SYNTHESIS OF PEMOLINE-$\underline{d}_5$: A METABOLIC PROBE FOR HYPERACTIVITY

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SUMMARY

The drug, pemoline-$\underline{d}_5$, 2-imino-5-phenyl-$\underline{d}_5$-4-oxazolidinone, was prepared by a four step synthesis from benzene-$\underline{d}_6$. The synthetic sequence required the preparation of dichloroacetophenone-$\underline{d}_5$, mandelic acid-$\underline{d}_5$ and ethyl mandelate-$\underline{d}_5$. Mass spectral as well as infrared data of the labelled drug are also presented.

Key Words: Pemoline, Deuterium Labelling, Dichloroacetophenone-$\underline{d}_5$, Mandelic Acid-$\underline{d}_5$, Mass Spectral and Infrared Absorption Data.

INTRODUCTION

Hyperactivity is a condition manifested by many learning disabled children which results in an impairment to their academic performance. Common characteristics often exhibited by hyperactive children include poor attention span, motor difficulties, low frustration tolerance, low perseverance, and organizational problems. This condition is diagnosed predominantly more often for boys than for girls. One of the current treatment methods for hyperactivity is drug therapy. Stimulant drugs, such as caffeine (1), amphetamines (2), methylphenidate (3), and pemoline (4), have been prescribed for both school age and preschool age children. These drugs are prescribed on the premise that hyperactivity is a function of attention deficiencies, therefore the stimulant drugs elevate the hyperactive individual to a normal threshold of attention at which he or she can concentrate.

The newest member of the group of drugs used to treat this learning impairment is pemoline. This drug is chemically different from amphetamine, caffeine or methylphenidate, but does possess some structural similarities to this group of compounds.

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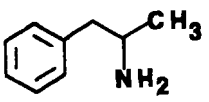
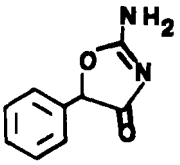
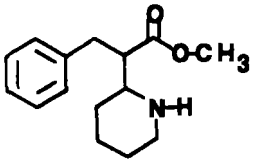


Amphetamine



Pemoline



Methylphenidate

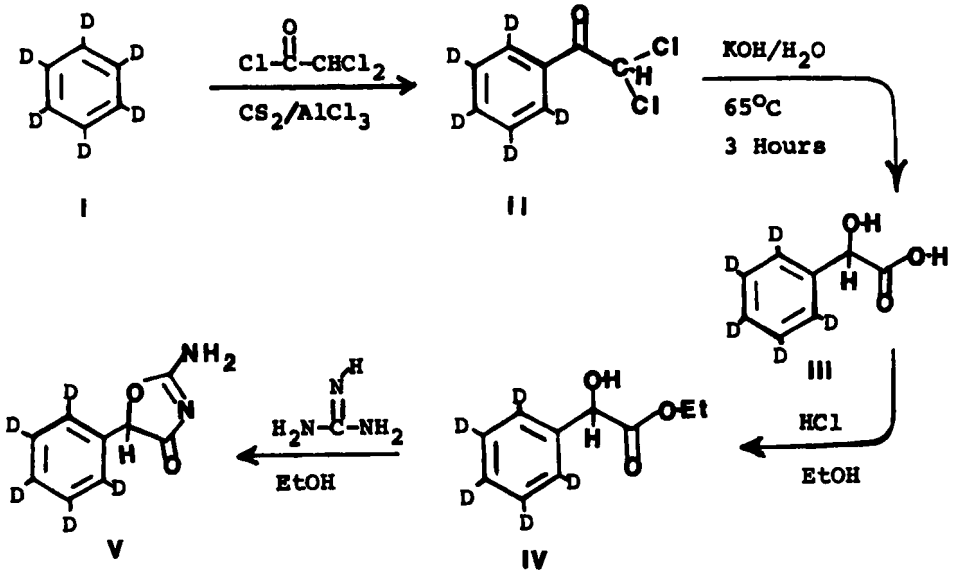
Currently considerable controversy exists in the literature regarding the effectiveness of pemoline in hyperactive children (5). Additionally, serious doubts and interest have developed concerning the claim that pemoline can reverse senility and enhance memory in adults (6). Literature articles have stated that pemoline was superior (7) to other hyperactive psychotropic drugs, ineffective (8), nontoxic (9), toxic (10), a drug of abuse (11), and decreased (12) as well as increased (13), RNA-synthesis. In addition, pemoline has been proposed as a radioresistive compound during cancer chemotherapy (14).

