Syntheses of 1-*O*-Methyl-^{14}C-DL-*myo*-Inositol (Methyl-^{14}C-Bornesitol) and 5-*O*-Methyl-^{14}C-*myo*-Inositol (Methyl-^{14}C-Sequoyitol)

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Received on February 3, 1970.

SUMMARY

1-O- *and 5*-O-*Methyl-^{14}C*-myo-*inositols (methyl-^{14}C-bornesitol and -sequoyitol) were synthesized by methylation of appropriately blocked* myo-*inositol derivatives with methyl = ^{14}C iodide and potassium hydroxide. The benzyl blocking groups of 1*-O-*methyl-^{14}C-3,4,5, 6-tetra*-O-*benzyl*-DL-myo-*inositol and 1,2,3,4,6-penta*-O-*benzyl -5*-O-*methyl-^{14}C*-myo-*inositol were cleaved by hydrogenolysis catalyzed by palladium on carbon-palladium chloride (5 : 1) to obtain the 1*-O- *and 5*-O-*methyl-^{14}C*-myo-*inositols respectively.*

INTRODUCTION

As part of our program of research on inositol metabolism, we have undertaken the synthesis of 1-*O*- and 5-*O*-methyl-^{14}C-*myo*-inositols [methyl-^{14}C-bornesitol (*1*) and -sequoyitol (*12*)].

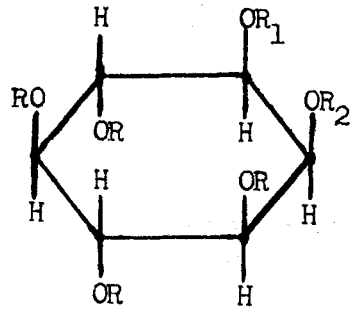
Methylation of the appropriately substituted *myo*-inositols was carried out with methyl-^{14}C iodide and potassium hydroxide in benzene. Removal of the benzyl substituents from the methylated inositols by hydrogenolysis gave the desired products *1* and *12*.

RESULTS AND DISCUSSION

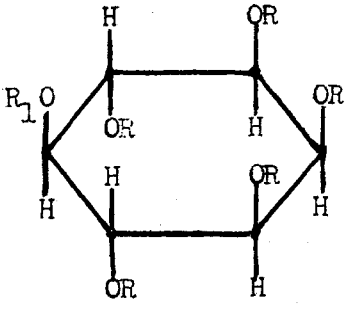
Methyl-^{14}C-bornesitol. — Methylation of 1,4,5,6-tetra-*O*-benzyl-DL-*myo*-inositol (*2*) with methyl-^{14}C iodide and potassium hydroxide was accomplished by modifying the procedure described for unlabeled methylation [1]. Thin-layer chromatography on silica gel of the product of methylation revealed

two radioactive components of which the faster moving (R_F 0.78) corresponded to the dimethyl compound *3* while the slower moving component (R_F 0.49) was attributable to the 1-*O*-methyl ether *4*. It is likely that *4* was accompanied by a small amount of the 2-*O*-methyl ether *5* [1]. The distribution of label in the 1-*O*- (0.677 mCi) and 1,2-di-*O*-methyl-^{14}C-DL-*myo*-inositols (0.450 mCi) (*4* and *3* respectively) was in the ratio of 1.5 : 1.

1-*O*-Methyl-^{14}C-3,4,5,6-tetra-*O*-benzyl-DL-*myo*-inositol (*4*) was hydrogenolyzed in acetic acid with a mixture of palladium on carbon-palladium chloride (5 : 1) catalysts to obtain methyl-^{14}C-DL-bornesitol (*1*) in 58 % radiochemical yield from *4* after chromatography on a cellulose column. Either one of the two catalysts alone was ineffective in cleavage of benzyl groups from *4*. Thin-layer chromatography of methyl-^{14}C *1* on cellulose showed only one radioactive spot (R_F 0.23) identical with that of unlabeled bornesitol. Four other radioactive products were obtained in the hydrogenation of *4* but were not characterized. The fastet moving component (R_F 0.96, TLC on cellulose) possessed *ca.* 5.6 % of the label from *4*, whereas the amount of label in the other three products was 7.5 % (R_F 0.73), 1.2 % (R_F 0.55), and 0.8 % (R_F 0.30). The last of these by-products (R_F 0.30) was most likely the 2-*O*-methyl-^{14}C-*myo*-inositol (*6*) since its observed $R_{BORNESITOL}$ value (1.35) on cellulose thin-layer plate resembled results obtained earlier by paper chromatography [2].



