SYNTHESIS AND CHARACTERIZATION OF 5-METHYL-1-PHENYL-3,4,5,6-TETRAHYDRO-1H-2,5-BENZOXAZOCINE-1,3,4,6-^{13}C HYDROCHLORIDE (NEFOPAM-^{13}C HYDROCHLORIDE)

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SUMMARY

Nefopam hydrochloride (I), an analgesic agent, was labelled with carbon-13 at C-1,3,4 and 6 positions for metabolic studies. The starting materials for the synthesis included commercially available phthalic acid-carboxyl-$^{13}C_1$ (II) and 2-methylaminoethyl-1,2-$^{13}C_2$ alcohol. The extent of isotope enrichment was determined by nuclear magnetic resonance and mass spectroscopy. The overall yield of I was 34% from phthalic acid-carboxyl-^{13}C.

Key Words: Analgesic, Benzoxazocine, Carbon-13

INTRODUCTION

Originally synthesized as one of a new class of centrally acting skeletal muscle relaxants,[1] 5-methyl-1-phenyl-3,4,5,6-tetrahydro-1H-2,5-benzoxazocine hydrochloride (Nefopam hydrochloride) was subsequently shown in clinical studies to be a potent analgesic pharmacologically distinct from the narcotics.[2-5] In order to investigate the metabolic fate of Nefopam in man, it was desirable to synthesize labelled compound with carbon-13 at the ring aliphatic carbon positions. This labelling pattern permitted observation of drug metabolites in the ^{13}C NMR spectrum without

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the need for complete purification since the labelled peaks were 30-fold stronger than background absorptions. Mass spectral analysis was facilitated by the labelling pattern since high mass fragments had a unique isotope pattern. Reports on the analysis of the enriched metabolites are in preparation.[6] The advantages of using nonradioactive labelled drugs for metabolic and biosynthetic analysis studies have been discussed.[7,8] Independently, Ebel and Schütz have isolated and identified the two major metabolites of Nefopam as the N-demethyl and N-oxide analogues.[9]

DISCUSSION

The reaction sequence for the synthesis of Nefopam-^{13}C hydrochloride (I) is illustrated in Figure 1.

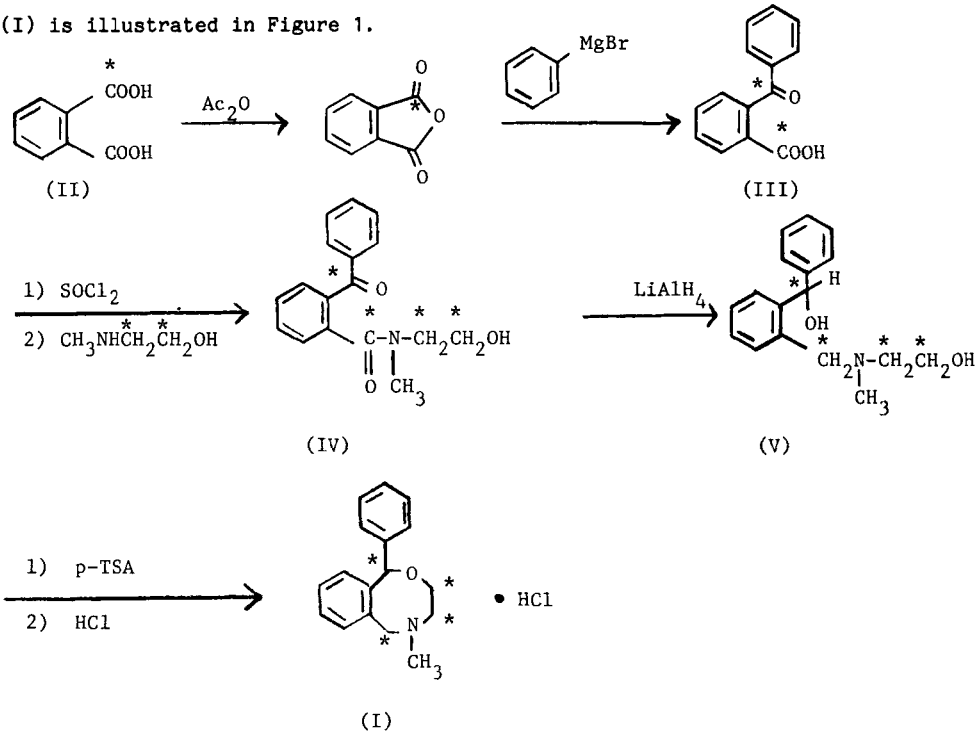

FIGURE I. The reaction sequence for the synthesis of Nefopam-^{13}C hydrochloride.

Phthalic acid-carboxyl-$^{13}C_1$ (II) was converted to the anhydride and reacted with phenyl Grignard to give benzoylbenzoic acid-1,2-^{13}C (III). Treatment with thionyl chloride followed by 2-methylaminoethyl-1,2-$^{13}C_2$ alcohol gave 2-benzoyl-N-methyl-N-(2-hydroxyethyl-1,2-^{13}C) benzamide-1,2-^{13}C (IV). The benzamide was reduced with lithium aluminum hydride to the corresponding amine diol (V) which was cyclized in the presence of para-toluene sulfonic acid to Nefopam-^{13}C and isolated as the hydrochloride salt (I).

