SYNTHESIS OF ISOTOPICALLY LABELLED SALICYLATES

D. R. Hawkins and R. W. Pryor
Department of Metabolism & Pharmacokinetics,
Huntingdon Research Centre,
Huntingdon,
England

SUMMARY

[^{13}C-carboxyl]Salicylic acid has been prepared by carbonation of 2-benzyloxybromobenzene followed by reductive debenzylation. Deuterium and tritium labelled salicylic acid and $^{2}H_{2}/^{13}C$-salicylic acid were prepared by reduction of the 3,5-dibromo derivatives using Raney Ni-Al. Deuterium labelled salicylic acid containing up to four deuterium atoms was prepared by catalytic exchange with Raney Ni-Al in 5% NaOD/D_2O.

Key words: Salicylic acid, carbon-13, carbonation, Raney Ni-Al, deuterium, tritium

INTRODUCTION

As part of a programme concerned with the use of stable isotopes in drug metabolism, it was necessary to obtain appropriate labelled forms of some model drugs. Synthesis of the antidepressant drug, amitriptyline, labelled with carbon-13 has been reported[1] and in this paper we now describe the synthesis of salicylic acid and acetylsalicylic acid labelled with carbon-13 and deuterium.

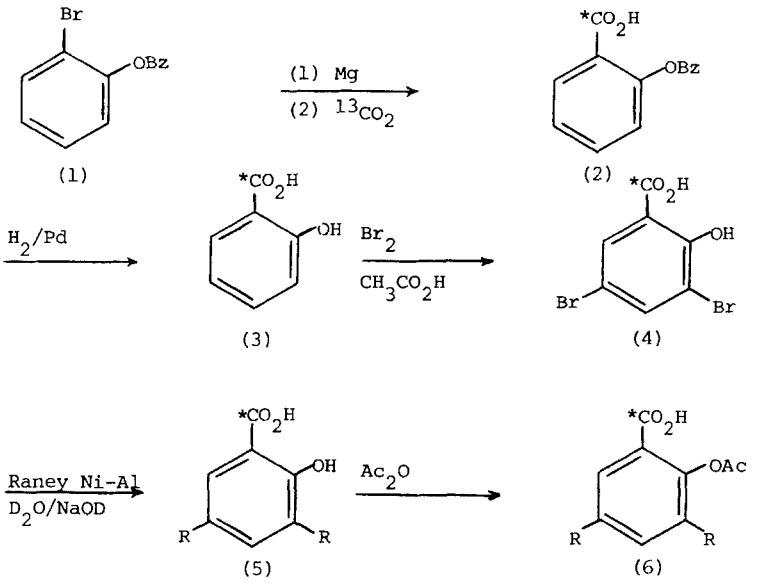
0362-4803/81/040593-10$01.00

Received December 20, 1979
Revised January 11, 1980

RESULTS AND DISCUSSION

The scheme shows the synthetic route employed for the synthesis of [^{13}C-carboxyl/3,5-^{2}H]salicylic acid (5, R = ^{2}H) and acetyl-[^{13}C/$^{2}H_2$]salicylic acid (6, R = ^{2}H). The carbonation of 2-benzyloxybromobenzene using $^{13}CO_2$ generated from barium [^{13}C]carbonate and hydrogenation of the resulting product gave [^{13}C-carboxyl]salicylic acid (3) in a yield of about 50%. Salicylic acid readily forms a dibromo derivative with substitution occurring at positions 3 and 5, ortho and para to the phenolic hydroxyl and meta to the electron-withdrawing carboxyl group.

Scheme



* = ^{13}C or ^{12}C R = ^{2}H or ^{3}H

Aromatic halides can be conveniently reduced in aqueous solution with Raney Ni-Al generated in situ[2]. The identity and extent of deuterium incorporation in all products was established by mass spectrometry. When dibromosalicylic acid in 5% w/v sodium deuteroxide and Ni-Al alloy was heated under reflux for 1 hour, salicylic acid was obtained in a yield of about 80%, but mass spectrometry showed that the product contained a mixture, di-, tri and tetra-deuterated compounds. However, when the reduction was carried out at 50°C for 1 hour, salicylic acid was obtained which contained mostly two deuterium atoms. Reduction was also shown to be complete after 30 minutes at room temperature. [3,5-^{3}H]Salicylic acid was similarly prepared via reduction of 3,5-dibromosalicylic acid using Raney Ni-Al in tritiated water.

The dibromo derivative of [^{13}C-carboxyl]salicylic acid (4) was readily reduced to [^{13}C-carboxyl/3,5-^{2}H]salicylic acid (5, R = ^{2}H) in a yield of 73% and this was converted to acetylsalicylic acid (6, R = ^{2}H) by acetylation with acetic anhydride. The mass spectrum of the product showed a molecular ion at m/e 183 and fragment ions m/e 166 (M^{+} −OH), m/e 141 and m/e 123, all three mass units higher than in the spectrum of authentic acetylsalicylic acid.

