# SYNTHESIS OF $^{14}$C-LABELLED 10-[3-(DIMETHYLAMINO)-PROPYL]-2-NITRODIBENZ [b,f] [1,4] OXAZEPIN-11-(10 H)-ONE (NITROXAZEPINE)HYDROCHLORIDE †*

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SUMMARY

For pharmacokinetic and metabolism studies in animals and humans, nitroxazepine, an antidepressant, was labelled with carbon-14 at the 11-position of the dibenzoxazepine ring system in an overall yield of 12% starting with potassium [$^{14}$C] cyanide. The labelled preparation had a specific activity of 0.95 μCi/mg. $^{14}$C Label was also introduced in the methyl group of the terminal nitrogen atom in the side chain of nitroxazepine, starting from its des-methyl derivative and using [$^{14}$C] para-formaldehyde. In this case the overall yield of the final compound was 20%, and its specific activity 1.84 μCi/mg.

Keywords: Synthesis, nitroxazepine, 10-[3-dimethyl-amino)propyl]-2-nitrodibenz [b,f] [1,4] oxazepin-11-(10 H)-one, carbon-14, antidepressant

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† Active ingredient of Sintamil®

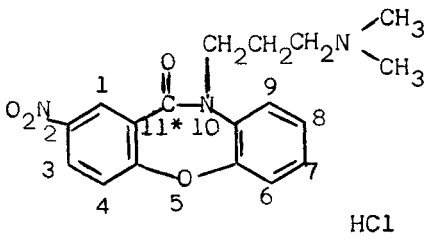
* Contribution No.695 from CIBA-GEIGY Research Centre.

0362-4803/83/121339-10$01.00

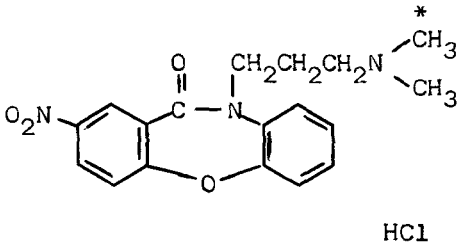

Received April 13, 1983
Revised July 26, 1983

## INTRODUCTION

Nitroxazepine, 10-[3-(dimethylamino)propyl]-2-nitrodibenz-[b,f][1,4] oxazepin-11 (10 H)-one is a novel dibenzoxazepine derivative which in the form of its hydrochloride, has been found to be a safe and useful drug for the treatment of depression[1,2,3]. For metabolic[4] and pharmacokinetic studies, it has been labelled with carbon-14 in the central seven membered ring at position C-11 (1a) and also on the methyl group in the side chain (1b), the label being indicated by the asterisk (*).




