SYNTHESIS OF ^{14}C-LABELED O-ACETYL-L-SERINE, O-BENZOYL-L-SERINE, β-N-PHENYL-L-ASPARAGINE, BENZYL GLYCINATE, AND α-DIMETHYLAMINOISOBUTYRIC ACID.

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SUMMARY

Syntheses and analyses are described for β-N-phenyl[^{14}C(U)]-L-asparagine, O-acetyl[1-^{14}C]-L-serine, O-benzoyl[7-^{14}C]-L-serine, benzyl[7-^{14}C]glycinate p-toluenesulfonate, benzyl glycinate[1-^{14}C] p-toluenesulfonate, and α-dimethylaminoisobutyric acid[1-^{14}C].

KEY WORDS: β-N-phenyl[^{14}C(U)]-L-asparagine, O-acetyl[1-^{14}C]-L-serine, O-benzoyl[7-^{14}C]-L-serine, benzyl[7-^{14}C]glycinate p-toluenesulfonate, benzyl glycinate[1-^{14}C] p-toluenesulfonate, α-dimethylaminoisobutyric acid[1-^{14}C].

INTRODUCTION

We have for some time been engaged in studies of the biochemistry and pathology of pancreatic cancer (1,2). An investigation into the structural requirements for pancreatic uptake from the blood of exogenous chemicals required that we prepare the title compounds labeled with carbon-14.

Of the literature methods for the preparation of β-N-phenyl-L-asparagine (3), we wished to choose one which would be certain to produce the β-amide uncontaminated by any α-isomer. We experienced considerable difficulty in reproducing Weygand's (3a) synthesis, and devised, instead, a synthesis similar to that of Michio and Ikua (3b) based on benzyl protection of the α-amino and carboxyl groups.

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Previous preparations of O-acyl serines have employed an acidic medium to block N-acylation (4,5,6). These procedures either use a large excess of acylating agent or require the use of volatile and moisture-sensitive acid chlorides, both disadvantages when dealing with small amounts of labeled compounds. Acylation of N-carbobenzoxy-L-serine benzyl ester with an acid anhydride, followed by unblocking via catalytic hydrogenation, avoids these difficulties, although half of the ^{14}C-label is wasted in the acylation step. Both O-acetyl and O-benzoyl serine were prepared in this way. In the preparation of the latter compound, the requisite benzoic anyhydride was formed and utilized *in situ* by reaction of benzoic acid with N,N'-dicyclohexylcarbodimide (DCC).

Benzyl glycinate *p*-toluenesulfonate has been prepared from glycine and benzyl alcohol by an azeotropic procedure (7). The ^{14}C material, labeled either in the benzyl or glycine portions, has apparently not been previously described. Using ^{14}C labeled glycine, we followed the literature procedure as described. However, to obtain the product with the ^{14}C label in the benzyl group, we modified this procedure by decreasing the amount of benzyl alcohol, increasing the amount of benzene, and using molecular sieves as a drying agent. The lack of solubility of the glycine salt in benzene may have been responsible for the poor yield obtained in the latter preparation.

α-Dimethylaminoisobutyric acid [1-^{14}C] has not been prepared before. We prepared it from labeled α-aminoisobutyric acid using the reductive amination procedure of Clarke et al. (8).

EXPERIMENTAL

The purity of all labeled products was based on comparisons of the chromatographic properties of the labeled and unlabeled components in at least three different thin layer chromatography solvent systems. Radiochromato-

grams were determined from the TLC plates, E. Merck, Silica Gel G, 5X20 cm X 0.25 mm, on a Packard Model 7201 radiochromatogram scanner. Unlabeled products, prepared via the same synthetic pathways, were analyzed by pmr, TLC and mp comparisons with the literature values. The radioactivity of the samples was determined in a Nuclear Chicago liquid scintillation counter. Vacuum evaporations were conducted in a Buchi rotary evaporator.

