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Method for Synthesis of 2-Azido-N(6)-m-tritiobenzylaminopurine, a Photoaffinity Label for Cytokinin-Binding Proteins in Plants

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Abstract:

A method for tritiation of 2-azido-N(6)-benzyladenine (AZBA), a photoaffinity analog of the plant hormone N(6)-benzyladenine (BA), was developed. The synthetic sequence involves condensation of 2,6-dichloropurine with m-iodobenzylamine to yield 2-chloro-N(6)-m-iodobenzyladenine, followed by selective hydrodeiodination with tritium gas over palladium catalyst in pyridine solution to give 2-chloro-N(6)-m-tritiobenzyladenine. The known conversion of this compound to AZBA enables preparation of the photoaffinity label compound with specific activity high enough to permit detection of cytokinin-binding proteins existing at low concentrations in plant tissues.

Introduction:

Mornet et al. (1) and Keim and Fox (2) have previously described the synthesis of 2-azido-N(6)-benzyladenine (AZBA) with the aim to use this compound for photoaffinity labelling of cytokinin-binding proteins in plant

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material. This compound exhibits the same biological (cytokinin) activity as the parent benzyladenine (BA), and undergoes photolysis under specified conditions (1,3). However, this photoaffinity analog was prepared with a 14C label (2) due to precursor availability, and the low specific activity obtainable with this isotope limited the sensitivity of the assay. In order to search for cellular receptors for cytokinins occurring in concentrations lower than that of the seed protein detected by Keim and Fox a more radioactive form of AZBA was needed.

Of the methods available for intoduction of tritium into the molecule we preferred to use hydrogenation with tritium gas for maximum specificity of labelling and minimum destruction of the compound during the labelling process. The synthesis of AZBA was originally (1) carried out by coupling benzylamine with 2,6-dichloropurine to yield 2-chloro-N(6)-benzyladenine, followed by displacement of the chloride with hydrazine, and nitrosation of the hydrazine substituent to afford the azide. Due to the probable lability of an azide or hydrazine functionality under conditions of catalytic hydrogenation, we planned to introduce the tritium by dehydrohalogenation of an iodo substituent in the presence of the chloropurine moiety, reasoning that we should be able to selectively cleave a more labile iodo group. The commercially available m-iodobenzylamine served as a convenient benzylamine analog.

Results and Discussion:

The condensation of 2,6-dichloropurine and m-iodobenzylamine proceeded smoothly in aqueous buffer solution, the coupling product crystallizing spontaneously from the reaction mixture in pure form. The 2-chloro-N(6)-m-iodobenzyladenine (IBA) was quite insoluble in the usual hydrogenation solvents but dissolved readily in pyridine. Atmospheric pressure hydrogenation of IBA in pyridine over 10% palladium/carbon catalyst yielded 2-chloro-N(6)-benzyladenine (CBA). HPLC analysis of the reaction product

showed that the conversion was better than 95% after 18 hours, and that none of the over-reduction product N(6)-benzyladenine was detectable.

The actual tritiation was carried out by DuPont NEN Research Products, Inc. using these conditions. The specific activity of the crude was found to be 16.56 Curies/mmole, assuming a tritium scintillation counting efficiency of 33%. HPLC analysis of the radiolabelled product showed that the about 70% of the tritium co-eluted with an authentic sample of CBA. No distinct peaks absorbing at 272 nm other than that of CBA were seen when undiluted crude tritiation product was assayed by HPLC. A sample of the crude material was purified to homogeneity and the specific activity determined, using UV absorptivity as a mass measurement. The final specific activity was calculated as 6.41 Curies/mmole. The known conversion of CBA to AZBA, and this method of introducing a tritium label, constitutes a formal synthesis of tritiated AZBA.

Materials and Methods:

NMR spectra were obtained on a Varian XL-300, and are reported as: delta values (number of resonating protons, multiplicity) for proton; delta values only are reported for 13C. UV spectra were taken on a Perkin-Elmer Lambda 5, and liquid scintillation counting done with a Beckman LS7500 in Aquasol 2 (DuPont). HPLC analyses were carried out on a Hewlett Packard 1084B with 1040A Diode Array Detector, using a Varian MCH-10 column (25 x 0.4 cm), with a linear solvent gradient of tetrahydrofuran vs. 1% aqueous acetic acid, 30 to 100% over 15 minutes, monitoring at 272 nm wavelength. Retention times were 9.3 minutes for IBA, 8.3 minutes for CBA, and 6.1 minutes for N(6)-benzyladenine. Thin layer chromatography analyses were done using Kodak silica gel TLC plates developed in chloroform/ethanol 9:1 (v/v).

