SYNTHESIS OF DEUTERIUM-LABELLED CHLORPROMAZINE AND CHLORPROMAZINE METABOLITES.

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SUMMARY

The synthesis of 2-chloro-10-(3'-dimethylaminopropyl)phenothiazine-3', 3'-d_2 (Chlorpromazine-d_2) and its four major metabolites (the mono- and didemethylated amines, chlorpromazine sulfoxide, and chlorpromazine N-oxide), all containing the 3',3'-d_2 label in > 95% isotopic purity, is described. Considerations leading to the optimum number and position of the labels are discussed, and the problem of coupling the aliphatic side-chain to the tricyclic system is avoided by utilizing the readily available 2-chloro-10-(2'-cyanoethyl)phenothiazine as starting material, so that the carbon skeleton of the side-chain is already in position before the isotope is introduced.

Key Words: Chlorpromazine, chlorpromazine metabolites, deuterium labelling.

INTRODUCTION

Chlorpromazine [2-chloro-10-(3'-dimethylaminopropyl)phenothiazine] 6a (CPZ) is one of the most widely used phenothiazine tranquilizers in clinical practice (1). Although a variety of chromatographic and spectrophotometric

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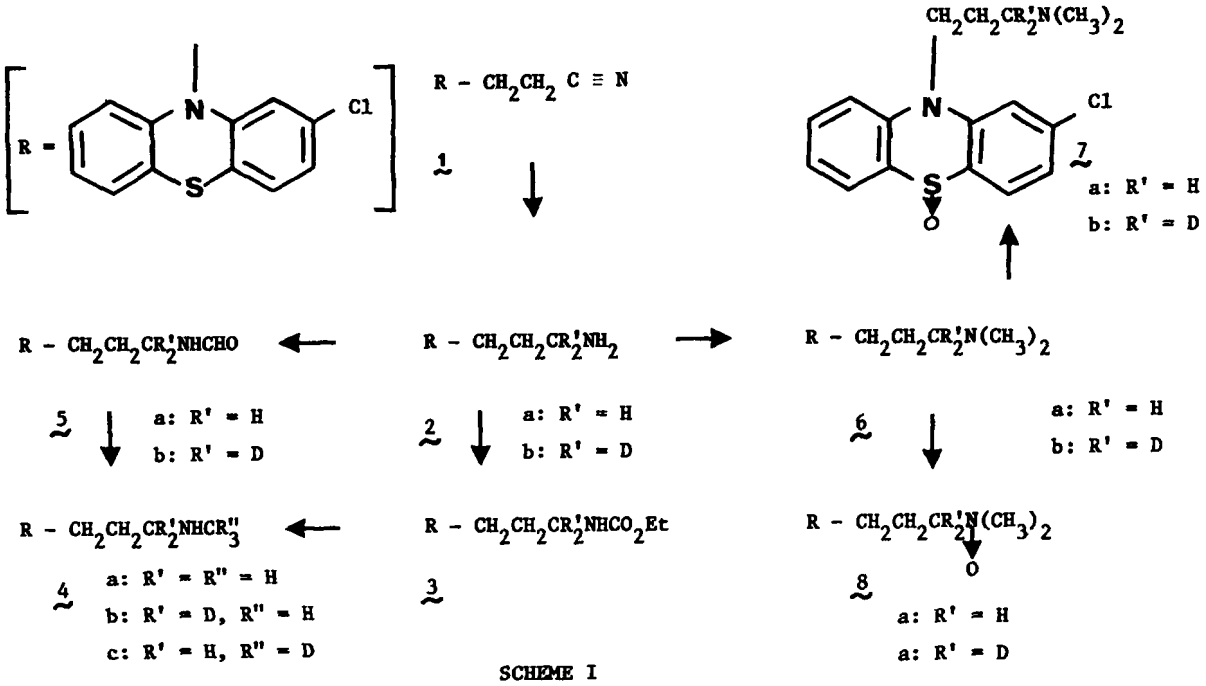
assay procedures have been developed for CPZ and its metabolites (1), the low plasma levels found, and the sensitivity of the metabolites to light and air, have resulted in a high degree of variability between different methods and laboratories (1). The development of an accurate method of quantitation of CPZ and its major metabolites by a combination of gas chromatography - mass spectrometry (gc/ms) and selected ion recording (sir) techniques (2) required the synthesis of stable-isotope-labelled variants of these compounds as internal standards.