1a

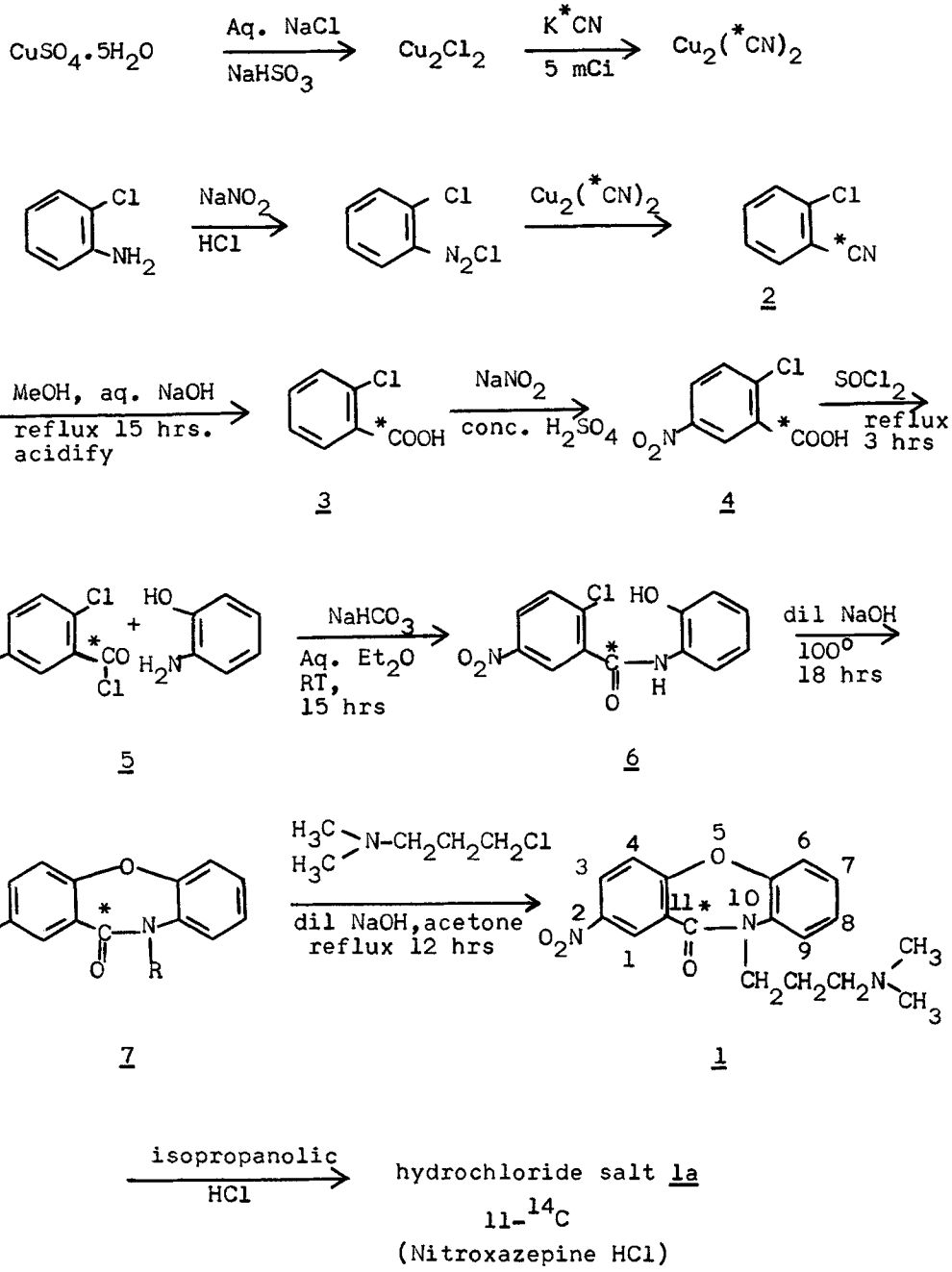



1b

The labelling at C-11 (1a) was performed on a 10 mM scale according to the Scheme A starting with potassium [$^{14}$C] cyanide (5 mCi). The side chain labelling, leading to (1b) was carried out on a 0.2 mM scale using [$^{14}$C] para-formaldehyde (0.5 mCi), as per Scheme B.

Scheme A : Reaction of cuprous [$^{14}$C] cyanide (generated in situ from potassium [$^{14}$C] cyanide and cuprous chloride) with 2-chlorobenzene diazonium chloride afforded

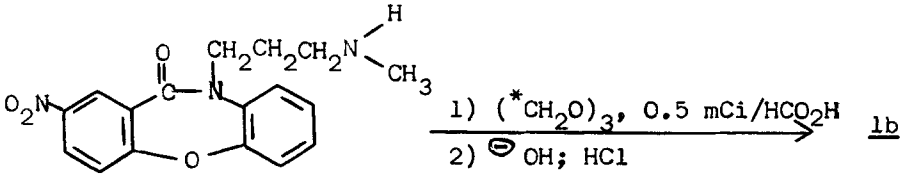
Scheme A


CuSO4.5H2O
Aq. NaCl
NaHSO3
Cu2Cl2
K*CN
5 mCi
Cu2(*CN)2
Cl
NH2
NaNO2
HCl
N2Cl
*CN
2
MeOH, aq. NaOH
reflux 15 hrs.
acidify
*COOH
3
conc. H2SO4
O2N
SOCl2
reflux 3 hrs
4
5
NaHCO3
Aq. Et2O
RT,
15 hrs
6
dil NaOH
100°
18 hrs
7
H3C
N-CH2CH2CH2Cl
dil NaOH, acetone
reflux 12 hrs
1
isopropanolic
HCl
hydrochloride salt 1a
11-14C
(Nitroxazepine HCl)

2-chloro[cyano-$^{14}$C]benzonitrile 2 which was transformed to the acid 3 by treatment with hot methanolic sodium hydroxide followed by acidification. Careful nitration of 3 using sodium nitrate and sulphuric acid led to the formation of 2-chloro-5-nitro[carboxyl-$^{14}$C]benzoic acid 4 which was converted to the acid chloride 5 and then by reaction with 2-aminophenol in the presence of sodium bicarbonate to yield the $^{14}$C-labelled amide 6. Cyclisation of 6 using hot aqueous sodium hydroxide gave lactone 7 carrying the $^{14}$C label at the 11-position. Alkylation of 7 with dimethylaminopropyl chloride in aqueous acetone and sodium hydroxide led to the formation of [$^{14}$C]nitroxazepine 1 which was converted to the crystalline hydrochloride salt 1a. The sequence of reactions gave 1a in an overall yield of 12%, based upon K$^{14}$CN used.

Scheme B : Clarke-Eschweiler reaction of des-methyl nitroxazepine 8 using [$^{14}$C] paraformaldehyde and formic acid introduced a labelled methyl group in the side chain. The resultant product was characterised as the hydrochloride 1b, which was obtained in an yield of 20% from 8.

