# LABELLED COMPOUNDS OF INTEREST AS ANTITUMOUR AGENTS I: N-METHYLFORMAMIDE AND N,N-DIMETHYLFORMAMIDE.

Michael D Threadgill and E Nicholas Gate
Cancer Research Campaign Experimental Chemotherapy Research Group
Department of Pharmacy, University of Aston in Birmingham
Birmingham B4 7ET, UK.

## SUMMARY

Efficient preparations of N-methylformamide (an active antitumour agent) have been developed in which the compound is labelled with $^{14}C$ in the formyl group, with $^{14}C$ in the methyl group and with deuterium in the methyl group. N,N-Dimethyl-$^{14}C$-formamide has also been synthesised.

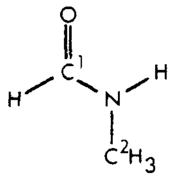
Key words: Antitumour, Carbon-14, Deuterium, Dimethylformamide, Methylformamide

## INTRODUCTION

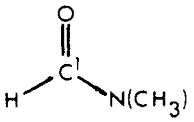
N-Methylformamide (1; NMF) is a compound which shows significant activity against a number of murine tumours |1-4| whereas that of its dimethyl analogue N,N-dimethylformamide (2; DMF) is, at best, marginal. In order to investigate both the in vivo distribution and the metabolic fate of the two different one-carbon fragments arising from NMF and DMF, we required NMF labelled with $^{14}C$ in each of the carbon positions separately (3 and 4) and DMF containing a $^{14}C$ methyl group (5). As it is possible that in vivo oxidation of the methyl group of NMF is responsible for furnishing the actual antitumour agent, we sought N-(trideutero methyl)-formamide (6) as a tool to examine any primary kinetic isotope effect in this putative metabolic activation; if such an effect were found, it may manifest itself, inter alia, as a decrease in antitumour activity upon deuteration.

0362-4803/83/040447-05$01.00

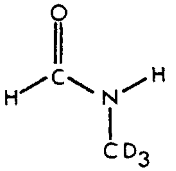
Received October 12, 1982



**1**: $C^1 = C^2 = {}^{12}C$
**3**: $C^1 = {}^{12}C ; C^2 = {}^{14}C$
**4**: $C^1 = {}^{14}C ; C^2 = {}^{12}C$



**2**: $C^1 = {}^{12}C$
**5**: $C^1 = {}^{14}C$



**6**

## RESULTS

The pyrolysis of ammonium salts of carboxylic acids has long been known to give carboxamides, albeit infrequently in modest yield. The synthesis of N,N-dimethyl-${}^{14}C$-formamide (5) by this route has been described by Vereš *et al* |5|; however, in our hands, in experiments using unlabelled material, this technique failed, giving a mixture of DMF (2) and dimethylammonium formate. A modification was therefore employed. Sodium ${}^{14}C$-formate was heated with an excess of methylamine hydrochloride in the absence of solvent in an apparatus designed to permit gentle reflux before allowing the product to distil. This served to ensure that all the volatile methylammonium ${}^{14}C$-formate had been thermally converted to the desired amide (3). The dimethyl analogue (5) was prepared similarly from sodium ${}^{14}C$-formate and dimethylamine hydrochloride.

The methyl-radiolabelled compound (4) was prepared from ${}^{14}C$-methylamine hydrochloride and ethyl formate in the presence of methanolic base. Chemical and radiochemical yields of compounds (3 - 5) were high.

Pure anhydrous N-(trideuteromethyl)-formamide (6) was prepared from trideuteromethylamine hydrochloride and ethyl formate in moderate yield. All the labelled NMF derivatives (3, 4, 6) are new compounds. Differences between the infra-red spectrum of (6) and that of its protio-analogue (1) permitted us to assign certain absorbance bands to vibrations of the methyl group of NMF ($\nu_{C-H}/\nu_{C-D} \sim 1.37$). These assignments complement the work of Suzuki [6], who used other deuterated NMF derivatives, but conflict with Sutherland's [7] proposals for assignment of the bands at 2880 cm$^{-1}$ and 960 cm$^{-1}$ in the spectrum of (1), which we have shown to be vibrations of the $CH_3$ group (see the Table).

Table. Infra-red Spectrum of 1 and 6

| $\nu$ (1) | $\nu$ (6) | $\nu$(1)/$\nu$ (6) | Assignment |
|---|---|---|---|
| 3400 | 3500-3200 | 1 | NH |
| 3060 | 3040 | 1.01 | CH |
| 3060 | 2230 | 1.37 | $CH_3$ |
| 2950 | 2110 | 1.40 | $CH_3$ |
| 2880 | 2080 | 1.38 | $CH_3$ |
| 1660 | 1660 | 1.00 | Amide I |
| 1550 | 1520 | 1.02 | Amide II |
| 1420 | 1050 | 1.35 | $CH_3$ |
| 1390 | 1385 | 1.00 | CH |
| 1240 | 1250 | 0.99 | Amide III |
| 1150 | 870 | 1.32 | $CH_3$ |
| 960 | 700 | 1.37 | $CH_3$ |

The outcome of the metabolic and pharmacokinetic investigations will be published elsewhere [8]; further studies on the physicochemical properties of this important antitumour agent (NMF, 1) are being actively pursued.