1 R = R_2 = H, R_1 = CH_3
2 R = $CH_2C_6H_5$, R_1 = R_2 = H
3 R = $CH_2C_6H_5$, R_1 = R_2 = CH_3
4 R = $CH_2C_6H_5$, R_1 = CH_3, R_2 = H
5 R = $CH_2C_6H_5$, R_1 = H, R_2 = CH_3
6 R = R_1 = H, R_2 = CH_3



7 R = Acetyl, R_1 = H
8 R = Acetyl, R_1 = 2-tetrahydropyranyl
9 R = $CH_2C_6H_5$, R_1 = 2-tetrahydropyranyl
10 R = $CH_2C_6H_5$, R_1 = H
11 R = $CH_2C_6H_5$, R_1 = CH_3
12 R = H, R_1 = CH_3

Although indistinguishable from *4* on a cellulose thin-layer plate, product of R_F 0.96 remained at the origin on a silica gel plate where the R_F of *4* was 0.49. The amount of palladium chloride present in the reaction mixture was critical. Prolonged hydrogenation with larger proportion of this catalyst formed the same side-products in much greater yields at the expense of the desired product *1*.

Methyl-^{14}C-sequoyitol. — 1,2,3,4,6-Penta-*O*-acetyl-*myo*-inositol (*7*), kindly supplied by Dr Angyal, was converted to its 5-*O*-(2-tetrahydropyranyl) derivative *8* as described [3]. Benzylation of *8* with benzyl chloride and potassium hydroxide gave the pentabenzyl ether *9* from which the 5-*O*-(2-tetrahydropyranyl) substituent was removed by hydrolysis with 80 % acetic acid to obtain crystalline 1,2,3,4,6-penta-*O*-benzyl-*myo*-inositol (*10*) in nearly 65 % over-all yield from the pentaacetate *8*.

An excess of the substrate *10* over methyl-^{14}C iodide was employed in the methylation reaction to assure maximum incorporation of the label. The 5-*O*-methyl-^{14}C ether *11* was homogeneous as indicated by thin-layer chromatography (R_F 0.73) on silica gel, and contained 0.750 mCi of radioactivity.

Removal of the benzyl groups from methyl-labeled *11* was achieved by hydrogenolysis with palladium on carbon-palladium chloride (5 : 1) catalysts as described above for methyl-^{14}C-bornesitol. 5-*O*-Methyl-^{14}C-*myo*-inositol (methyl-^{14}C-sequoyitol, *12*) was obtained in 59 % radiochemical yield from *11* after purification on a cellulose column. Methyl-^{14}C-sequoyitol (*12*) was identified by thin-layer chromatography (cellulose) and gas-liquid chromatography with authentic sequoyitol. Approximately 19 % of the label from *11* was present in a product whose R_F on a cellulose thin-layer plate was *ca.* 0.8; this material was not investigated further.

EXPERIMENTAL

Melting points were observed on a Fisher-Johns apparatus and are corrected. A Perkin-Elmer Model 257 spectrophotometer was employed for the determination of infrared spectra. Thin-layer chromatography (T.L.C.) was run either on silica gel G or cellulose (MN-Polygram Cel 300, Brinkmann Instruments, Inc.). Unless otherwise stated, solvent for T.L.C. on silica gel was 3 : 1 benzene-ether. Solvent for T.L.C. on cellulose was 4 : 1 acetone-water. Detection on silica gel plates was accomplished by exposure to iodine vapor or by heating plates which had been sprayed beforehand with 10 % methanolic sulfuric acid. Silver nitrate-potassium hydroxide spray [4] was used to detect spots on cellulose plates. Thin-layer plates were scanned with a Packard Model 7201 radiochromatogram scanner to detect radioactive components. A Packard Model 7821 gas chromatograph equipped with flame ionization detector and a temperature programmer was used for gas-liquid chromato-

graphy (G.L.C.). The stationary phase, a 3 % silicone polymer, OV-1, on Gas Chrom Q, 100-120 mesh (Applied Science Labs., State College, Pa.) was packed in a six-foot, 4 mm internal diameter, coiled glass column through which a stream of nitrogen as carrier gas was passed. Retention times were calculated from the beginning of the solvent peak. Column chromatography was performed either on silica gel (grade 950, Will Scientific Corp.) or Whatman Cellulose Powder (CF 11). Methyl-^{14}C iodide was purchased from New England Nuclear Corp. and contained *ca.* 1 mCi of radioactivity. The amount of label in methyl-^{14}C iodide was not accurately determined prior to methylation, hence percent radiochemical yields in methylation reactions are not given. To assay for ^{14}C, aliquots of samples were dissolved either in a mixture of water (0.5 ml) and liquid scintillation mixture A (15 ml) (prepared by dissolving naphthalene (100 g), PPO (7 g), and dimethyl POPOP (0.3 g) in *p*-dioxane to make 1 liter of solution) or in liquid scintillation mixture B (10 ml) (prepared by dissolving PPO (4 g) and dimethyl POPOP (0.3 g) in toluene to make 1 liter of solution). Samples were then counted in a Packard Model 3324 scintillation spectrometer with an efficiency of 85 % for ^{14}C. All measurements were converted to dpm on the basis of standardization with external standards.

