SYNTHESIS OF 3-(HYDROXYMETHYL-^{14}C)-8-METHOXYCHROMONE-2^{14}C AND 3-HYDROXYMETHYL-8-METHOXYCHROMONE-4-^{14}C.

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Received January 26, 1976
Revised July 15, 1976

SUMMARY

A simple route to 3-(hydroxymethyl-^{14}C)-8-methoxychromone-2-^{14}C using formaldehyde-^{14}C is described. However, this material was considered to be unacceptable for most biological studies because about 16% of the radioactivity was expired as $^{14}CO_2$ in the mouse. The synthesis of 3-methoxysalicylic (carboxyl-^{14}C) acid is described which was converted in several steps to 3-hydroxymethyl-8-methoxychromone-4-^{14}C. Only 0.03% of the radioactivity in this compound was expired as $^{14}CO_2$.

Key Words: 3-Methoxysalicylic acid, 3-(hydroxymethyl)-8-methoxy-3-methylsulfinyl-4-chromone, biostability.

INTRODUCTION

3-Hydroxymethyl-8-methoxychromone has been shown to be a new, orally active, specific prophylactic inhibitor of Type I hypersensitivity reactions (1). The drug was labeled with ^{14}C in order to more easily investigate the absorption and excretion rates, blood levels, tissue distribution and to determine its metabolites (2,3,4,5).

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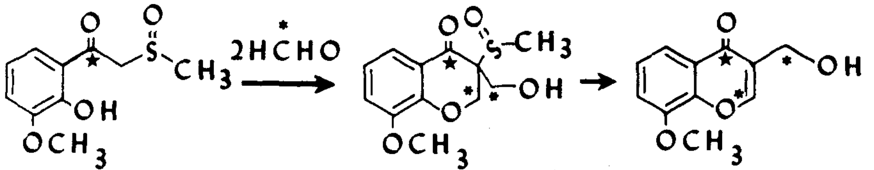
DISCUSSION

The synthesis of this drug has been described (6), and as represented in Scheme A will produce 3-(hydroxymethyl-^{14}C)-8-methoxychromone-2-^{14}C (III). This synthesis was simple, inexpensive and gave II in good yield even when the molar ratios of the 3-methoxy-2-(α-methyl-sulfinylacetyl) phenol (I), formaldehyde and potassium carbonate were reduced to 1:2.2:2.25. Elimination of the methylsulfinyl group from II with heat gave the desired III. The chemical yield was 43% while the radiochemical yield was 25%. However, when III was administered orally to a mouse, 16% of the radioactivity was expired as $^{14}CO_2$. This biological instability of the ^{14}C made III unacceptable for extensive metabolic use.



I

Ia ★ denotes ^{14}C

II ∗ denotes ^{14}C

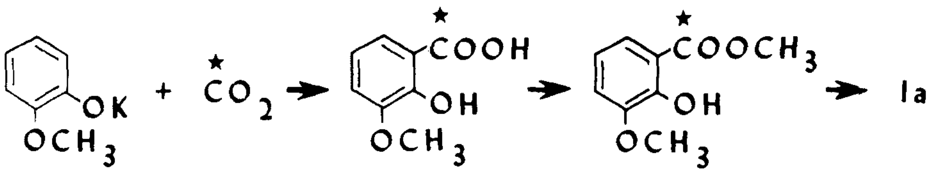
IIa ★ denotes ^{14}C

III ∗ denotes ^{14}C

IIIa ★ denotes ^{14}C

SCHEME A

Incorporation of ^{14}C into the carbonyl group of III appeared to offer the best location for a biologically stable label. The synthesis of 3-hydroxymethyl-8-methoxychromone-4-^{14}C (IIIa) is shown in Scheme B.



IV

V

VI

SCHEME B

The 3-methoxy salicylic-(carboxyl-^{14}C) acid (V) was prepared via the Kolbe-Schmitt Reaction (7) from the potassium salt of guiacol (IV) and $^{14}CO_2$ at 150°C for 4 hours at about 600 psi pressure. Yields of about 65% were obtained using only a 30% excess of $^{14}CO_2$. The methyl ester (VI) was then prepared, which gave Ia, when allowed to react with the anion of dimethylsulfoxide. When Ia, formaldehyde and potassium carbonate were allowed to react in a 1:7:2.25 molar ratio, the product was IIa, which when heated gave the desired IIIa. The chemical yield was 10%, while the radiochemical yield was 27%. When IIIa was administered orally to a mouse, 0.03% of the radioactivity was expired as $^{14}CO_2$.

