SYNTHESIS OF SODIUM [1,4-^{14}C] AUROTHIOMALATE

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SUMMARY

[1,4-^{14}C] Acetylthiomalic anhydride was prepared from [1,4-^{14}C] maleic anhydride and thioacetic acid. Removal of the acetyl group was achieved by sodium hydroxide hydrolysis and the resultant [1,4-^{14}C] thiomalic acid was extracted into ethyl acetate. Sodium [1,4-^{14}C] aurothiomalate was prepared by the reaction of gold(I) iodide with [1,4-^{14}C] thiomalic acid and precipitation of the sodium salt from alkaline solution by the addition of ethanol. The products were demonstrated to have a radiochemical purity of > 98% by radio-scanning of thin-layer chromatograms and scintillation counting of segments of the chromatogram.

KEYWORDS

Sodium [1,4-^{14}C] aurothiomalate, [1,4-^{14}C] thiomalic acid, [1,4-^{14}C] acetylthiomalic anhydride.

INTRODUCTION

Sodium aurothiomalate (Myochrysin) was introduced for the treatment of rheumatoid arthritis in 1932. An extensive trial conducted by the British Empire Rheumatism Council in 1961 demonstrated the clinical value of this drug (1) and it continues to be widely used. Despite the clinical improvement evident in many patients, treatment is unsuccessful in 30-40% of cases because of either

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poor response or, more usually, the development of gold toxicity. A number of trials have shown that there is no correlation between the concentration of gold in the blood and either remission of the disease or appearance of toxicity (2-4). Thus it has proved impossible to make dose adjustments in order to achieve beneficial response.

Since experimental work has hitherto been focussed upon gold itself, ignoring any contribution that the organic thiomalate moiety might have upon its absorption and distribution, it was proposed to investigate the metabolism of the complete drug. For this purpose, sodium aurothiomalate was synthesised with the carrier molecule radioactively labelled.

EXPERIMENTAL

The reaction sequence for the synthesis of sodium [1,4-^{14}C] maleic anhydride and thioacetic acid is outlined in Figure 1. The synthesis of [1,4-^{14}C] thiomalic acid was based upon reactions described by Holmberg and Schjanberg (5) who prepared acetylthiomalic acid from maleic acid. Since ^{14}C-labelled maleic acid is not commercially available, [1,4-^{14}C] maleic anhydride was used to prepare [1,4-^{14}C] acetylthiomalic anhydride which was then hydrolysed to 1,4-^{14}C thiomalic acid.

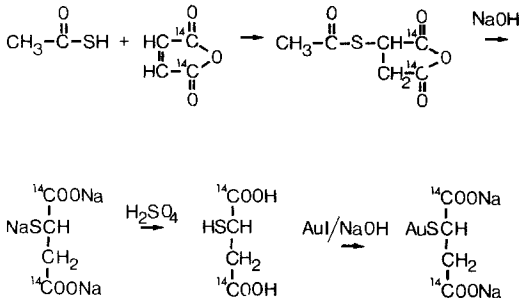

FIGURE 1. Synthesis of Sodium [1,4-^{14}C] aurothiomalate

[1,4-^{14}C] Acetylthiomalic anhydride

[1,4-^{14}C] Maleic anhydride (specific activity 20 mCi/mmol) was obtained from Amersham International plc (Amersham, Bucks, U.K.). The radioactive compound (1 mCi), dissolved in dichloromethane (approximately 0.2 ml), was added to 98 mg (1 mmol) non-radioactive maleic anhydride. The dichloromethane was evaporated under a stream of nitrogen at 0°C.

Thioacetic acid (Sigma Chemical Co., Poole, Dorset, U.K.) (0.1 ml) was added slowly over approximately 30 seconds to the solid [1,4-^{14}C] maleic anhydride at 0°C. Air in the reaction tube was displaced by nitrogen, the tube stoppered and gently mixed until the reactants went into solution. On standing at 4°C, the solution solidified to a white crystalline mass. The tube was gently heated until the mass melted and then slowly cooled to 4°C to effect crystallization. The product was recrystallized from benzene (approx. 1 ml), centrifuged, the sediment washed with cold benzene (1 ml) and dried in a stream of nitrogen to yield crystals of [1,4-^{14}C]acetylthiomalic anhydride (145 mg, 83%).