1-O-*Methyl-^{14}C-3,4,5,6-tetra*-O-*benzyl*-DL-myo-*inositol* (*4*). Procedure of Angyal and Tate [1] was slightly modified for methylation with methyl-^{14}C iodide. Powdered potassium hydroxide (2.6 g) was added to a solution of *2* [1] (0.210 g, 0.39 mmole) in benzene (8 ml). Into this solution was transferred methyl-^{14}C iodide (0.0309 g, 0.22 mmole, *ca.* 1 mCi) in a closed system, and the mixture was refluxed with stirring for 1 hour. Additional unlabeled methyl iodide (0.014 ml, 0.22 mmole) was added (flask must be cooled in ethanol-dry ice bath before addition) and the reflux was continued for one more hour. The mixture was washed with water (8 ml) and the aqueous layer was extracted with benzene (4 × 5 ml). The combined benzene extracts were dried with potassium carbonate and evaporated to dryness. Thin-layer chromatography (silica gel, 10 : 7 : 4 : 0.2 benzene-chloroform-petroleum ether-methanol) revealed two radioactive spots, one of R_F 0.49 corresponding to 1-*O*-methyl-3,4,5,6-tetra-*O*-benzyl-DL-*myo*-inositol (*4*) and the other of R_F 0.78 corresponded with 1,2-di-*O*-methyl-3,4,5,6-tetra-*O*-benzyl-DL-*myo*-inositol (*3*).

The crude mixture was resolved on a column (2 cm diameter) of silica gel (50 g) with 10 : 7 : 4 benzene-chloroform-petroleum ether into its two components. The 1-*O*-methyl-^{14}C derivative *4* contained 0.677 mCi of radioactivity while the amount of label in the dimethyl ether *3* was 0.450 mCi.

1-O-*Methyl-^{14}C*-DL-myo-*inositol* (*Methyl-^{14}C-DL-bornesitol*) (*3*). 1.-*O*-Methyl-^{14}C,3,4,5,6-tetra-*O*-benzyl-DL-*myo*-inositol (*4*) (0.137 mCi) was hydrogenolyzed at ambient pressure in glacial acetic acid (20 ml) with a mixture of 10 % palladium on carbon (200 mg) and palladium chloride (40 mg). The catalyst was removed by filtration through Celite and washed with acetic acid and

water. Removal of solvent from combined filtrate and washes left a residue which contained 0.100 mCi of ^{14}C, and showed three radioactive spots on a cellulose thin-layer plate, one major spot (R_F 0.28) identical with bornesitol (*1*), and two minor spots (R_F 0.73 and 0.96) containing approximately equal amounts of label.

Chromatography of the crude debenzylated mixture on a cellulose column (2 × 40 cm) separated the major product from minor constituents. Fractions (20 ml) were monitored for ^{14}C by spotting 10 μl aliquots on filter paper and assaying with an end-window counter. Besides the two minor components mentioned above, two additional products were detected. The desired product, 1-*O*-methyl-^{14}C-DL-*myo*-inositol (*1*), was obtained from fractions 15-19 and contained 0.079 mCi (57.6 %). T.L.C. (cellulose): R_F 0.23 (identical with unlabeled bornesitol).

Other labeled components, identified by T.L.C. on cellulose, appeared as follows: fractions 5-6, 0.0077 mCi (5.6 %), R_F 0.96; fractions 7-9, 0.0102 mCi (7.5 %), R_F 0.73; fractions 10-12, 0.00175 mCi (1.2 %), R_F 0.55; fractions 13-14, 0.00107 mCi (0.8 %), R_F 0.30.

1,2,3,4,6-Penta-O-*acetyl-5*-O-*(2-tetrahydropyranyl)*-myo-*inositol* (*8*). Compound *8* was prepared as described by Angyal and Gero [3]. Yield, 87 %; m.p. 219-221° C (lit. [3] m.p. 225-226°C); ν_{max}^{KBr}: 1750 (C = O), 1250 (acetate C-O-C) cm^{-1}; T.L.C. (silica gel): R_F 0.20; G.L.C. (200-275° C, 5°/min): retention time, 10 min.

1,2,3,4,6-Penta-O-*benzyl-5*-O-*(2-tetrahydropyranyl)*-myo-*inositol* (*9*). A solution of *8* (512 mg, 1 mmole) in benzyl chloride (7.5 ml) was stirred with powdered potassium hydroxide (4.84 g) at 100° C for 20 hours after which time benzene (20 ml) was added *. The organic layer was washed with water (5 × 20 ml) and then evaporated to dryness. Removal of traces of benzyl chloride and dibenzyl ether from the residue was effected by co-evaporation with water at 65° C under reduced pressure, and finally by drying at 90° C at 0.05 mm for several hours. The residual yellow gummy syrup (641 mg, 94 %) was used in the next step without purification. ν_{max}^{KBr}: 735, 730, and 698 (aromatic) cm^{-1}; T.L.C. (silica gel): R_F 0.69 (major), 0.61, 0.45, 0.35, and 0.05 (traces).