EXPERIMENTAL

Potassium Salt of Guiacol (IV): A mixture of 62.0 g (0.5M) guiacol and 28 g (0.5M) curshed potassium hydroxide, in toluene, was allowed to reflux for 4 hours protected (8) from CO_2. The calculated amount of water was collected in a Dean-Stark trap during this time. The reaction was cooled, filtered, and the solid washed with three 125 ml portions of anhydrous ether. The solid, after drying in vacuo at 110°C for 20 hours, was ground in a mortar with pestle in a N_2 atmosphere and weighed 73 g (90%).

3-Methoxysalicylic-(carboxyl-^{14}C) acid (V): The $CaCl_2$ dried $^{14}CO_2$ which was liberated at 10 mm pressure by the dropwise addition of 50 ml of 70% perchloric acid (over 15 minutes) to a mixture of 8.33 g of barium carbonate-^{14}C and 11.41 g of non-radioactive barium carbonate (total of 200 mCi and 100 mM), was condensed in a 75 ml capacity stainless steel, valve-equipped bomb (9), previously charged with 12.3 g (75 mM) of IV by immersion in a liq. N_2bath. The valve on the bomb was closed 15 min. after addition of the perchloric acid was complete. When at room temperature, the increase in weigh of 4.2 g (95%) was due to the $^{14}CO_2$.

A pressure gauge was added to the valve and the bomb was heated for four hours at 150°C and then allowed to cool to room temperature overnight. The maximum pressure was 575 psi. The following morning, the valve on the bomb was closed, the pressure gauge removed, and the bomb attached to a rubber balloon connected to a flask containing 200 ml of a freshly prepared and filtered solution of 5% barium hydroxide. The needle valve was repeatedly opened to allow a small amount of $^{14}CO_2$ to collect in the balloon and then to be trapped as barium carbonate-^{14}C until the bomb was at atmospheric pressure.

The valve was removed and the solid in the bomb dissolved in five 50-ml portions of hot water. The aqueous phases were combined, bone-blacked (10), and to the filtrate in a 500-ml R.B. flask equipped with an equilibrated addition funnel was added 18 ml of 6N HCl dropwise over ca. 15 minutes, and any liberated $^{14}CO_2$ allowed to bubble through the same barium hydroxide solution used to collect the excess $^{14}CO_2$ from the bomb. The precipitated barium carbonate-^{14}C was filtered, washed with water and acetone, dried and weighed 4.23 g (19.2% recovered).

The acidic aqueous solution was cooled in an icebath and the precipitated V was filtered, washed with water, and dried in vacuo at 61° C for 2 hours to give 9.7 g. This was recrystallized from 250 ml boiling water, bone-blacked, and cooled. The precipitate was filtered, washed with water, and dried as above to give 7.52 g (60%) which melted 149-151°C. A pure sample melted 149-151°C (7,11). A thin-layer chromatogram (TLC)(12)gave a single UV absorbing spot at an R_f = 0.95, identical with an authentic sample, and when scanned (13) contained all the radioactivity on the plate. The specific activity was 1.70 mCi/mM (theory required 2 mCi/mM). This material was used to prepare VI.

Methyl 3-methoxysalicylic-(carboxyl-^{14}C)acid (VI): A solution of 7.52 g (44.6 mM) V in 53.7 ml methanol, containing 2.86 ml conc. sulfuric acid, was refluxed for 8 hours, cooled, and the solvent removed. The residue was partitioned between 82 ml benzene and 41 ml water. The aqueous phase was extracted with an additional two 20-ml portions of benzene. The organic phases were combined, dried with magnesium sulfate, filtered and the solvent removed. This gave 8.43 g of VI, which melted 61.5-64.5°C. A pure sample melted 62-65°C. This material was used to prepare Ia.