2-chloro-N(6)-m-Iodobenzyladenine (IBA)

A mixture of 2,6-dichloropurine (2.25 gm, 11.9 mmole) and m-iodobenzylamine hydrochloride (1.59 gm, 5.9 mmole) in disodium hydrogen phosphate solution (0.1 M, 950 ml) was heated at 80 degrees C for two hours,

then chilled to 4 degrees overnight. The white crystalline precipitate was filtered, washed with water and dried under vacuum to yield 0.89 gm (39%) IBA better than 95% pure by TLC, HPLC and NMR.

Melting point 252-255 degrees (needles from ethanol).

PMR(d6-DMSO): 8.708(1H, s(br)); 8.164(1H, s); 7.746(1H, s); 7.618,7.593(1H, d); 7.378,7.353(1H, d); 7.157,7.130,7.106(1H, t); 4.599(2H,s); 3.357(1H, s(br)).

13CMR(d6-DMSO): 154.347, 152.951, 142.196, 140.359, 136.240, 135.762, 130.756, 127.092, 125.791, 117.600. 95.057, 42.849.

Mass Spectrum: 385 (M+), 350 (M-Cl), 258 (M-I), 232 (M-I-Cl), 182, 154, 119.

Found 384.958481, -1.7 ppm from 384.959125 calculated for C12H9N5ClI (35Cl).

2-chloro-N(6)-benzyladenine (CBA): Reduction of IBA with H2

A sample of 2-chloro-N(6)-m-iodobenzyladenine (38.5 mg, 0.10 mmole) was dissolved in dry pyridine (5 mL) and 10% palladium on carbon (12 mg) was added. The mixture was stirred at room temperature overnight under hydrogen (atmospheric pressure), then was filtered through Celite. The flask and Celite were washed with an additional 2 mL pyridine, and the combined washes were reduced under nitrogen stream to 3 mL. This solution was poured into chilled water (15 mL), and the precipitate separated by centrifugation (9000 G, 30 minutes, 5 degrees C). The supernatant was discarded and the pellet resuspended twice in ice water. The crude product was better than 95% pure by HPLC, and could be further purified by recrystallization from water. The material was identical with a sample of CBA prepared from benzylamine and 2,6-dichloropurine (1,2).

Melting point 282-284 degrees (platelets from water).

PMR(d6-DMSO): 8.750(1H, s(br)); 8.145(1H, s); 7.336(5H, m); 4.652(2H, s); 3.371(1H, s(br)).

13CMR(d6-DMSO): 155.124, 153.162, 150.848, 139.857, 128.630, 127.668, 127.430, 127.151, 118.191, 43.567.

Mass spectrum: 259 (M+), 224 (M-Cl), 154, 119, 106, 91, 69.

Found 259.062393, -0.3 ppm from 259.062473 calulated for C12H10N5Cl (35Cl).

UV: Lambda max=272 nm, molar extinction coefficient=18,900/M.

2-chloro-N(6)-m-tritiobenzyladenine (3H-CBA): Reduction of IBA with T2

The reaction was carried out as described above using carrier-free tritium gas (by DuPont NEN products). After removal of the catalyst, exchangeable tritium was removed with ethanol, and 500 millicuries of the crude product was returned to us. To assay radiochemical purity, a 10 uL sample of the labelled material was mixed with a 50 uM solution of cold standard and analyzed by HPLC. A total of 94% of the total radioactivity was recovered from the column, and 67.8% of the radioactivity co-eluted with the carrier. When an undiluted sample of the radioactive sample was subjected to HPLC analysis, the UV absorptivity trace monitoring at 272 nm showed a peak with retention time identical to the cold standard, with no detectable amounts of the precursor or over-reduced product present. Collection of fractions indicated that 75.4% of the total counts were contained in the peak corresponding to the desired product. Measurement of the UV absorptivity of this fraction indicated that the specific activity of the purified material was 6.41 Curies/mmole. The remaining counts were not concentrated in any single other fraction, suggesting that slowly exchanged tritiums that were not removed by the ethanol treatement were labile in the HPLC solvents.

Conclusion

The selective catalytic hydrodeiodination of 2-chloro-N(6)-m-iodobenzyladenine to yield 2-chloro-N(6)-m-tritiobenzyladenine using palladium on carbon in pyridine solvent under hydrogen (tritium) gas at atmospheric pressure has been shown to be an effective route to a known precursor of the cytokinin photoaffinity analog 2-azido-N(6)-benzyladenine.

References:

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