Mass spectroscopy indicated that the phthalic acid-carboxyl-$^{13}C_1$ was 90% monolabelled. The average labelling of each methylene of 2-methylaminoethyl-1,2-$^{13}C_2$ alcohol (86%) was determined by integration of the ^{13}C satellites relative to the center band in the proton spectrum. The ^{13}C spectrum showed a pair of doublets, $^1J_{CC} \equiv 37$ Hz. Peaks for the two monolabelled alcohols, at the 8% level relative to the dilabelled doublet, were observed as singlets in the middle of the doublets. Thus, the alcohol is 77% dilabelled and 9% monolabelled each at C-1 and C-2. Structures of the enriched intermediates were confirmed by comparison of their proton NMR and mass spectra to authentic unlabelled samples.

The four aliphatic carbons in the eight-membered ring of I were labelled during the course of the synthesis. Theoretical enrichment for C-1 and C-6 in I based on the 90% enrichment (single carbonyl carbon) of the phthalic acid and the 25% dilution with cold benzoylbenzoic acid is 36%. The proton spectrum shows 1:4:1 triplets in which the outside peaks are due to coupling to the ^{13}C ($^1J_{CH}$ = 142 Hz). Integration of the outside peaks relative to the center band indicates a 33% enrichment, and is within experimental error of the calculated value. The $-OCH_2CH_2N-$ portion of I was not diluted and these carbons (C-3 and 4) show a pair of doublets (J = 35 Hz) in the ^{13}C spectrum (see Figure 2). Singlets for the two

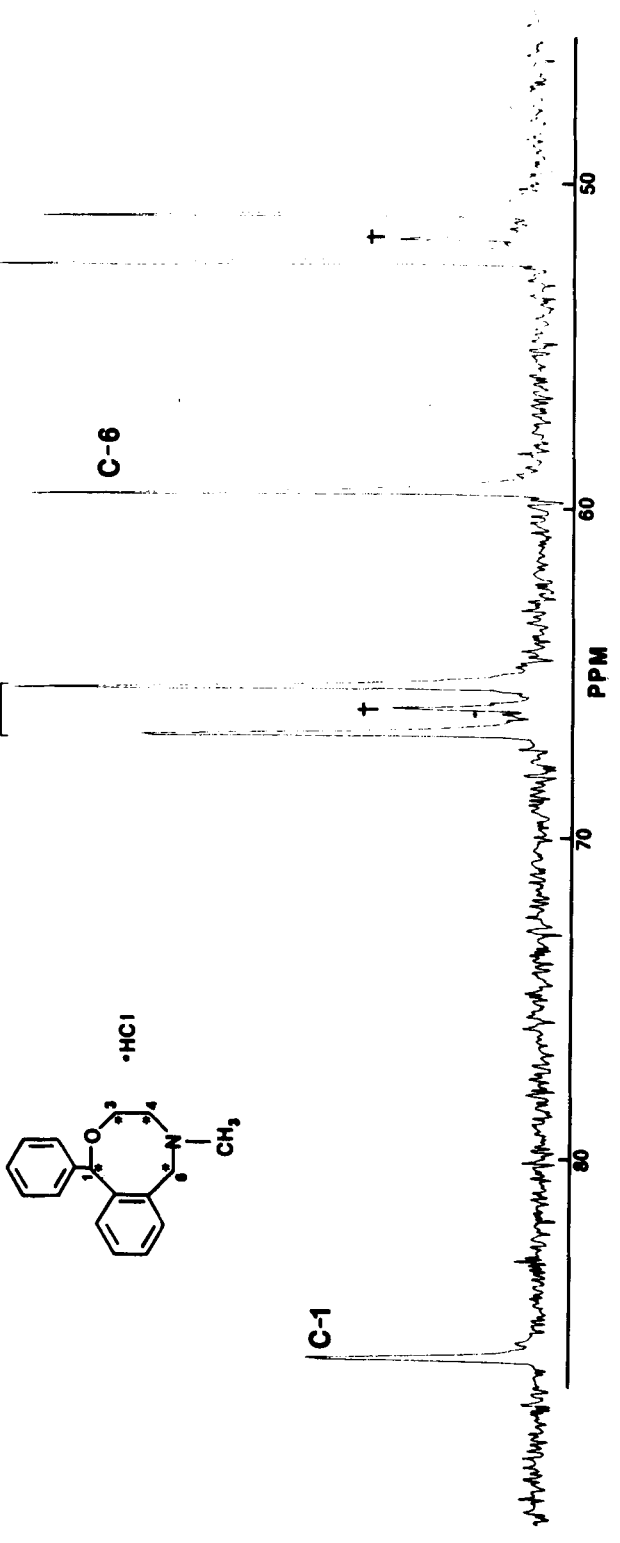

FIGURE 2. Proton noise decoupled ^{13}C NMR spectrum of Nefopam-^{13}C hydrochloride. Peaks marked with a dagger are due to monolabelled C-3 and 4.

monolabelled portions are observed with the same relative intensity as in the 2-methylaminoethyl-1,2-$^{13}C_2$ alcohol. Although the enrichment of this ethylene moiety is twice that of the other carbons, the splitting from the one-bond coupling reduces the peaks 2-fold and results in comparable intensities for all the enriched carbon peaks in I. No long range carbon-carbon coupling was observed.