With such a diversity of biological activity reported, concerning this compound, it would appear reasonable to first consider the metabolic pathways of this drug and confirm whether the metabolites of the parent drug are active, inactive, or toxic. However, little has been reported concerning the metabolism and identification of metabolites of this controversial drug. A comprehensive review of the literature concerning this compound reveals only two sources identifying metabolites of pemoline. The first source, a drug monograph (7), regarding Cylert®, pemoline, revealed pemoline-dione, mandelic acid and other polar metabolites. A second report (11), revealed that 35% of the administered drug is unaccounted for after 48 hours.

Therefore, because such a scant amount of information was available pertaining to the metabolism of a compound used extensively in children and the

intriguing reports (6) of memory enhancement in adults, a labelled pemoline-d_5 probe, 2-imino-5-phenyl-d_5-4-oxazolidione, was prepared to aid in metabolic studies of the parent drug.

The labelled pemoline-d_5 (V) was prepared by condensation of ethyl mandelate-d_5 (IV) with guanidine (free base) in ethanol (15). The precursor ethyl mandelate-d_5 (IV) was prepared from benzene-d_6 by a three step sequence. This required a Friedel-Crafts alkylation of hexadeuterobenzene (I) with dichloroacetylchloride, treatment of the generated dichloroacetophenone-d_5 (II) with KOH to form mandelic acid-d_5, and finally esterification of the mandelic acid-d_5 in ethanol with dry HCl. Crystals of pemoline-d_5 (V) precipitated from solution and were easily isolated after condensation of ethyl mandelate-phenyl-d_5 (IV) with guanidine in ethanol.



EXPERIMENTAL

Dichloroacetophenone-d_5 (II) - Five grams (60 mmoles) of benzene-d_6, I, (Stohler Isotope Chemicals, Inc.)were added slowly over 15 minutes to 8.77 g (59 mmoles) of dichloroacetylchloride and 7.94 g (60 mmoles) $AlCl_3$ in 25 ml CS_2. The reaction mixture was protected from atmospheric moisture using a rubber septum and a 15 gauge needle to exclude moisture and allow the release of DCl. The reaction mixture was stirred at 25°C for two hours and then poured into 400 ml ice and 20 ml concentrated HCl. The oil and CS_2 which separated were washed with 1N NaOH, and then with water. Evaporation of the CS_2 yielded 7.35 g (63% yield) of an oil. IR, film (cm^{-1}): 3000 (CH), 2270 (phenyl-d_5), 1705 (C=O), 1555 (sharp), 1155 (sharp), 810 (multiplet) and 765 (sharp); M.S. (rel. int.): M^+ = 193, 195, 197 (<1%), m/e 132 (2%), m/e 130 (5%), m/e 110 (100%), m/e 82 (35%) and m/e 54 (11%).

Mandelic acid-d_5 (III) - All 7.35 g (38 mmoles) of dichloroacetophenone-d_5 were slowly added to 6.0 g (150 mmoles) of NaOH in 50 ml H_2O maintained at 65°C for three hours. At the end of that time 6.2 ml of 12 M HCl was added to the reaction mixture. The acidic solution was placed in a liquid-liquid extractor and the mandelic acid-d_5 extracted with ethyl ether for 24 hours. After evaporation of the ether and recrystallization from benzene and ethanol 5.9 g (>99%) of mandelic acid-d_5 were obtained. M.p. 120-121°C, IR, KBr (cm^{-1}): 3330 (C-OH), 2800 (CO_2H), 1630 (broad, CO_2H), 1230 (broad), 1010 (sharp), and 825 (broad); UV (λ_{max} ethanol): 257 nm; M.S. (rel. int.): M^+ = 157 (9%), m/e 127 (30%), m/e 112 (100%), m/e 110 (34%), m/e 84 (38%), m/e 82 (27%) and m/e 54 (12%); M*,m/e 63.0 (112→84).

