylmagnesium bromide was prepared in the usual manner from vinyl bromide (2.6 g), magnesium (0.53 g) and THF (25 ml). This solution was treated with a solution of the labelled dibenzosuberone (IX, 1.7 g) in THF (15 ml) over 15 minutes, then stirred under reflux for a further 2 hours. After cooling, a saturated solution of ammonium chloride (20 ml) was added dropwise and the resulting two layers were separated. The aqueous phase was extracted with ether and the combined organic phases washed with water, dried ($MgSO_4$) and evaporated to dryness. TLC (C_6H_6, R_f 0.3) indicated that the desired product was contaminated with a small amount of more polar material, but was nevertheless of sufficient purity to use in the next stage.

[5,10(11)-$^{13}C_1$]-5-(β-Chloroethylidene)-5H-dibenzo[a,d]-10,11-dihydrocycloheptene (XI)

Dry hydrogen chloride gas was passed into a refluxing solution of the above carbinol (X; 2 g) in chloroform (30 ml) for 30 minutes. The mixture was then cooled and excess HCl removed by bubbling nitrogen through it. Evaporation and trituration of the residue with hexane provided the desired chloride as colourless crystals. Yield 1.75 g (81%), m.pt. 93-95°C. TLC (C_6H_6) R_f 0.55.

[5,10(11)-$^{13}C_1$]-5(β-^{13}C-cyanoethylidene)-5H-dibenzo[a,d]-10,11-dihydrocycloheptene (XII)

The above chloro-compound (XI; 1.75 g) and sodium cyanide - ^{13}C (95 atom %; 0.36 g; Prochem, London) in acetonitrile (25 ml) were heated together under reflux for 24 hours. The mixture was evaporated to dryness and the residue partitioned between ether and water. The organic phase was separated, dried ($MgSO_4$) and evaporated to dryness to yield the crude product (1.6 g) as a colourless solid. A solution of this material in benzene was chromatographed on a column of silica gel. Elution with hexane provided a less polar impurity whereas benzene eluted the desired product (1.3 g, 78%) as pale yellow crystals. TLC (C_6H_6) R_f 0.35.

[5,10(11)-$^{13}C_1$]-5-(γ-^{13}C-γ-N-acetylaminopropylidene)-5H-dibenzo[a,d]-10,11-dihydrocycloheptene (XIII)

A solution of the above nitrile (XII; 1.3g) in acetic anhydride (50 ml) was hydrogenated in the presence of Raney nickel catalyst at one atmosphere for 24 hours. The catalyst was removed by filtration and the solution poured onto excess water with stirring. The resulting mixture was basified with dilute sodium hydroxide solution and extracted well with ether. Evaporation of the combined, dried extracts yielded an orange oil, which, upon trituration with hexane, provided the product as a colourless solid (0.6 g).

[5,10(11)-$^{13}C_1$]-5-(γ-^{13}C-γ-aminopropylidene)-5H-dibenzo[a,d]-10,11-dihydrocycloheptene (III; $^{13}C_2$-Desmethylnortriptyline)

A mixture of the labelled N-acetate (XIII; 0.44 g) and a 30% solution of potassium hydroxide in aqueous ethanol (1:1; 15 ml) was heated under reflux for 20 hours, then evaporated to a small volume, diluted

with water (30 ml) and extracted with ether. The organic extracts were combined, washed with water and extracted with 10% hydrochloric acid (2 x 5 ml). The combined aqueous extracts were basified and extracted with ether, to afford the product as a colourless oil (0.26 g: 68%). 35 mg of this material were converted to the hydrochloride salt using ethereal HCl, and recrystallised from methanol-ethyl acetate as colourless needles. Yield 27 mg, m.pt. and mixed m.pt. 255-259°C, TLC ($CHCl_3$- MeOH-NH_4OH, 60:8:1; Rf 0.64) showed that the product ran as a single spot and was identical with an authentic sample.