DISCUSSION

The major metabolites of CPZ are the products of N-demethylation, N-oxidation, and S-oxidation, giving respectively the secondary and primary amines 4a and 2a, the N-oxide 8a, and the sulfoxide 7a. Due to the nature of the mass spectrometric assay, the molecular weights of the internal standards must be at least two mass units greater than those of the unlabelled compounds in order to avoid contributions from the natural abundance of ^{13}C in these molecules. The ready metabolic loss of the N-methyl groups, and the greater synthetic problems of introducing labels into the aromatic rings, indicated carbons 1, 2 and 3 of the sidechain as the ideal sites for the labels. The thermolytic decomposition of the N-oxide 8a on gas chromatography (3) and mass spectrometry (4) to give 10-allyl-2-chlorophenothiazine removes one of the hydrogen atoms in the 2'-position, while introduction of label by hydride reduction of the 10-(2'-aminopropionyl) derivatives frequently leads to hydrogenolysis and loss of sidechain (5). These reasons make the 3'-position the optimum site for the label, which would thus be retained fully in all metabolites including the thermolysis product of 8a.


R = N S Cl
R - CH2CH2 C ≡ N
1
CH2CH2CR'2N(CH3)2
N S Cl
O
7
a: R' = H
b: R' = D
R - CH2CH2CR'2NHCHO
R - CH2CH2CR'2NH2
R - CH2CH2CR'2N(CH3)2
5
a: R' = H
b: R' = D
2
a: R' = H
b: R' = D
6
a: R' = H
b: R' = D
R - CH2CH2CR'2NHCR''3
R - CH2CH2CR'2NHCO2Et
R - CH2CH2CR'2N(CH3)2
O
4
a: R' = R'' = H
b: R' = D, R'' = H
c: R' = H, R'' = D
3
8
a: R' = H
a: R' = D

SCHEME I

In view of the considerable natural abundance of the ^{37}Cl and ^{34}S isotopes, it appears at first sight necessary to have internal standards four mass units heavier than the unlabelled substances in order to avoid interference at (M+2) from ^{37}Cl and ^{34}S. However, this problem can in fact be avoided by the simple expedient of focusing the mass spectrometer on the ion containing the ^{35}Cl isotope for the d_o compound, while for the d_2 internal standard the focus is on the ^{37}Cl isotope ion. There will thus be no interference from natural abundance of Cl and S at (M+4), except for a very minor contribution due to the simultaneous ^{37}Cl ^{34}S abundances in the d_o compound, which is compensated for in the normal manner by the standard curve (2).

Normal synthetic procedures involve the coupling of the sidechain to the aromatic system (6). However we found it more efficient to base the synthesis on a precursor in which the carbon skeleton of the sidechain is already in position before the isotope is introduced, thus obviating the coupling step. We therefore chose to proceed from 2-chloro-10-(2'-cyanoethyl)-phenothiazine 1, readily available by a condensation of 2-chlorophenothiazine and acrylonitrile (7). Although attempted reduction of 1 with lithium aluminum hydride (LAH) in tetrahydrofuran gave only a reverse Michael reaction with complete loss of sidechain, the use of a Soxhlet extractor to contain the cyanide 1, and ether as solvent, successfully minimized the elimination and led to the primary amine 2 in good yield. Repetition of the reduction with lithium aluminum deuteride (LAD) gave the d_2-compound 2b in high isotopic purity by eims (99% d, of which 95.5% was d_2 and 4.5% d_1). This high level of isotopic purity was maintained throughout the synthetic sequence shown in Scheme I.