Ethyl mandelate-d_5 (IV) - All 5.9 g (38 mmoles) of mandelic acid-d_5 were dissolved in 75 ml absolute ethanol to which anhydrous HCl was bubbled (1 hr) into the solution until saturated with gas. After 60 minutes some ethanol and most HCl rotary evaporated away. To the remaining ethanol, 20 ml of an aqueous saturated $NaHCO_3$ solution was added and the mixture extracted with

excess ethyl ether. Evaporation of the ether yielded 5.0 g (71%) of an oil, ethyl mandelate-d_5. M.S. (rel. int.): M^+ = 185 (14%); m/e 168 (4%), m/e 112 (100%), and m/e 84 (44%).

Pemoline-d_5 (V) - All 5.0 g (26 mmoles) of ethyl mandelate-d_5 were added to guanidine (free base) in ethanol. The guanidine was prepared from 2.58 g (27 mmoles) of guanidine hydrochloride in 22 ml absolute ethanol to which was added 1.10 g (27.5 mmoles) NaOH in 1.2 ml H_2O and 12 ml ethanol. NaCl was filtered off and the ethanol evaporated to a clear 10 ml solution. Crystals, 0.9 g (18.4% yield) of pemoline-d_5 were filtered after the guanidine and ethyl mandelate-d_5 were allowed to react at 25°C overnight. M.p. 235-236°C; IR, KBr (cm^{-1}): 2270 (phenyl-d_5), 1650 and 1550 (broad), 1230 and 700; NMR (δ), DMSO-d_6: 5.75 δ (s,-CH); UV, λ_{max} (H_2O): 217 and 262 nm, λ_{max} ($H_2O/HClO_4$): 217 nm, 262; M.S. (rel. int.): M^+ = 181 (100%), m/e 112 (97%), m/e 110 (24%), m/e 95 (42%), m/e 84 (26%).

DISCUSSION

The preparation of 2-imino-5-phenyl-4-oxazolidinone was first reported by Traube and Ascher in 1913 (15). The synthesis required only the condensation of ethyl mandelate with guanidine. The reaction occurs spontaneously at room temperature. Therefore, to prepare the deuterium labelled drug it was necessary to synthesize ethyl mandelate-phenyl-d_5. An initial attempt to utilize a Grignard condensation of phenyl-d_5-magnesium bromide with ethyl glyoxalate proved unsuccessful. However, a very good yield of mandelic acid-phenyl-d_5 and its ester was obtained following standard procedures (16,17) utilizing a base hydrolysis reaction with phenyl-d_5-dichloroacetophenone. The precursor, phenyl-d_5-dichloroacetophenone, was prepared conveniently by the Friedel-Crafts alkylation of benzene-d_6 (Stohler Isotope Chemicals, Inc.) in carbon disulfide. In an additional study dichloroacetophenone-phenyl-d_5 was also prepared by the Friedel-Crafts alkylation of benzene-d_6 using acetyl chloride. Dichloroacetophenone-phenyl-d_5 was generated by bubbling excess chlorine gas into glacial acetic acid containing acetophenone-d_5, following standard procedures (16).

Figures 1-A and 1-B reveal the mass spectra of nonlabelled pemoline and pemoline-$\underline{d}_5$, respectively. A comparison of the molecular ion regions (M^+ 176 and M^+ 181) of both spectra clearly show that the four step synthesis, using benzene-$\underline{d}_6$, has incorporated five deuterium atoms into the parent compound.