The proton NMR spectrum of authentic acetylsalicylic acid shows a singlet at δ2.4 ($\underline{CH}_3CO$) and complex multiplets in the aromatic region including a quartet at δ8.1 attributable to the proton (H-6) adjacent to the carboxyl group (Figure 1). The spectrum of acetyl[$^{2}H_2$]salicylic acid shows two singlets in the aromatic region at δ8.1 and 7.6. The small coupling of about 2Hz associated with each indicates that the

protons are meta to each other. Since the signal at δ8.1 is due to H-6, the other signal at δ7.6 must be due to H-4. This provides unambiguous evidence that the deuterium atoms were incorporated at positions 3 and 5. The spectrum of acetyl[$^{2}H_2$/^{13}C]salicylic acid shows a singlet aromatic proton at δ7.56 (H-4) and a doublet at δ8.1 (H-6) due to coupling between the ^{13}C-carboxyl and H-6.

Table 1

The incorporation of deuterium in salicylic acid during treatment with Raney Ni-Al in 5% NaOD in D_2O at 50°C.

Reaction time (hours)	Number of deuterium atoms (relative %)				
	0	1	2	3	4
0.5	48	39	13	-	-
3	27	47	21	5	-
4.5	18	40	32	10	-
24	-	10	27	46	18

Since during treatment with Raney Ni-Al deuterium was introduced at positions besides 3 and 5 in 3,5-dibromosalicylic acid, presumably as a result of exchange, the rate and extent of deuterium incorporation in salicylic acid treated under the same conditions was investigated. The extent of exchange was measured by mass spectrometric analysis of aliquots of the reaction mixture and the results are summarised in Table 1. At 30 minutes, more than 50% of the salicylic acid contained

at least one deuterium atom. The extent of deuterium incorporation increased with time and occurred at every available position since at 24 hours the product contained about 18% of the tetradeuterated species. In a separate experiment, when the reaction mixture was heated under reflux for 30 hours, the isolated salicylic acid contained about 90% of the tetradeuterated species.

Other workers[2] have used both Raney Ni-Al and Raney Cu-Al for the preparation of specifically deuterated phenols from corresponding halogenated derivatives. Using 10% w/v NaOD in D_2O at room temperature, chloro and bromo phenols were readily reduced to the deuterated derivatives using Raney Ni-Al, although deuterium was also incorporated at other positions. Raney Cu-Al was less active and did not readily reduce chloro phenols[2]. Raney Ni-Al has been used for the preparation of deuterium and tritium labelled aromatic amino acids by catalytic exchange in 0.5N NaOD/D_2O at room temperature[3]. It was shown that hydrogen atoms meta and para to the alkyl side-chain in tyrosine and phenylalanine exchanged much more readily than the ortho protons. A Zn-Cu couple has been used to prepare deuterated compounds from organic halides, the reactions being carried out in water-miscible ethers such as dioxan or tetrahydrofuran using D_2O as deuterium source[4].

Thus, these metal catalysts provide a very mild, convenient method of preparing deuterium and tritium labelled compounds from aryl halides and are particularly useful for compounds with acidic functions since the reductions are carried out in aqueous basic media. The same metal catalysts can also be used for the direct introduction of deuterium and tritium by exchange and it may be possible to obtain specific exchange of some aromatic protons by appropriate choice of reaction conditions and catalyst activity.