1,2,3,4,6-Penta-O-*benzyl*-myo-*inositol* (*10*). The tetrahydropyranyl group of *9* (640 mg) was hydrolyzed with 80 % acetic acid by heating in a 110° C-bath for 2 hours. The solvent was removed under reduced pressure and the residue was crystallized from methanol to give colorless crystals of *10*; yield, 382 mg (67 %, 63 % from *8*); m.p. 175-177° C; ν_{max}^{KBr}: 3535 (OH), 732 and 692 (aromatic) cm^{-1}; T.L.C. (silica gel): single spot (R_F 0.62).

* This reaction was run at the suggestion of Dr Angyal.

1,2,3,4,6-Penta-O-benzyl-5-O-methyl-^{14}C-myo-inositol (*11*). Methyl-^{14}C iodide (12.4 mg, 0.088 mmole, *ca.* 1 mCi) was transferred in a closed system into a suspension of powdered potassium hydroxide (560 mg) in a solution of *10* (83 mg, 0.132 mmole) in benzene (3 ml). The mixture was stirred under reflux for 5 hours after which time benzene was distilled off in a closed system. The residue was partitioned between benzene (25 ml) and water (5 ml). The benzene layer was washed further with water (4 × 5 ml) and evaporated to give syrupy *11* which crystallised on standing; yield, 0.75 mCi; T.L.C. (silica gel): single radioactive spot (R_F 0.73).

5-O-Methyl-^{14}C-myo-inositol (Methyl-^{14}C-sequoyitol) (*12*). 1,2,3,4,6-Penta-*O*-benzyl-5-*O*-methyl-^{14}C-*myo*-inositol (*11*) (0.25 mCi) was hydrogenolyzed at atmospheric pressure in glacial acetic acid (20 ml) in the presence of 10 % palladium on carbon (200 mg) and palladium chloride (40 mg) catalysts. The catalysts was removed by filtration through Celite and washed with acetic acid and water. The combined filtrates were evaporated to dryness, the residue was dissolved in water, and the solution was successively passed through small columns of Dowex 50 (H^+) and Dowex 1 ($HCOO^-$) resins. The eluates were concentrated to dryness under reduced pressure; yield, 0.175 mCi (70 %); T.L.C. (cellulose): R_F *ca.* 0.3 (major radioactive spot identical with sequoyitol) and *ca.* 0.8 (minor radioactive spot).

The remainder of *11* was similarly hydrogenolyzed and the product was combined with the one obtained above. The combined crude products containing methyl-^{14}C *12* were chromatographed on a cellulose column (2 × 38 cm). Fractions (20 ml) were monitored for ^{14}C as described above for bornesitol. The desired product, methyl-^{14}C-sequoyitol (*12*), was recovered from fractions 11-14; yield, 0.443 mCi (59 %); T.L.C. (cellulose): single radioactive spot (R_F 0.30) identical with sequoyitol. The radioactivity of the trimethylsilyl derivative of methyl-^{14}C *12* eluted simultaneously with unlabeled 1,2,3,4,6-penta*akis*-*O*-trimethylsilyl-5-*O*-methyl-*myo*-inositol when separated by gas-liquid chromatography (3 min at 170° C, then programmed to 240° C at 5°/min). Methyl-^{14}C *12* from fraction 10 was not included in the final recovery since it was contaminated with the component of R_F 0.45 (see below).

Other labeled components, identified by T.L.C. on cellulose, eluted off the column as follows: fractions 5-8, 0.139 mCi (18.6 %), R_F 0.78; fraction 9, R_F *ca.* 0.45.

Acknowledgments

The authors thank the National Institute of General Medical Sciences, National Institutes of Health, United States Public Health Service for a Grant (No. GM 12422) in support of this work. Able technical assistance of Miss Anne Golebiewski is gratefully acknowledged.

REFERENCES

1. ANGYAL, S. J. and TATE, M. E. — *J. Chem. Soc.*, 6949 (1965).
2. ANGYAL, S. J., MCHUGH, D. J. and GILHAM, P. T. — *J. Chem. Soc.*, 1432 (1957).
3. ANGYAL, S. J. and GERO, S. D. — *J. Chem. Soc.*, 5255 (1965).
4. TREVELYAN, W. E., PROCTOR, D. P. and HARRISON, J. S. — *Nature*, **166** : 444 (1950).