2'-Hydroxy-3'-methoxy-2-(methylsulfinyl)acetophenone-(carbonyl-^{14}C)(Ia): To a suspension of 7.18 g (170 mM) 57% oil dispersion of sodium hydride in 52.2 ml dry benzene, was added 35.6 g (45.6 mM) dimethylsulfoxide over 15 min. with magnetic stirring. The internal temperature was raised rapidly to 70°C and kept there for 1 hour. At the end of this time, the foaming had subsided and the temperature was lowered to room temperature. A solution of 8.43 g (46.3 mM) of VI was added dropwise over 1 hour and stirring continued overnight. A solution of 13.7 ml glacial acetic acid in 32 ml water was added dropwise over 4 hours keeping the internal temperature between 7-10°C. The resulting solid was filtered, washed with two 25-ml portions of cold water, and two 25-ml portions of Skelly B (20), and air dried to give 8.80 g (83.3%) of Ia which melted 137-140°C. A pure sample melted 140-142°C. A TLC (12) exhibited two spots, one at R_f= 0.34 which was identical with a pure sample of Ia and another at R_f = 0.74. The material at R_f = 0.34 contained 95% of the radio-activity on the plate when scanned (13). This material was used to prepare IIa.

3-Hydroxymethyl-8-methoxy-3-methylsulfinyl-4-chromanone-4-^{14}C (IIa): The 8.80 g (38.6 mM) of Ia was added to a solution of 11.97 g (86.7 mM) potassium carbonate in 126 ml of water with magnetic stirring. To this was added 21.9 ml (269 mM) of 37% formaldehyde, dropwise, over 30 minutes. After an additional 30 minutes, a precipitate appeared which increased with time. After three hours, the precipitate was filtered, washed with four 30 ml portions of cold water, and then magnetically stirred with 70 ml of water for 30 min. After filtering, the solid was air dried for several hours and then for 1 hr. at 22°C in vacuo to give 7.68 g (73.6%) of IIa which melted 120-123°C. A TLC (12) exhibited two spots, one at R_f = 0.78 which was identical with a pure sample of IIa, and another at R_f = 0.82. The spot at R_f = 0.78 contained 95% of the radioactivity on the plate when scanned (13). This material was used to prepare IIIa.

3-(Hydroxymethyl)-8-methoxychromone-4-^{14}C (IIIa): A solution of 7.68 g (28.4 mM) of IIa in 236 ml of xylene was distilled at a rate such that 118 ml of distillate was collected in 2 hrs. The remaining solution was cooled and stirred overnight at room temperature. The precipitate was filtered, washed with two 10 ml portions of xylene and two 10 ml portions of cold solox (14), and air dried to give 3.25 g (55%) of crude IIIa. This was added to 65 ml refluxing absolute ethanol. When all the solid had dissolved, 0.5ml of 0.1N aqueous sodium hydroxide was added and the solution cooled in an icebath. The precipitate was filtered, washed with one 5 ml portion of cold ethanol and air dried. The resulting solid was dissolved in 45 ml boiling xylene and bone blacked (10). When cooled, a precipitate formed which was filtered and dried in vacuo at 61°C for 2 hours to give 2.05 g (34.8%) of IIIa which melted at 167-169°C. An authentic sample (15) melted at 167-169°C. An IR as a mull exhibited maxima at the same wavenumbers as an authentic sample. The absorptivity was 118 at 225 nm which was 98.8% of the authentic sample when similarly measured in ethanol. A quantitative TLC (16), containing 100 micrograms of IIIa, exhibited a main spot at R_f = 0.50 identical with a spot of pure material and about 2% of a second spot at R_f = 0.77. When scanned (13), the TLC demonstrated that 98.2% of the radioactivity on the plate was contained in the spot at R_f = 0.50 and 1.8% in the spot at R_f = 0.77. The specific activity of IIIa was determined to be 1.70 mCi/mM (8.26 mCi/g) (17).

3-(Hydroxymethyl-^{14}C)-8-methoxy-3-methylsulfinyl-4-chromanone-2-^{14}C (II): To a solution of 0.602 g (4.37 mM) of potassium carbonate in 0.75 ml of water was added 0.442 g (1.94 mM) of I. To this was added 0.0144g (0.21 mM) of formaldehyde-^{14}C (18) in 0.31 ml of water dropwise over 5 min. The formaldehyde-^{14}C was quantitatively transferred into the reaction with 0.334 ml (4.0 mM) of 37% formaldehyde in 3 ml of water and then with 1 ml of water. A total of 4.21 mM of formaldehyde-^{14}C, containing 500 μ Ci of ^{14}C (specific activity = 119 μCi/mM) was added over 30 minutes. The reaction proceeded as described in IIa. The product weighed 0.365 g (69.7%). A TLC (19) exhibited a main spot at 0.08 which was identical with a pure sample, and much weaker spots at R_f = 0.20, 0.29, 0.38 and 0.52. When scanned (13), the main peak contained 93% of the radioactivity while the remainder was distributed about equally among the other spots. This material was used to prepare III.