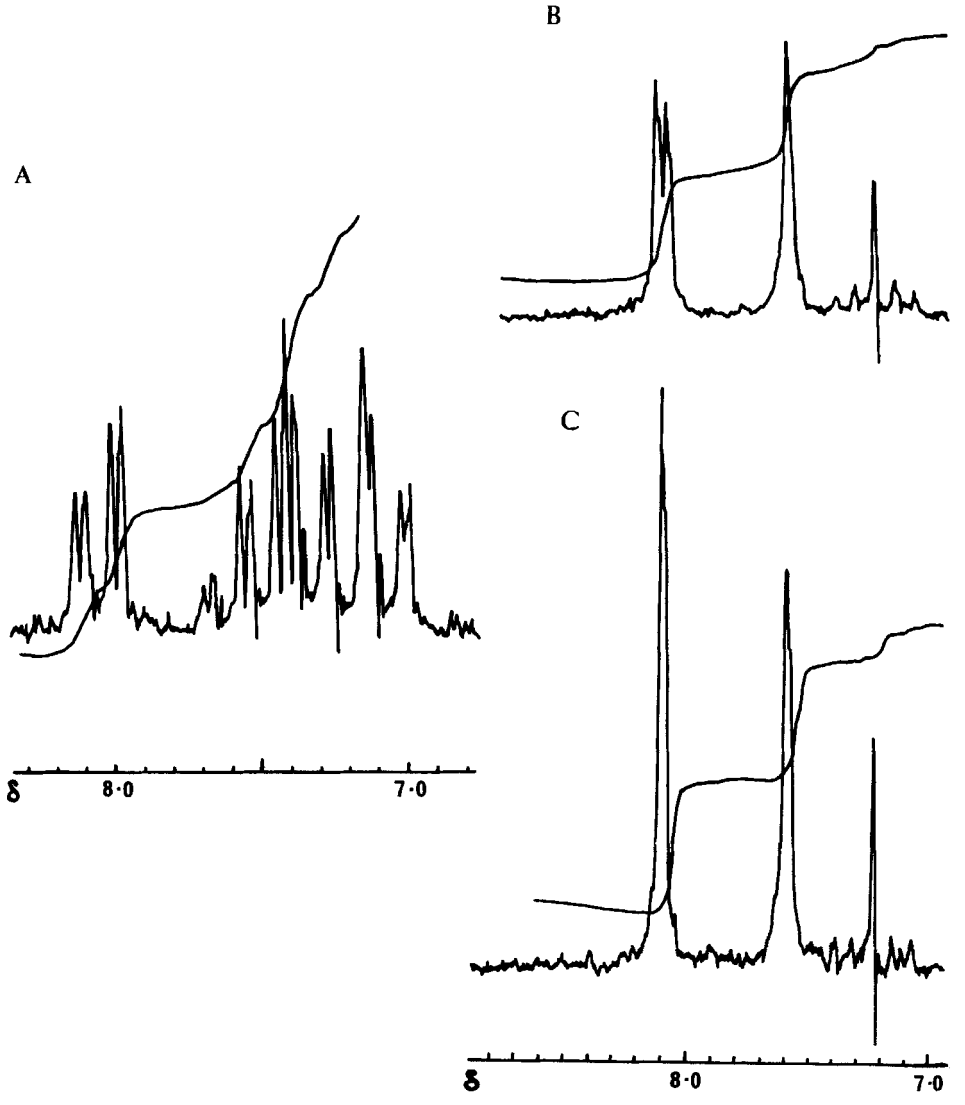


Figure 1. Partial proton NMR spectra (aromatic protons) of A) acetylsalicylic acid B) acetyl[$^{13}C/^{2}H_2$]salicylic acid and C) acetyl[$^{2}H_2$]salicylic acid.

EXPERIMENTAL

2-Benzyloxybromobenzene (1)

A solution of o-bromophenol (10 g, 0.058 mole) and benzyl bromide (9.9 g, 0.058 mole) in acetone (50 ml) was heated under reflux with potassium carbonate (8.3 g, 0.06 mole) for 4 hours. The mixture was filtered and the filtrate concentrated to give a brown oil which was distilled *in vacuo* to give the product as a colourless oil (9.3 g, 60%) b.p. 100°/0.06 mm. The product appeared as a single component (R_f 0.75) on tlc using the solvent system acetic acid:benzene:ether:methanol (18:120:60:1, v/v) and had an infrared spectrum consistent with the structure of the required product.

o-Benzyloxy[^{13}C-carboxyl]benzoic acid (2)

A solution of o-benzyloxybromobenzene (5.26 g, 20 mmole) in dry tetrahydrofuran (20 ml) was added dropwise to magnesium turnings (530 mg, 22 mg atoms). The reaction was initiated by the addition of an iodine crystal. The resulting solution of the Grignard reagent was used directly. An aliquot of this solution (4 ml, 2.32 mmole) was carbonated in a manifold apparatus at -25°C with $^{13}CO_2$ liberated from barium [^{13}C]carbonate (454 mg, 0.23 mmole) by the addition of conc. sulphuric acid. Dilute sulphuric acid was added to the reaction mixture and the crude product was extracted with ether. After back extraction of the carboxylic acid into aqueous M-sodium hydroxide, the solution was acidified and the product extracted with ether. The dried ($MgSO_4$) extract was evaporated to dryness to give a brown solid (271 mg, 51%). The product appeared as a single component (R_f 0.55) on tlc using the solvent system

acetic acid:benzene:ether:methanol (18:120:60:1, v/v). The mass spectrum showed a molecular ion at m/e 229 and a base peak at m/e 91 consistent with the structure of the title compound.