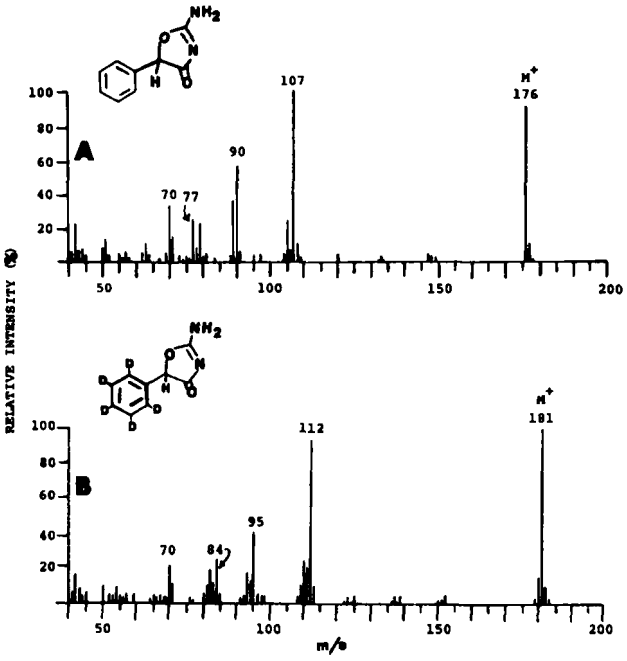


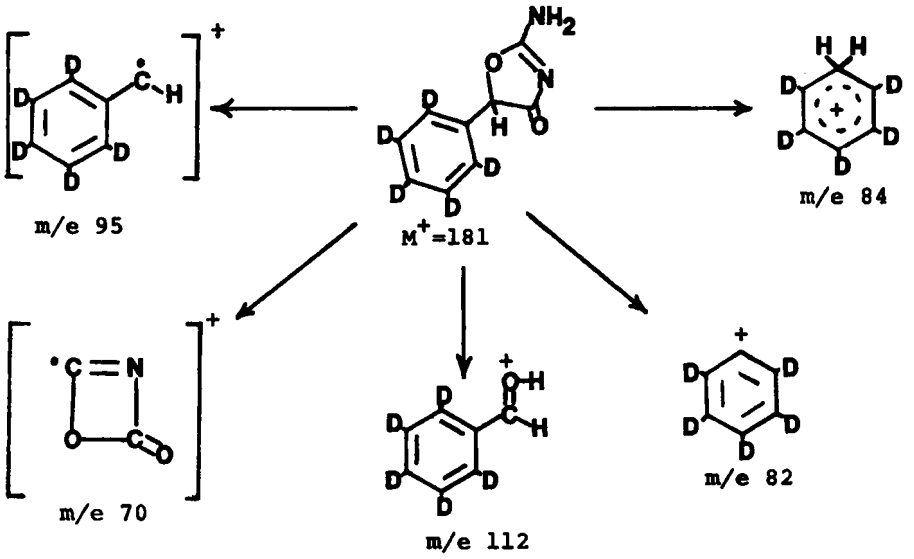
Figure 1-A: Mass Spectrum of 2-imino-5-phenyl-4-oxazolidinone.

Figure 1-B: Mass Spectrum of 2-imino-5-phenyl-$\underline{d}_5$-4-oxazolidinone.

(Both spectra were obtained by probe distillation directly into the ion source of the mass spectrometer (DuPont 490-F, magnetic sector, single focusing, 70 ev).

The shift of significant fragment ions of the nonlabelled drug (Figure 1-A), to new fragment ions at m/e 84, m/e 95, and m/e 112, for the labelled compound (Figure 1-B) indicate that the phenyl group has been uniformly labelled with five deuterium atoms. Also, by directly comparing the significant mass spectral fragment ions of the nonlabelled drug (Figure 1-A), that either remain or shift

specific amounts in the spectrum of the labelled drug (Figure 1-B), a proposed fragmentation scheme can be presented for the labelled compound. This scheme should give added insight into the elucidation of the structure of metabolites of the parent drug following feeding experiments using pemoline-$\underline{d}_5$.



