NOTE

SYNTHESIS OF ^{14}C-LABELED INDOLE-3-ACETYLASPARTIC ACID

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SUMMARY

The synthesis of indole-3-acetyl-2-^{14}C-N-aspartic acid from carrier-free indole-3-acetic acid-2-^{14}C (54.5 mCi/mmole) and bis-t-butyl-l-aspartic acid was accomplished by coupling with dicyclohexylcarbodiimide followed by removal of the ester blocking groups by treatment with 2 N NaOH.

Key Words: Dicyclohexylcarbodiimide, indole-3-acetic acid, indole-3-acetylaspartic acid.

INTRODUCTION

All plants studied form indole-3-acetylaspartic acid (IAA-Asp) following application of indole-3-acetic acid (IAA) to the plant tissue (1). Perhaps analogously, the metabolite indole-3-acetylglutamine has been found in human urine in patients with an inherited aberation in indole metabolism (2). Quantitative determination of IAA-Asp, and studies of its metabolism will be facilitated by an easy radiochemical synthesis of this commercially unavailable IAA-amino acid conjugate.

The synthesis of unlabeled IAA-Asp has been accomplished utilizing indole-3-acetyl chloride (3), a mixed anhydride of IAA (4) or an ester of IAA such as the p-nitrophenyl ester (5) as the acylating reagent. These reactions, with variations, were found to be unsuccessful in the micromolar synthesis of IAA-Asp. Good (6) described the synthesis of unlabeled IAA-Asp by dicyclohexylcarbodiimide coupling of IAA with the dibenzyl ester of aspartic acid followed by hydrogenolysis. A simplification of this method, with modifications

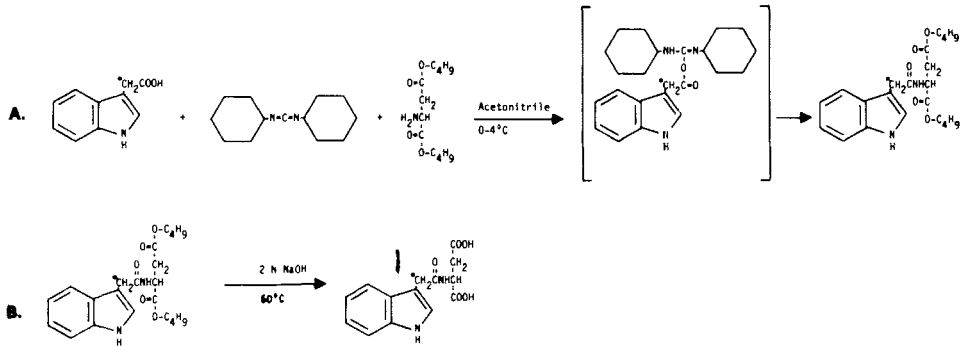
0362-4803/81/091393-04$01.00

Received July 28, 1980
Revised September 19, 1980

permitting manipulation of the highly radiosensitive IAA, is now reported which allows the micromolar synthesis of ^{14}C-IAA-Asp.

DISCUSSION

The reactions leading to IAA-Asp consist of coupling the IAA to bis-t-butyl-aspartate with dicyclohexylcarbodiimide, as described by Sheehan and Hess (7,8), followed by base hydrolysis of the blocking ester groups (Scheme 1, Reactions A and B).



Reaction conditions were such that the synthesis gave maximum yields at the concentration of ^{14}C-IAA and in the solvent - acetonitrile - supplied, thus eliminating the purification steps for IAA previously reported (9). The presence of up to 1% (v/v) of water in the acetonitrile did not influence the final yield.

The identity of the product was established by a number of criteria. First, a parallel synthesis was run on a larger scale starting with 3.5 mg of unlabeled IAA and the identity of the product of this synthesis confirmed as follows. The synthetic product had the same R_f value on Silica Gel thin layer chromatograms as authentic IAA-Asp prepared by the method of Mollan et al. (5). The methylated sample was analyzed by gas chromatography/mass spectrometry on a DuPont 321 GC/MS using a 3% SE-30 4 ft column, temperature programmed from 220-290°C at 10°/min. Ions at m/z 318 (m^+), 259, 173, 158, 130 (base peak), 103 and 77 confirmed the structure to be bis-methyl-IAA-Asp. The ^{14}C-labeled com-

pound was then compared with the unlabeled compounds. The ^{14}C-t-butyl ester, free acid and methyl ester had the same R_f values on Silica Gel thin layer chromatograms as did the unlabeled compounds. The methyl ester of the ^{14}C compound had the same retention time isothermally at 265°C on a 3% OV-17 6 ft gas chromatograhic column as the methyl esters of the unlabeled compound and authentic standard. Finally, an injection of a mixture of the ^{14}C compound and the authentic standard gave a single peak which, when collected, contained the majority of the radioactivity.

EXPERIMENTAL

Indole-3-acetic acid-2-^{14}C specific activity 54.5 mCi/mmole was obtained form New England Nuclear as an acetonitrile solution containing 100 μCi/ml and was used without purification. To a 2 ml vial equipped with a teflon-lined screw cap and containing 0.5 mg (2.0 μmol) l-aspartic acid-bis-t-butyl ester HCl (Serva Feinbiochemish, Heidelberg) was added 500 μl of the acetonitrile solution of ^{14}C-IAA (50 μCi, 0.92 μmol, 0.16 mg). After chilling on ice to 0°C, 0.62 mg (3.0 μmol) of dicyclohexylcarbodiimide (Aldrich Chemical Co., Milwaukee, WI) was added. The progress of the reaction was monitored by thin layer chromatography on Silica Gel 60 (E. Merck, Darmstadt) using chloroform: methanol:water (85:14:1) as solvent and Ehmann's reagent for indole detection (10). The reaction was complete after 8 hrs at 0-4°C and the mixture was transferred to a 5 ml vial containing 2 ml of 2 N NaOH and heated to 60°C. Removal of the t-butyl blocking groups was monitored by Silica Gel 60 thin layer chromatography with development in methyl ethyl ketone:ethyl acetate: ethanol:water (3:5:1:1). After 14 hr at 60°C, ester hydrolysis was complete and the mixture was chilled to 0°C, acidified to approximately pH 2.0 with phosphoric acid and this acid solution chromatographed on a 2 x 14 cm column of Sephadex LH-20 using 2-propanol:water (1:1) as solvent. ^{14}C-IAA-Asp eluted as a single peak between 48 and 55 ml. Yield calculated from 10 μl of the final solution and based on the specific radioactivity of the sample was 0.12 mg of

^{14}C-IAA-Asp, 22.5 μCi, or 45% of initial ^{14}C-IAA. Radiochemical purity was determined in two ways. First, by cochromatography on Silica Gel 60 with 10 μg of authentic standard, greater than 89% of the radioactivity was at the R_f of IAA-Asp and, second, by C_{18} reverse phase high pressure liquid chromatography. Elution was with 20% ethanol:water plus 1% acetic acid and 96% of the radioactivity was contained in a single peak coincident with carrier IAA-Asp.

ACKNOWLEDGEMENTS

I thank Dr. M. Zabik for the use of his mass spectrometer, Mr. R. Hangarter for the gift of the authentic indole-3-acetylaspartic acid and Miss M. La Haine for assistance with manuscript preparation. This work was supported by National Science Foundation grant PCM 79-04637 and National Aeronautics and Space Administration grant NAGW-97, ORD 25796 to Dr. R. S. Bandurski and is journal article 9541 from the Michigan Agricultural Experiment Station.

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