5,10(11)-$^{13}C_1$-5-(γ-^{13}C-γ-N-methylaminopropylidene)-5H-dibenzo[a,d]-10,11-dihydrocycloheptene (II; $^{13}C_2$-Nortriptyline)

A solution of $^{13}C_2$-desmethylnortriptyline (III; 110 mg) in dry pyridine (1 ml) was treated with ethyl chloroformate (redistilled; 45 µl) and allowed to stand at room temperature for one hour. It was then evaporated to dryness and the residue partitioned between water and ether. The organic layer was separated, washed with dilute hydrochloric acid and water, dried and evaporated to provide the carbamate (XIV; 85 mg) as a brown oil, ν_{max} 1710 cm^{-1}, M^+ 323. This was dissolved in dry ether (3 ml) and added dropwise to a stirring slurry of lithium aluminium hydride (280 mg) in ether (12 ml). After stirring the resulting mixture at reflux temperature overnight, 0.3 ml each of ethanol, water and 20% aqueous sodium hydroxide were added, followed by 1 ml of water. The resulting mixture was filtered, washed well with ether and the filtrate was extracted twice with dilute hydrochloric acid. The combined acid extracts were basified and extracted with ether, to provide 48 mg (69%) of $^{13}C_2$-nortriptyline base, which was chromatographically homogeneous by TLC ($CHCl_3$-MeOH-NH_4OH, 60:8:1; R_f 0.65). It was converted to the hydrochloride and recrystallised as described above. Yield 40 mg, m.pt. and mixed m.pt. 214-216°C.

[5,10(11)-$^{13}C_1$]-5-(-^{13}C- -N,N-dimethylaminopropylidene)-5H-dibenzo[a,d]-10,11-dihydroxyxloheptene (I; $^{13}C_2$-Amitriptyline)

A mixture of $^{13}C_2$-desmethylnortriptyline (III; 138 mg), formic acid (98%; 0.4 ml) and formaldehyde solution (37%; 0.45 ml) was heated at 100°C for 2 hours. Concentrated hydrochloric acid (0.25 ml) was added and the mixture evaporated to dryness in vacuo. The residue was dissolved in water, washed twice with ether, basified and extracted in the usual way to yield 105 mg (68%) of $^{13}C_2$-amitriptyline base. TLC ($CHCl_3$-MeOH-NH_4OH, 60:8:1) R_f 0.78. This was converted to the hydrochloride and recrystallised as above. Yield 77 mg, m.pt. and mixed m.pt. 193-196°C.

Mass spectrometry and ^{13}C-NMR spectroscopy

The chemical ionisation (isobutane) mass spectra of ^{13}C-labelled amitriptyline, nortriptyline and desmethylnortriptyline showed quasimolecular (M+1) ions at m/e 280, 260 and 252 respectively, each two mass units higher than the corresponding non-labelled compounds. The relative intensities of these ions and those at one and two mass units lower indicated that about 80% of the molecules contained two carbon-13 atoms.

The proton-decoupled ^{13}C-NMR spectra of each $^{13}C_2$-labelled compound showed four single resonance signals assigned to carbons 5, 10 and 11 in the tricyclic ring and the γ side chain carbon. The 10 and 11 carbons can be individually distinguished due to their different spatial orientation relative to the side chain. The chemical shifts, relative to TMS, of these carbons (33.0 and 34.8 ppm)

were identical for all three compounds. Similarly the C-5 signals had almost identical chemical shifts occurring at 148.2 to 148.5 ppm. The γ side-chain carbon exhibited the predicted upfield shift in the series $-CH_2N(CH_3)_2$ (58.3 ppm), $-CH_2NHCH_3$ (50.0 ppm), $-CH_2NH_2$ (40.6 ppm). The relative intensities of the resonance signals was consistent with the isotopic incorporation at each labelled position and the hybridisation state of the carbon atom. Thus, the γ-carbon produced the most intense signal, while the signals due to the sp^3 C-10 and 11 atoms were greater than that due to the sp^2 C-5, despite the greater isotope incorporation at the latter position.

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