Scheme 1: Interpretation of significant mass spectral fragment ions observed in Figure 1-B for 2-imino-5-phenyl-$\underline{d}_5$-4-oxazolidinone.

From Figure 1-B the mass spectrum reveals that only 86% phenyl-$\underline{d}_5$ labelled pemoline had been obtained. A synthetic deviation from a 100% labelled phenyl moiety seems to have been realized during the first step of the four step synthesis in which the Friedel-Craft alkylation of benzene-$\underline{d}_6$ (100% labelled) utilized dichloroacetylchloride. This acid chloride possessed one exchangeable proton, a source for hydrogen, contributing to the decrease in isotopic purity of the labelled drug. However, this slight decrease in isotopic purity should not interfere with feeding experiments in animals aimed at the identification of new metabolites of the parent drug.

Figures 2-A and 2-B show the infrared absorption spectra of nonlabelled pemoline and pemoline-$\underline{d}_5$, respectively. Both spectra are reasonably similar with respect to absorption bands associated with the oxazolidinone ring system. However, noticeable differences between the two spectra are observed at 3040 cm^{-1} and 2270 cm^{-1}. The former band reveals an aromatic C-H stretching absorption for the nonlabelled drug (Figure 2-A), while the later band indicates an aromatic C-D stretching absorption for the labelled drug (Figure 2-B). The absorption band at 2270 cm^{-1} is observed in the infrared region calculated for C-D stretching frequencies (18). This band appears to be very specific for the phenyl-$\underline{d}_5$ labelled drug and may also aid in the identification of new metabolites of the parent drug.

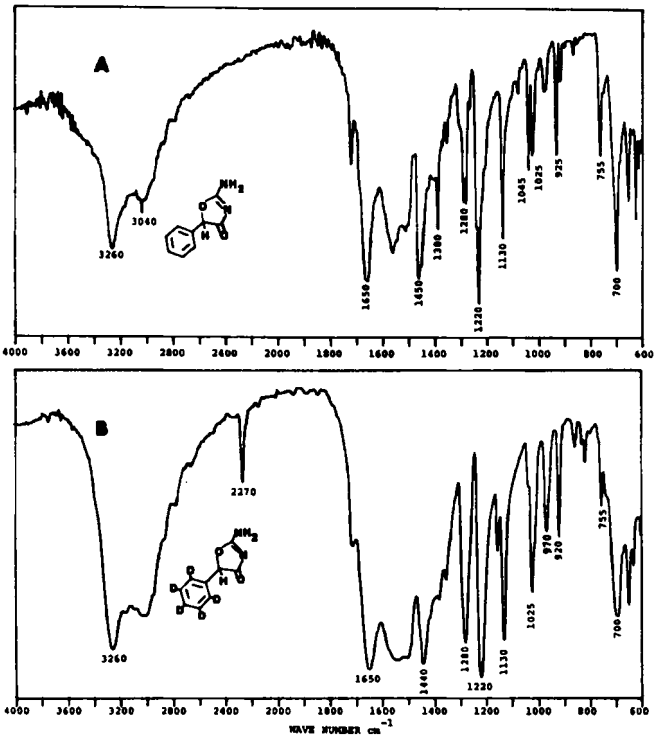


Figure 2-A: Infrared absorption spectra (KBr) of 2-imino-5-phenyl-4-oxazolidinone.

Figure 2-B: Infrared absorption spectra (KBr) of 2-imino-5-phenyl-$\underline{d}_5$-4-oxazolidinone.

CONCLUSION

A relatively inexpensive scheme has been developed for the preparation of pemoline-d_5. This labelled probe appears well suited for metabolism studies in which new metabolites can be identified by mass spectrometry. In addition, this labelled drug would appear well suited for use as an internal standard while monitoring blood levels of the parent drug in body fluids by combined GC-MS-computer techniques. Currently, work is in progress using this labelled probe to identify new metabolites of the parent drug in rats.

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