β-N-Phenyl[^{14}C(U)]-L-asparagine. Aniline[^{14}C(U)] hydrochloride (250 μCi from New England Nuclear, 4.0 mg, 0.031 mmol) and freshly recrystallized (from isopropanol) aniline hydrochloride (125.6 mg, 0.969 mmol) were mixed in methanol. The methanol was evaporated and the solid dried in the drying pistol. The β-anhydride of N-carbobenzoxy-L-aspartic acid α-benzyl ester was prepared by adding DCC (206.3 mg, 1.00 mmol) to the acid (714.7 mg, 2.00 mmol) dissolved in CH_2Cl_2 (1.75 mL). After stirring 30 min , a mixture prepared from the aniline[^{14}C(U)] hydrochloride and diisopropylethyl amine (129.3 mg, 1.00 mmol) in CH_2Cl_2 (1.2 mL) was added to the anhydride. After stirring overnight, the reaction mixture was filtered. The filtrate was diluted with EtOAc (20 mL), washed with 1 M HCl, saturated $NaHCO_3$, twice with saturated NaCl, dried with anhydrous $MgSO_4$, and evaporated to give a solid. The crude intermediate was hydrogenated at 60 psi in a mixture of MeOAc (17 mL) and water (2 mL) in the presence of Pd/C (50 mg). When hydrogen uptake had ceased, the catalyst was removed by filtration through Celite, which was then washed with water and EtOAc. The aqueous layer of the filtrate was evaporated in vacuum and the residue was recrystallized from warm aq NH_3 to give 126.4 mg (58.2%) and 66.1 μCi (26.4%) of the hemihydrate of β-N-phenyl[^{14}C(U)]-L-asparagine (114 μCi/mmole) with radiochemical purity of 99+% as determined by TLC: PhOH:H_2O (3:1), R_f = 0.59; CH_3CN:0.1 M NH_4OAc (3:1), R_f = 0.31; n-BuOH: AcOH:H_2O:pyridine (15:3:12:10), R_f = 0.34; n-BuOH:Me_2CO: AcOH:3 M NH_3:H_2O (9:3:2:2:4), R_f = 0.40.

O-Acetyl[1-^{14}C]-L-serine. To a stirred solution of acetic[1-^{14}C] anhydride (250 μCi from Amersham Searle, 0.92 mg, 8.9 μmol), acetic anhydride (63.5 mg, 0.622 mmol), and 4-dimethylaminopyridine (6.11 mg, 0.050 mmol) in PhH (2 mL) and CH_2Cl_2 (1 mL) was added N-carbobenzoxy-L-serine benzyl ester (164.7 mg, 0.500 mmol) in CH_2Cl_2 (2 mL). The solution was stirred for 50 min. The reaction mixture was diluted with EtOAc (25 mL), washed with saturated $NaHCO_3$, H_2O, 1 M HCl and saturated NaCl, dried with anhydrous Na_2SO_4 and evaporated in vacuum to give a white waxy solid. The crude O-acetyl[1-^{14}C]-N-carbobenzoxy-L-serine benzyl ester (167.5 mg) was hydrogenated in MeOAc (3.8 mL) - H_2O (1 mL) with Pd/C catalyst (27 mg) as described above. The product was recrystallized from H_2O-EtOH to give 50.0 mg (68%) and 47.1 μCi (48%) of O-acetyl[1-^{14}C]-L-serine (139 μCi/mmole). The radiochemical purity was determined as 99+% based on the following TLC solvent systems: n-BuOH:AcOH:H_2O (4:1:1), R_f = 0.19; CH_3CN: 0.1 M NH_4OAc (3:2), R_f = 0.43; PhOH:H_2O (3:1), R_f = 0.40.

O-Benzoyl[7-^{14}C]-L-serine. Benzoic[7-^{14}C] acid (500 μCi, 4.5 mg, 37 μmol, from New England Nuclear) and benzoic acid (266.6 mg, 2.18 mmol) were dissolved in PhH (1.5 mL). DCC (229.0 mg, 1.11 mmol) in PhH (0.4 mL) was added. After 30 min stirring, a solution of N-carbobenzoxy-L-serine benzyl ester (329.3 mg, 1.00 mmol) and 4-dimethylaminopyridine (13.1 mg, 0.11 mmol) in CH_2Cl_2 (1 mL) was added. After stirring overnight, the mixture was filtered. The filtrate was diluted with EtOAc (25 mL), washed with saturated $NaHCO_3$, H_2O, 1 M HCl and saturated NaCl, dried with anhydrous Na_2SO_4 and evaporated to dryness. The white O-benzoyl[7-^{14}C]-N-carbobenzoxy-L-serine benzyl ester was hydrogenated in MeOAc (35 mL) - H_2O (7 mL) in the presence of Pd/C (250 mg), as described above. The crude product was recrystallized from water to give 47.2 mg (22.6%) and 48.3 μCi (21.4%) of O-benzoyl[7-^{14}C]-L-serine. The radiochemical purity was determined as 99+% based on the following TLC solvent systems: PhOH:H_2O (3:1), R_f = 0.52; n-BuOH:Me_2CO:3 M NH_3:AcOH: H_2O

(9:3:2:2:4) R_f = 0.41; n-BuOH:AcOH:H_2O (4:1:1), R_f = 0.39; MeEtCO:pyridine:H_2O:AcOH (70:15:15:2), R_f = 0.30.