The ^{13}C chemical shifts of the aliphatic and carbonyl carbons were determined on non-enriched samples and are given in Table 1. The $^1J_{CC}$ determined on labelled samples I and IV are also given. The methine and methyl carbons were assigned using off-resonance continuous wave spin decoupling. The lowfield methylene carbon in I is assigned to C-3 since oxygen is much more deshielding than nitrogen.[10] The C-6 was assigned to the middle methylene resonance because the aromatic ring is more deshielding than a CH_2.[11] The latter assignment was confirmed by the doublet splitting pattern in enriched I.

TABLE 1. ^{13}C Chemical Shifts of Aliphatic Carbon Atoms

STRUCTURE	C-1	C-3	C-4	C-6	NCH_3	$^1J_{CC}$
I	86.4	65.9	51.6	59.4	42.6	35
IV (anti)	197.5[a]	59.4	53.7	170.7[a]	32.8	37
(syn)	196.5[a]	60.1	50.6	171.4[a]	38.6	37
V	75.3	61.3	59.3	58.7	41.2	b

[a] analogous carbonyl resonance

[b] not determined

EXPERIMENTAL

Benzoylbenzoic acid-1,2-^{13}C (III): Phthalic acid-carboxyl-$^{13}C_1$ (II) (18 mmole) in acetic anhydride (10 ml) was refluxed two hours and concentrated *in vacuo*. The solid residue was dissolved in ether (250 ml), filtered, and again concentrated to dryness to give a quantitative yield of pure phthalic anhydride. The phthalic anhydride (18 mmole) was dissolved in benzene (50 ml) and treated with phenyl Grignard (1.275 molar in ether) (15.4 ml, 18.8 mmole). After stirring at 50-60°C for three hours, the cooled mixture was decomposed with saturated ammonium chloride (20.4 ml) and 6N hydrochloric acid (1.4 ml). The combined organic layer and solid were separated and extracted with dilute sodium hydroxide. The basic solution was acidified and extracted with ether. Solvent removal gave an oily solid which after recrystallization gave a 59% yield (10.6 mmole).

2-Benzoyl-N-methyl-N-(2-hydroxyethyl-1,2-^{13}C) benzamide-1,2-^{13}C (IV): The above product (III) was diluted with benzoylbenzoic acid (2.7 mmole) to give a total of 13.3 mmole. The acid was refluxed in benzene (10 ml) and thionyl chloride (7 ml) for three hours and concentrated. The residual oil was dissolved in benzene (20 ml) and treated with triethylamine (20 mmole) followed by 2-methylaminoethyl-1,2-$^{13}C_2$ alcohol (13.3 mmole). The reaction mixture was stirred and refluxed overnight, cooled, and concentrated. The residue was dissolved in chloroform, washed successively with dilute hydrochloric acid, dilute sodium hydroxide and water, and then dried over potassium carbonate. The chloroform was removed to give an oil in quantitative yield.

2{N-(2-hydroxyethyl-1,2-^{13}C)-N-methylaminomethyl-^{13}C-}benzhydrol-1-^{13}C (V): The preceding amide (IV) (13.3 mmole) was dissolved in tetrahydrofuran (25 ml) and added dropwise to a slurry of lithium aluminum hydride

(40 mmole) in tetrahydrofuran (25 ml). The stirred mixture was refluxed overnight, cooled, and decomposed by the successive addition of water (1.5 ml), 6N NaOH (1.5 ml), and water (4.5 ml). The resulting suspension was filtered and the filter cake was washed with warm tetrahydrofuran. The combined filtrates were concentrated to an oil, dissolved in ether, and extracted into dilute hydrochloric acid. The acid layer was basified and then re-extracted into ether. Concentration of the dried organic layer gave an oil in 87.5% yield.

5-Methyl-1-phenyl-3,4,5,6-tetrahydro-1H-2,5-benzoxazocine-1,3,4,6-^{13}C hydrochloride (Nefopam-^{13}C hydrochloride) (I): The above amine diol (V) (11.6 mmole), p-toluene sulfonic acid (17.4 mmole), and toluene (30 ml) were vigorously stirred and refluxed under a water separator for six hours. The reaction mixture was cooled and basified with 6N NaOH. The organic layer was separated, dried, and concentrated to an oil. The oil was redissolved in ether and treated with ethereal-hydrogen chloride to give a crystalline solid product. Recrystallization from methanol-ether gave a recovery of 2.2g (66%) of I.

The ^{13}C NMR spectra were obtained on a Varian XL-100 spectrometer with an acquisition time of 0.8 sec. (1.25 Hz/point) using a 60 μ sec (40° tip angle) pulse and 8K transform. All spectra were obtained in $CDCl_3$ except for I, which was run in D_2O (dioxane reference). Mass spectra were obtained on a CEC 21-110C spectrometer.

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