Scheme B




Both the synthetic sequences were carried out several times with non-radioactive material to optimise the yield and purity at each step.

The relevant data are given below :

TABLE

| Compound No. | Mol. wt. | M.P. °C | Yield % |
|---|---|---|---|
| 1.HCl | 377.8 | 226-7 | 50 (1a)<br>70 (1b) |
| 2 | 137.5 | liquid | 84 |
| 3 | 156.5 | 140-42 | 47 |
| 4 | 201.5 | 164-6 | 89 |
| 5 | 220.0 | liquid | 93 |
| 6 | 292.6 | 186-8 | 73 |
| 7 | 256.2 | 258-60 | 98 |
| 8.HCl | 363.7 | 215-7 | - |

## EXPERIMENTAL

Melting and boiling points are uncorrected.

Potassium cyanide-$^{14}C$ (8.1 mg; sp. acty. 40.3 mCi/m.mole, 5 mCi) was procured from Bhabha Atomic Research Centre, Trombay, Bombay 400 085, INDIA. Nonradioactive potassium cyanide was of Anala R grade.

Paraformaldehyde $^{14}C$ (sp. acty. 325 μCi/mg; 0.5 mCi) was obtained from the Radiochemical Centre, Amersham, England.

2-Chloroaniline and 2-aminophenol were purchased from Fluka AG, Buchs, Switzerland and purified before use.

Silica gel (100-200 mesh) of E. Merck was used for column chromatography and all other chemicals (reagent grade) were procured locally.

Radioactivity measurements were made with a Nuclear-Chicago, Mark I, Liquid Scintillation Counter.
The Scintillator fluid (cocktail) contained 4 g PPO and 0.5 g POPOP per litre of toluene.
Reversed isotope dilution analyses (RIDA) involving repeated crystallisations from methanol-ether and TLC comparisons were with analytically pure unlabelled nitroxazepine hydrochloride. 5 mg of RIDA samples were dissolved in methanol (5 ml) and mixed with cocktail (15 ml) before counting.
Radiometric TLC was done on 20 cm. glass plates precoated with a layer of silica gel $F_{254}$ (TLC grade) 150 μ in thickness. Measurement of radioactivity of sections of the radiochromatograms was carried out as suspensions in a mixture of methanol (5 ml) and cocktail (10 ml).

Benzene diazonium chloride

To a suspension of 2-chloroaniline hydrochloride (from 2-chloroaniline 1.4 g, conc. HCl 2.75 ml and crushed ice 10 g) in a 50 ml RB flask was added a solution of sodium nitrite (0.77 g) in water (2-3 ml) cautiously over a period of 5 min. Then the mixture was made neutral by careful addition of sodium carbonate (0.65 g) in water (2 ml) (turbidity - sign of neutralization).

2-Chloro[cyano-$^{14}$C]benzonitrile (2)

To a hot aqueous solution of $CuSO_4.5H_2O$ (800 mg) in water (2.5 ml) was added sodium chloride (200 mg) and sodium bisulphite (165 mg) and the mixture stirred vigorously. The precipitated cuprous chloride was collected by centrifugation, washed with 3 ml $H_2O$ twice and transferred with water (2 ml) to a RB flask (50 ml) containing potassium

[$^{14}$C] cyanide 5 mCi, (642 mg), in water (1 ml) kept under a layer of toluene (2 ml). The mixture was stirred magnetically and to the cuprous cyanide thus formed in situ was added freshly prepared neutral benzene diazonium chloride solution. After the addition was over, the mixture was stirred at 0° for 30 min, at RT for 1½ hr and later at 70-100° for a few minutes. After cooling, the mixture was extracted thoroughly with ether, to give the crude o-chlorobenzonitrile 2 which was chromatographed on a silica column. Elution with benzene gave the pure [$^{14}$C] nitrile (2) 1.26 g. Yield 92%.

2-Chloro[carboxyl-$^{14}$C]benzoic acid (3)

Hydrolysis of 2 (1.16 g) was carried out with a mixture of aqueous sodium hydroxide (10%; 10 ml) and methanol (5 ml) under reflux, over a water bath for 15 hrs. Removal of methanol on a rotary evaporator followed by acidification with conc. HCl (1.8 ml) precipitated the chlorobenzoic acid 3, which was filtered off, washed with water and dried thoroughly; 620 mg. Yield 47%.

2-Chloro-5-nitro[carboxyl-$^{14}$C]benzoic acid (4)

A mixture of 3 (620 mg) and sodium nitrate (440 mg) was treated under stirring at 0° with conc. $H_2SO_4$ (4.35 ml), added cautiously, dropwise, over 20 min. Stirring in the cold was continued for 1½ hrs. Crushed ice was added; the precipitated chloronitrobenzoic acid 4 was filtered, washed with water and dried; 712 mg. Yield 89%.