The primary amine was readily converted, via the carbamate 3, to the secondary amine metabolite 4c by using LAD. A more efficient method utilized

conversion of the primary amine 2b to the crystalline N-formyl derivative 5b followed by LAD reduction to 4b.

The Eschweiler-Clarke reduction (8) of the primary amine 2b led to CPZ-d_2 6b, the hydrochloride of which was readily converted to the sulfoxide 7b, while oxidation of the base 6b afforded the N-oxide 8b. All compounds gave nmr and eims spectra in full agreement with their structures.

The method described above thus offers a convenient and efficient synthesis for stable-isotope-labelled CPZ and its four major metabolites from a readily accessible starting material. With the increasing application of stable-isotope-labelled variants as internal standards by the use of gc-ms-sir techniques (9), the availability of these compounds makes possible the simultaneous quantitation of the drug and four metabolites in body fluids, and these results will be reported separately.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Literature melting points refer to the undeuterated compounds. Nmr spectra were measured on a Varian A-60A spectrometer using tetramethylsilane as the internal reference. Electron impact mass spectra were recorded on an AEI MS-12 spectrometer by direct insertion. Deuterium analysis by mass spectrometry was performed on the molecular ions.

2-Chloro-10-(3'-aminopropyl)-phenothiazine (2a). 2-Chloro-10-(2'-cyanoethyl)-phenothiazine (1, 14.3 g, 50 mmol) was placed in the thimble of a Soxhlet extractor. The reaction flask was charged with lithium aluminum hydride (3.8 g, 100 mmol) in 1400 ml of anhydrous ether. After 72 hours reflux 40 ml of ice water and 60 ml of 20% aqueous sodium hydroxide were

added followed by an additional 60 ml of ice water. The aqueous layer was filtered and both the filter cake and filtrate were extracted repeatedly with ether. The combined ether layers were dried (anhydrous magnesium sulfate), a saturated solution of HCl gas in ether was added and the product precipitated as the hydrochloride of 2a (12.0 g, 71%), m.p. 239-242°. Evaporation of the ether solution gave 3.3 g (28%) of 2-chlorophenothiazine. Recrystallization of the primary amine hydrochloride from ethanol gave the product as white crystals, m.p. 242-243.5° [lit. (7) m.p. 235-237°]. Nmr (free base, $CDCl_3$) δ 1.2 (broad s, 2H, NH_2), 1.87 (p, 2H, CH_2-CH_2-CH_2), 2.70 (t, 2H, CH_2-NH_2), 3.90 (t, 2H, phenothiazine N-CH_2), 6.8-7.3 (m, 7H, aromatic); eims (70 ev) m/e (rel. intensity) 292 (36%), 290 (100%), 248 (15%), 246 (42%), 235 (24%), 234 (39%), 233 (68%), 232 (83%), 58 (34%).

2-Chloro-10-(3'-aminopropyl)-phenothiazine-3',3'-d_2 (2b). By the same method, using lithium aluminum deuteride, 1 was converted into the hydrochloride salt of 2b, m.p. 241-243°; a mixed mp with authentic undeuterated salt was not depressed. Nmr (free base, $CDCl_3$) δ 1.1 (broad s, 2H, NH_2), 1.87 (t, 2H, -CH_2-CD_2-) 3.90 (t, 2H, phenothiazine N-CH_2-), 6.8-7.3 (m, 7H, aromatic); eims (70 ev) (rel. intensity) 294 (36%), 292 (100%), 291 (5.0%), 248 (17%), 246 (40%), 235 (22%), 234 (51%), 233 (56%) 232 (88%), 60 (34%).