ACKNOWLEDGEMENTS: This work was supported by a grant from the Medical Research Council. We thank Dr J Grieg for his helpful advice and Mrs K Farrow for the mass spectrometric studies.

## Experimental

The radioactivity was measured by the scintillation method using a Packard Tricarb 2660 intrument and radiochemical purity was checked using thin-layer chromatography on silica gel plates (Silica Gel 60 $F_{254}$, Merck). Infrared spectra were obtained from thin films of liquid sample using a Perkin-Elmer 1310 spectrometer. Proton magnetic resonance spectra were obtained at 60 MHz using a Varian EM-360A spectrometer with tetramethylsilane as internal standard. Radiochemicals ($^{14}$C-methylamine hydrochloride and sodium $^{14}$C-formate) were obtained from Amersham International PLC and trideuteromethylamine hydrochloride (98%D) from Aldrich Chemical Co.Ltd.

### N-$^{14}$C-Methylformamide (3)

A solution of sodium methoxide in methanol (2.0ml) (prepared from sodium metal (69 mg)) was added slowly to a cooled solution of $^{14}$C-methylamine hydrochloride (202.5 mg; 1.0 mCi) in methanol (1.0 ml). Anhydrous sodium carbonate (318 mg) and ethyl formate (3.0 ml) were added and the mixture was stirred at 25°C for 18 hours. Ethyl formate (4.0 ml) was added and the salts were filtered off using a cotton-wool plug. The filtrate and ethyl formate washings were combined and the solvents evaporated carefully under reduced pressure to give N-$^{14}$C-methylformamide (163 mg; 0.92m Ci; chemical yield 92%) as a colourless liquid. It had chromatographic properties identical to a commercial sample of unlabelled N-methylformamide.

### N-Methyl-$^{14}$C-formamide (4)

Methylamine hydrochloride (270 mg) and sodium $^{14}$C-formate (136 mg; 1.7m Ci) were placed in the side-arm of a 7cm x 1cm test-tube equipped with 10cm x 1cm side-arm and with a reflux condenser. Gentle heating of the solids over a flame for 8 minutes caused the volatile materials to boil under reflux within the side-arm. The mixture was then heated more strongly and a pale straw-coloured liquid distilled into the main tube. The distillate was extracted with acetone (6x4 ml) Careful evaporation of the solvent furnished N-methyl-$^{14}$C-formamide monohydrate (122 mg; 1.35 mCi; chemical yield 79%) as a pale yellow liquid. It had properties identical to an equimolar mixture of authentic N-methylformamide and water.

N,N-Dimethyl-$^{14}$C-formamide (5)

This compound was prepared using a technique similar to that used for (4) from dimethylamine hydrochloride (326 mg) and sodium $^{14}$C-formate (136 mg; 870 µCi) N,N-Dimethyl $^{14}$C-formamide monohydrate (120 mg; 560µCi; chemical yield 66%) was obtained as a pale straw-coloured liquid having properties identical to an equimolar mixture of authentic N,N-dimethylformamide and water.

N-(Trideuteromethyl)-formamide (6)

Sodium (660 mg) was added to absolute ethanol (30 ml) at -5°C. When all the metal had dissolved, (trideuteromethyl)amine hydrochloride (2.0g) was added, followed by ethyl formate (25 ml) and anhydrous sodium carbonate (10g). The mixture was stirred at -5°C for 1 hour then 12 hours at 20°C. A clear solution was obtained after filtration and the filtrate and ethyl formate washings were concentrated in vacuo to 10ml. Distillation using the bulb-to-bulb technique gave N-(trideuteromethyl)-formamide (860 mg; 49%) a colourless liquid. $BPt_3$ 65-70°C $\nu_{max}$ (liquid film): (see Table). $\delta(CDCl_3)$: 7.3 (1H) br (NH, 8.03) 0.1H) s CHO cis-form, 8.27 (0.9H) s CHO trans-form. m/z: 62 ($M^+$).

## REFERENCES

1. Clarke, D.A., Philips, F.S., Sternberg, S.S., Barclay, R.K. and Stock, C.C.- Proc.Soc.Exp.Biol.Med. 84: 203 (1953).
2. Skipper, H.E. Schabel, F.M. Jnr., Binns, V., Thomson, J.R. and Wheeler, G.P.- Cancer Research 15: 143 (1955).
3. Furst A., Cutting, W.C. and Gross, H.-Cancer Research 15: 294 (1955).
4. Gescher, A., Gibson, N.W., Hickman, J.A., Langdon, S.P., Ross, D. and Atassi, G.-Brit. J. Cancer 45: 843 (1982).
5. Vereš, K., Kozel, J. and Procházka, Ž.-Coll. Czech. Chem.Commun: 28, 750 (1963).
6. Suzuki, I.-Bull. Chem. Soc. Japan. 35: 540 (1962).
7. De Graaf, D.E. and Sutherland, G.B.B.M.-J. Chem. Phys. 26: 716 (1957).
8. Brindley, C., Gescher, A., Harpur, E.S., Slack, J.A., Threadgill, M.D. and Whitby, H.M.-Cancer Treat. Rep. in press.