[1,4-^{14}C]Thiomalic Acid

[1,4-^{14}C]Acetylthiomalic anhydride (145 mg) was dissolved in 2.7 M sodium hydroxide (1.5 ml) and the solution kept under nitrogen. The mixture was kept at 4°C for 16 hours to complete the hydrolysis. The hydrolysate was acidified with 1.7 M sulphuric acid (1.5 ml) and extracted by continuous liquid/liquid extraction with 75 ml ethyl acetate for 45 minutes. The extract was evaporated to dryness under nitrogen to yield 124 mg (99%) of [1,4-^{14}C] thiomalic acid, mpt 149°C. Thin-layer chromatography on silica gel G1500, developed with methyl isobutyl ketone: formic acid: water (40:20:20) and sprayed with 0.1% (w/v) potassium permanganate, revealed a single spot of Rf 0.84, identical to that of thiomalic acid standard. Radioactive scanning of the chromatogram showed radioactivity to be associated only with the thiomalic acid

spot. The specific activity of the [1,4-^{14}C]thiomalic acid was determined as 732μCi/mmol. Comparisons of NMR and IR spectra of the synthesised compound and authentic thiomalic acid showed them to be identical.

Sodium [1,4-^{14}C]aurothiomalate

Attempts to incorporate gold(I) from gold cyanide (6) gave poor yields of about 10% and therefore the alternative procedure using gold(I) iodide was adopted (Figure 1).

Gold metal (20 mg) was dissolved in aqua regia, evaporated just to dryness and the residue dissolved in 1 ml concentrated hydrochloric acid. Potassium chloride (9.5 mg) was added followed by potassium iodide (49.8 mg in 1 ml water) to produce a brown, flocculent precipitate of gold(I) iodide. The precipitate was washed with distilled water (x2) and with ethanol (x2) and collected by centrifugation, all operations being conducted under nitrogen. The precipitate was dried under a stream of nitrogen to yield gold(I) iodide (32 mg, 97%).

[1,4-^{14}C]Thiomalic acid (15 mg, 73.2μCi) dissolved in distilled water (1 ml) was added to the gold iodide. 4 M sodium hydroxide was added dropwise until the gold iodide just dissolved. Ethanol (40 ml) saturated with sodium acetate was added and after standing for 16 hours the precipitate was centrifuged, washed with ethanol and dried under nitrogen.

The sodium [1,4-^{14}C]aurothiomalate (19.0 mg, 48.9%) contained 9.6 mg gold and had a ^{14}C content of 36.5μCi. The specific activity, 746μCi/mmol, was similar to that of the thiomalic acid precursor. Thin-layer chromatography on silica gel G1500 using solvent systems, ethanol:water (90:10) and butanol: acetone: acetic acid: water (70:70:20:40) revealed a compound containing gold and carbon-14 which had Rf values in the two systems of 1.0 and 0.47 respectively. These compared favourably with the Rf values (1.0 and 0.48) found with authentic sodium aurothiomalate (May & Baker Limited) and were quite distinct

from those of thiomalic acid (0.0 and 0.84 respectively). Radio-scanning of the chromatograms and scintillation counting of scraped segments of the chromatograms demonstrated that the product had a radiochemical purity of > 98%.

DISCUSSION

The synthesis of [1,4-^{14}C]thiomalic acid was performed on a considerably reduced scale compared with that of Holmberg and Schjanberg (5) and therefore certain modifications to their procedure were required. Because of the small amount of material used, air was routinely displaced by nitrogen to limit oxidation. When adding thioacetic acid to [1,4-^{14}C]maleic anhydride, a poor yield of [1,4-^{14}C]acetylthiomalic anhydride was obtained if heating occurred, the sample was therefore kept at 0^{o}C. Recrystallization of [1,4-^{14}C] acetylthiomalic anhydride from benzene was important to remove excess thioacetic acid and ensure radiochemical purity. Precipitation of [1,4-^{14}C]thiomalic acid did not take place following acidification and extraction of the product into ethyl acetate was found to be effective.

The specific activity of [1,4-^{14}C] thiomalic acid was low but the overall yield was good (83%). The loss of radioactivity was shown to occur by volatization of maleic anhydride during the removal of dichloromethane.

The intermediate and final products of this synthesis were prepared in sufficient amounts and with necessary radiochemical purity for use in metabolic experiments (7).

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REFERENCES

1. Empire Rheumatism Council. Ann. Rheum. Dis. 20 315 (1961)

2. Billings, R., Grahame, R., Marks, V., Wood, P. and Taylor A. Rheum. Rehab. 14, 13, (1975)

3. Jessop, J.D. and Johns, R.G.S. Ann. Rheum. Dis. 32, 228 (1973)

4. Mascarenhas, B.R., Gronda, J.L. and Freyberg, R.H. Arth. Rheum. 15, 391 (1972)

5. Holmberg, B. and Schjanberg, E. Arkiv. Kemi. Mineral Geol. 14A, part 7 (1940)

6. Trenner, N.R. and Bacher, F.A. - U.S. Patent No. 2370493, (1945)

7. Taylor, A. - Ph.D. thesis, University of Surrey (1982)