Benzyl glycinate[^{14}C(U)], p-toluenesulfonate. The procedure of Greenstein and Winitz (7) was modified in the following manner for ^{14}C labeled glycine. Glycine (75.1 mg, 1.00 mmol) and 1.5 mL of a solution of glycine[^{14}C(U)] (150 μCi from New England Nuclear, 0.1 mg) in 0.01 N HCl were mixed and lyophilized. To this mixture was added p-toluenesulfonic acid monohydrate (194.4 mg, 1.02 mmol), benzyl alcohol (1.6 mL) and benzene (0.8 mL), and the mixture refluxed under a small Dean-Stark trap. After water ceased to distill, the solution was cooled, diluted with ether (2 mL) and stored in the refrigerator overnight. Recrystallization from ether-methanol (4:1) gave 246.3 mg (73%) and 93.8 μCi (63%) of benzyl glycinate[^{14}C(U)] p-toluenesulfonate (129 μCi/mmol) with radiochemical purity of 98+% as determined by TLC in the following solvent systems: PhH:EtOAc:Et_2NH (10:10:1); $CHCl_3$:MeOH:HCO_2H (1:1:1); n-BuOH:AcOH:H_2O (5:1:2).

Benzyl[7-^{14}C] glycinate, p-toluenesulfonate. Benzyl[7-^{14}C] alcohol (250 μCi from Amersham-Searle, 1.84 mg, 0.02 mmol) and benzyl alcohol (214.4 mg, 1.98 mmol) were mixed in benzene (4.2 mL) in the presence of p-toluenesulfonic acid monohydrate (194.0 mg, 1.02 mmol) and glycine (75.1 mg, 1.00 mmol). The mixture was heated under reflux and the distillate was returned to the reaction mixture through a Teflon thimble which contained 3A molecular sieves. After 8 h at reflux, the solution was cooled, diluted with ether (3 mL) and stored in the refrigerator overnight. The crude solid was collected by filtration and partitioned between aqueous $NaHCO_3$ and EtOAc. The EtOAc phase was washed with saturated NaCl, dried with anhydrous $MgSO_4$, filtered, and treated with 5 mL of a 0.2 M solution of p-toluenesulfonic acid in ether. Solvents were removed in vacuum, the crude solid was triturated with ether, filtered, and recrystallized from isopropanol to give 132.3 mg (39.2%) and 28.9

μCi (23.1%) of benzyl[7-^{14}C] glycinate p-toluenesulfonate (74 μCi/mmol) with radiochemical purity of 99+% as determined in the following TLC solvent systems: n-BuOH:AcOH: H_2O (5:1:2); n-BuOH:HCO_2H:H_2O (5:1: 2); PhH:EtOAc:Et_2NH (10:10:1); $CHCl_3$:MeOH:HCO_2H (1:1:1).

2-Dimethylaminoisobutyric[1-^{14}C] acid. A solution of α-aminoisobutyric[1-^{14}C] acid (100 μCi from New England Nuclear, 0.2 mg, 2 μmol) and α-aminoisobutyric acid (51.4 mg, 0.498 mmol) in 90% formic acid (0.57 mL, 13.3 mmol) and 37% formalin (0.43 mL, 5.72 mmol) was refluxed for 2 h. Excess formaldehyde and formic acid were removed by evaporating three times with water, then paraformaldehyde was removed by filtration. After evaporation of the filtrate, the product was recrystallized from isopropanol and dried in a drying pistol over P_4O_{10} to give 50.8 mg (76.3%) and 76.5 μCi (76.5%) of 2-dimethylaminoisobutyric[1-^{14}C] acid (200 μCi/mmol). The radiochemical purity was 99+% as determined by TLC: CH_3CN:0.1M NH_4OAc (3:2), R_f = 0.65; $CHCl_3$: MeOH:17% NH_3 (2:2:1), R_f = 0.41; PhOH:H_2O (3:1), R_f = 0.50; n-BuOH:Me_2CO:3M NH_3:AcOH:H_2O (9:3:2:2:4), R_f = 0.17.

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