2-Chloro-10-(3'-methyl-d_3-aminopropyl)-phenothiazine (4c). A solution of the primary amine hydrochloride 2a (6.0 g, 18.3 mmol) and pyridine (1.45 g, 18.3 mmol) in 70 ml of methylene chloride was cooled to 0° and ethyl chloroformate (1.98 g, 18.3 mmol) in 10 ml of methylene chloride was added dropwise. After 24 hr stirring at room temperature, water was added, the mixture was filtered, and the methylene chloride layer was dried and evaporated to give 3.6 g (71%) of the ethyl carbamate 3 as a viscous liquid. Nmr ($CDCl_3$) δ 1.18 (t, 3H, CH_3), 3.27 (q, collapses to a triplet with added D_2O, 2H, NH-CH_2-CH_2)

4.00 (p, 4H, overlapping phenothiazine N-CH_2- at 3.88 and (-CH_2-CH_3 at 4.07), 5.0 (broad s, 1H, NH), 6.8-7.4 (m, 7H, aromatic); eims (70 ev) m/e (rel. intensity) 364 (16%), 362 (44%) 234 (37%), 232 (90%), 130 (55%), 102 (62%), 78 (100%).

Without further purification the ethyl carbamate (1.2 g, 3.3 mmol) in 15 ml of anhydrous ether was added slowly to lithium aluminum deuteride (0.30 g, 6.6 mmol) in 50 ml anhydrous ether and the reaction mixture was refluxed for 24 hr. After cooling, 5 ml of ice water and 10 ml of 20% aqueous sodium hydroxide were added. The ether layer was separated, dried (magnesium sulfate), and treated with anhydrous HCl in ether. The hydrochloride of 4c separated on standing and crystallized from ethanol to give 450 mg (39%) of white crystals, mp 184.5-185.5° [lit.(7) mp 180-182°]. Nmr (free base, $CDCl_3$) 1.47 (s, 1H, NH), 1.92 (p, 2H, CH_2-CH_2-CH_2), 2.68 (t, 2H, -CH_2-NH-) 3.88 (t, 2H, phenothiazine N-CH_2-) 6.8-7.3 (m, 7H, aromatic); eims (70 ev) m/e (rel. intensity) 309 (9.6%), 307 (25.8%), 306 (1.3%), 75 (29.7%), 47 (100%).

2-Chloro-10-(3'-formamidopropyl)-phenothiazine (5a). The primary amine 2a (1.15 g, 4.0 mmol), formic acid (0.30 g, 6.5 mmol) and 50 ml of benzene were refluxed overnight with a Dean-Stark trap, which was then replaced with a Soxhlet extractor filled with dry benzene and 25 g of 4 A molecular sieves, and the solution was again refluxed overnight. The total benzene solution was extracted with 50 ml 0.1 N-HCl and 50 ml 5% aq. sodium carbonate, and on evaporation gave 950 mg (75%) of 5a, crystallizing from toluene as white crystals, mp 114.5-115.5°, previously reported (7) as an oil. Nmr ($CDCl_3$) 1.97 (p, 2H, -CH_2-CH_2-CH_2-), 3.35 (q, 2H, NH-CH_2-), 3.90 (t, 2H, phenothiazine N-CH_2-), 6.4 (broad s, 1H, NH), 6.8-7.3 (m, 7H, aromatic), 8.00 (d, J = 1 Hz, 1H, CHO); eims (70 ev) m/e (rel. intensity) 320 (28%), 318 (76%), 248 (13%), 246 (37%), 235 (28%), 234 (47%), 233 (74%), 232 (100%), 86 (87%), 58 (41%).

Anal. Calcd for $C_{16}H_{15}ClN_2OS$: C, 60.27; H, 4.74; N, 8.78; S, 10.03. Found: C, 60.14; H, 4.82; N, 8.95; S, 10.05%.

2-Chloro-10-(3'-formamidopropyl)-phenothiazine-3',3'-d_2 (5b). The same method was used to prepare 5b from 2b. The product had mp 113-115°; eims (70 ev) m/e (rel. intensity) 322 (33%), 320 (88%), 319 (3.6%), 248 (13%) 246 (35%), 235 (30%), 234 (46%), 233 (82%), 232 (100%), 88 (85%), 60 (42%).