2-Chloro-5-nitro[carboxyl-$^{14}$C]benzoylchloride (5)

Refluxing the above carboxylic acid for 3 hrs with thionyl-chloride (2.2 ml), followed by the removal of the excess of

reagent on the rotary evaporator gave the acid chloride 5; 725 mg. Yield 93%.

N-(2'-Chloro[carboxyl-$^{14}$C]benzoyl)-2-hydroxyaniline (6)

A solution of the foregoing acid chloride 5 in dry ether (4 ml), 2-aminophenol (360 mg), sodium bicarbonate (512 mg), ether (4.5 ml) and water (3 ml) was stirred in an ice-bath for 15 hrs; then the ice was allowed to melt, with stirring being continued for another 6 hrs. Ether was removed on a rotary evaporator, water was added and the solid anilide 6 was filtered off, washed with water and dried; 706 mg; yield 73%.

2-Nitro[11-$^{14}$C]dibenz [b,f] [1,4] oxazepin-11 [10 H]-one (7)

Cyclisation of 6 was effected by stirring with dilute aqueous alkali (17 ml $H_2O$ containing 3 ml N aq. NaOH) over a water bath at 100° for 18 hrs. The precipitate was filtered off and washed with water to afford lactam 7 ; 607 mg. Yield 98%.

10-[3-(Dimethylamino)propyl]-2-nitro[11-$^{14}$C]dibenz [b,f][1,4] oxazepin-11 (10 H)-one hydrochloride (Nitroxazepine HCl) (1a)

To a stirred mixture of above nitrolactam 7, acetone (7.5 ml) and aq. NaOH (2N, 1.35 ml) was added dropwise a solution of 3-dimethylaminopropylchloride (906 mg of freshly prepared base) in acetone (9 ml) in three equal aliquots over 1 hr intervals and the mixture refluxed with stirring over an oil bath at 75° for 12 hrs. Cooling and extraction with ether afforded the free base 1 as an oil, 850 mg. Conversion of this base to its hydrochloride by treatment with isopropanolic HCl and subsequent crystallisation from methanol-ether afforded the pure $^{14}$C-labelled 1a, 448 mg, sp. acty. 0.95 μCi/mg.

Reversed isotope dilution analyses and Radiometric TLC Silica/ solvent systems a, b, c - Rf

a. Butanol(38) : HOAc(8) : Pyridine(24) : $H_2O$(30) - 0.7

b. Butanol(4) : HOAc(1) : $H_2O$(1) - 0.6

c. Chloroform(3) : MeOH(1) - 0.55

indicated the purity of the $^{14}$C-labelled preparation as being >98%.

10-[3-(Dimethylamino)propyl]-2-nitro[methyl-$^{14}$C]dibenz [b,f] [1,4]oxazepin-11 (10H)-one hydrochloride (1b)

To a freshly prepared sample of des-N-methylnitroxazepine base 8 (liberated from 72.8 mg of corresponding hydrochloride[4]) was added [$^{14}$C] paraformaldehyde and an appropriate amount of nonradioactive paraformaldehyde (15.8 mg). Formic acid (98%; 2.5 ml) was added cautiously and the mixture heated over a boiling waterbath for $4\frac{1}{2}$ hrs after attaching a reflux condenser to the reaction flask. The mixture was cooled and crushed ice (3 g) added. The solution was basified with aqueous sodium hydroxide (10%; 7.5 ml) and the liberated base extracted thoroughly with ether. This was converted to its hydrochloride 1b, with 6M isopropanolic HCl and 1b crystallised from methanol-ether. Yield 53 mg; sp. acty. 1.84 μCi/ mg. The radiochemical purity when assessed by the methods outlined earlier was > 99%.

ACKNOWLEDGEMENTS

We thank Dr. B. Anjaneyulu for his cooperation and help in the preparation of the manuscript and Dr. A. Venkateswarlu for experimental assistance in carrying out cold experiments.

## REFERENCES

1. Nagarajan, K., David, J., Kaul, C. L., Maller, R.K., Rao, R. R. and Grewal, R. S., Indian J. Physiol. Pharmac., 19, 39 (1975).

2. Nagarajan, K., David, J., Grewal, R. S. and Govindachari, T. R., Indian J. Exptl. Biol., 12, 217 (1974).

3. Nagarajan, K. and Arya, V. P., J. Sci. Ind. Res., 41, 232 (1982).

4. Nagarajan, K., Maller, R. K., Anjaneyulu, B., Nagana Goud, A. and Venkateshwarlu, A., Indian J. Chem., 12, 270 (1974).