[^{13}C-carboxyl]salicylic acid (3)

A solution of o-benzyloxy[^{13}C-carboxyl]benzoic acid (750 mg, 3.3 mmole) in ethanol (20 ml) was hydrogenated at room temperature under hydrogen at atmospheric pressure in the presence of 10% palladium on charcoal catalyst. After 1 hour, when the uptake of hydrogen was complete, the solvent and catalyst were removed to yield the product (418 mg, 91%) as a pale brown solid. The product appeared as a single component (R_f 0.2) on tlc using the solvent system benzene:methanol (9:1, v/v). The mass spectrum showed a molecular ion at m/e 139 and fragment ions at m/e 121 and m/e 93, all being one mass unit higher than the corresponding ions in the spectrum of authentic salicylic acid.

3,5-Dibromo[^{13}C-carboxyl]salicylic acid (4)

[^{13}C]Salicylic acid (1.7 g, 1.22 mmole) was dissolved in acetic acid (25 ml) and the stirred solution cooled in ice. Bromine (3.9 g, 2.45 mmole) was added dropwise and when the addition was complete, water (175 ml) was added. The resulting precipitate was filtered off, washed with water and dissolved in ether. The carboxylic acid was extracted into sodium bicarbonate solution, the ether layer discarded and re-extracted into ether after acidification with sulphuric acid. The dried ($MgSO_4$) extract was evaporated to dryness to give a white solid (2.3 g, 63%).

[^{13}C-carboxyl-3,5-dideutero]salicylic acid (5, R = ^{2}H)

3,5-Dibromo[^{13}C]salicylic acid (689 mg, 2.3 mmole) was dissolved in 5% sodium deuteroxide (6 ml) and evaporated to dryness. Deuterium oxide (3 ml) was added and then evaporated under reduced pressure. This process was repeated three times. Finally, the material was dissolved in deuterium oxide (6 ml) and Ni-Al alloy (300 mg) was added and the mixture was heated at 50°C for 1 hour. After cooling, water (10 ml) was added, the catalyst filtered off and the solution acidified with sulphuric acid. The product was extracted with ether (3 x 20 ml) and the combined dried extracts evaporated to dryness to yield the title compound as a white solid (237 mg, 73%). This product appeared as a single component on tlc corresponding to authentic salicylic acid.

Acetyl[^{13}C-carboxyl-3,5-dideutero]salicylic acid (6, R = ^{2}H)

[^{13}C, $^{2}H_2$]Salicylic acid (230 mg. 1.63 mmole) was dissolved in acetic anhydride (0.5 ml), and conc. sulphuric acid (1 drop) was added and the mixture was heated at 50 - 60°C for 15 minutes. After cooling, water (10 ml) was added and the mixture was extracted with ether (3 x 10 ml). The dried ether extracts were evaporated to dryness to give the title compound as a solid (180 mg, 60%) which appeared as a single component on tlc corresponding to authentic acetylsalicylic acid. The mass spectrum showed a molecular ion at m/e 183 and fragment ions at m/e 166, 141 and 123, all three mass units higher than the corresponding fragments in the spectrum of authentic unlabelled compound.

Acetyl[3,5-^{3}H]salicylic acid (6, R = ^{3}H)

3,5-Dibromosalicylic acid (248 mg, 0.84 mmole) was dissolved in 10% aqueous sodium hydroxide (1 ml) and tritiated water (1 ml, 5 Ci) was added. Nickel-aluminium alloy (100 mg) was added to the mixture which was heated to 50°C for 30 minutes. After cooling, water (10 ml) was added and the catalyst filtered off and washed with water. The filtrate was acidified and extracted with ether (3 x 50 ml). The combined dried ($MgSO_4$) extracts were evaporated to dryness and the resulting solid residue dissolved in methanol and the solvent evaporated off. This procedure was repeated three times to remove labile tritium. The product (118 mg, 2.03 mCi, 17.2 μCi/mg) and non-radioactive salicylic acid (60 mg) were dissolved in acetic anhydride (0.35 ml), conc. sulphuric acid (1 drop) was added and the mixture was heated at 50 - 60°C for 15 minutes. After cooling, water (6 ml) was added with stirring and the resulting solid was filtered off, washed and dried. The product (54 mg, 2.77 μCi/mg) appeared as a single radioactive component (detected by tlc scanning) using silica gel tlc (solvent system benzene:ether:acetic acid:methanol, 120:60:18:1, v/v) which corresponded to authentic acetylsalicylic acid.

REFERENCES

1. I. Midgley, R. W. Pryor and D. R. Hawkins, J. Labelled Cpds. Radiopharm., XV, 511 (1978).
2. M. Tashiro, A. Iwasaki and G. Fukata, J. Org. Chem., 43, 196 (1978).
3. R. C. Woodworth and C. M. Dobson, Febs Letters, 101, 329 (1979).
4. L. M. Stephenson, R. V. Gemmer and S. P. Current, J. Org. Chem., 42, 212 (1977).