2-Chloro-10-(3'-methylaminopropyl)-phenothiazine-3',3'-d_2 (4b). Prepared from 5b by the method reported (7) for the protio compound, 4b hydrochloride had mp 182-184° [lit. (7) 180-182°]; a mixed mp with authentic undeuterated hydrochloride was not depressed. Nmr ($CDCl_3$ + D_2O) δ 1.91 (t, 2H, $-CH_2-CH_2-CD_2-$), 2.73 (s, 3H, $-CH_3$), 3.88 (t, 2H, phenothiazine N-CH_2-), 6.7-7.3 (m, 7H, aromatic); eims (70 ev) m/e (rel. intensity) 308 (18%), 306 (43%), 305 (3.7%), 74 (45%), 46 (100%).

2-Chloro-10-(3'-dimethylaminopropyl)-phenothiazine-3',3'-d_2 (6a). The amine 2b (1.22 g, 4.2 mmol), formic acid (1.5 g, 33 mol), and 37% aqueous formaldehyde (1.5 g, 19 mmol) were heated at reflux for 3 hr under a nitrogen atmosphere. After cooling, the excess reagents were removed under high vacuum, the residue was dissolved in 100 ml of dil. aqueous hydrochloric acid and this was extracted with ether (5 x 25 ml). The aqueous layer was made basic with potassium carbonate and was extracted with ether (3 x 50 ml). The combined basic ether extracts were dried (sodium sulfate) and evaporated under reduced pressure. The residue (1.05 g) after passing through a 20 x 150 mm silica gel (Baker Analyzed Reagent) column with ether gave 915 mg (68%) of 6b as a colorless liquid. Nmr ($CDCl_3$) δ 1.88 (t, 2H, $-CH_2-CD_2-$), 2.18 (s, 6H, $-N(CH_3)_2$), 3.87 (t, 2H, phenothiazine N-CH_2-) 6.8-7.3 (m, 7H, aromatic); eims (70 ev) m/e (rel. intensity), 322 (7.7%), 320 (21%), 319 (0.8%), 88 (23.9%), 60 (100%). The hydrochloride formed white crystals (from ethanol) mp 194.5-196°

[lit. (10) mp 194-195°]; mp with authentic undeuterated salt was not depressed.

2-Chloro-10-(3'-dimethylaminopropyl)-phenothiazine sulfoxide-3',3'-d_2 (7b). A solution of m-chloroperbenzoic acid (145 mg, 85%, 0.71 mmol) in 5 ml of methylene chloride was added dropwise over 10 min to a stirred solution of 6b hydrochloride (200 mg, 0.56 mmol) in 5 ml of methylene chloride. After standing at room temperature for 1 hr, the mixture was extracted with 5% aqueous sodium carbonate (2 x 50 ml). The methylene chloride layer was dried (sodium sulfate) and evaporated, affording 7b as a white solid (135 mg, 70%), crystallizing from toluene-hexane as white crystals, mp 114.5-115.5° [lit.(11) mp 114-115°]; a mixed melting point with authentic undeuterated material was not depressed. Eims (70 ev) m/e (rel. intensity) 338 (2.1%), 336 (5.2%, 248 (14%), 246 (34%), 60 (100%).

2-Chloro-10-(3'-dimethylaminopropyl)-phenothiazine N-oxide-3',3'-d_2 (8b). The tertiary amine 6b (156 mg, 0.49 mmol) in 0.8 ml ethanol was heated with 57 µl (0.60 mmol) 30% aqueous hydrogen peroxide in a closed reaction vial at 75° for 3 hr. After cooling, the solvent was removed under reduced pressure and the residue dissolved in 2.5 ml of water. The water solution was extracted with benzene (3 x 5 ml), and the benzene layer then saturated with anhydrous potassium carbonate and extracted with methyl acetate (5 x 5 ml). The combined methyl acetate extracts were placed on a 20 x 150 mm silica gel (Baker Analyzed Reagent) column and elution with methanol gave the product 8b (35 mg, 21%) as white crystals, mp 93-95° from ethyl acetate [lit. (12) mp 94-95°]; eims (70 ev) m/e (rel. intensity) 322 (1.1%), 320 (2.8%), 277 (9.5%), 275 (25%), 234 (43%), 232 (100%), 60 (21%).

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