3-(Hydroxymethyl-^{14}C)-8-methoxychromone-2-^{14}C (III): The 0.365 g (1.35 mM) of II was converted into 0.162 g (58%) of unrecrystallized III using the same procedure as described for IIIa. When 0.12 g was recrystallized from xylene, 0.090 g of 98% radiochemically pure III resulted (16).The remainder of the radio-activity, 0.7% and 1.3%, was contained in spots at R_f = 0.54 and 0.78, respectively. A mixture of 10 mg of recrystallized III (98.0% radiochemical purity) and 90 mg. of non-radioactive III was recrystallized from xylene to give 90 mg. of III, which melted 167-169°C. An authentic sample (15) melted 167-169°C. An IR as a mull exhibited maxima at the same wave numbers as an authentic sample. The absorptivity was 122 at 226 nm, which was 102% of the authentic sample when similarly measured in ethanol. A TLC (16) exhibited a single spot at R_f = 0.46 which, when scanned (19), contained all of the radioactivity on the plate. The specific activity was determined to be 0.102 μCi/mg (21.0 μCi/mM) (17).

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Mrs. N. Eltvedt. We are also indebted to Dr. R. C. Greenough for providing the spectal data.

REFERENCES

1. Herzig D.J., Giles R.E., Schumann P.R., Kusner E. J., and Dubnick B. - Fed. Proc. 34, 760 (1975).
2. Crew M.C., Melgar M.D., George S., Szpiech J.M., Greenough R.C., and DiCarlo F.J., - Fed. Proc. 34, 733 (1975).
3. Crew M.C., Szpiech J.M., and DiCarlo F.J. - Xenobiotica, 6,83(1976)
4. Crew M.C., Melgar M.D., George S., Grennough R.C., Szpiech J.M., and DiCarlo F.J. - Xenobiotica, 6,89 (1976)
5. Crew M.C., Melgar M.D., George S., Szpiech J.M., and DiCarolo F.J. - Xenobiotica, In press.
6. (a) Shavel, J., Jr., von Strandtmann M., Klutchko S., and Cohen M.P. - U.S. Patent 3,798,240 (3/19/74).
   (b) Klutchko S., Cohen M.P., Shavel, J., Jr., and von Strandtmann M. - J. Heterocyclic Chemistry 11, 183 (1974)
7. Lindsey A.S., and Jeskey H. - Chem. Rev. 57, 583 (1957)
8. Ascarite, purchased from Arthur H. Thomas Co.,Philadelphia, Pa., and magnesium perchlorate were used.
9. Available from Matheson Gas Products, East Rutherford, N.J. 07073
10. Darco G60, available from Atlas Chemical Industries, Wilmington, De. 19899, was used.
11. Mandel H.G., and Smith P.K. - J. Amer. Pharm. Assoc., Sci. Ed. 39, 479 (1950).
12. Silica gel GF; ethyl acetate/methanol (9:1) visualized under 253.7 nm light.
13. A Packard Radiochromatogram Scanner Model 7200 was used.
14. Solox is a mixture containing ethanol as the principal ingredient and is available from U.S. Industrial Chemicals, New York, N.Y. 10016
15. The authentic sample was provided by M. Goodenough and A. D. Lewis of these laboratories.
16. Silica gel GF; chloroform/acetone (85:15) visualized under 253.7 nm light.
17. A Packard Tri Carb Liquid scintillation Spectrometer Model 3310, equipped with automatic external standardization, was used. The composition of the cocktail was either 4 g PPO and 0.1 g POPOP dissolved in 1 liter toluene. or 7 g PPO, 0.3 g diMePOPOP and 100 g naphthalene dissolved in 1 liter 1,4-dioxane.
18. Purchased from New England Nuclear Corp., Boston, Mass. 02118.
19. Silica gel GF; benzene/ethyl acetate (1:1) visualized under 253.7 nm light.
20. Skellysolve B is principally n-hexane and is available from Skelly Oil Co., El Dorado, Texas.