SYNTHESIS OF ^{14}C- AND ^{3}H-LABELLED 3-INDOLYLACETYL-ASPARTIC ACID

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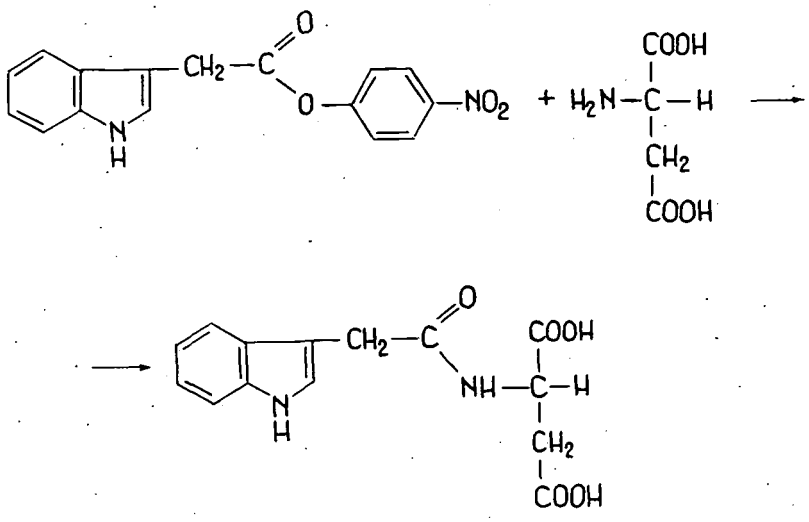
The paper describes the syntheses of 3-indolylacetyl-carboxy-^{14}C-aspartic acid, 3-indolylacetyl-aspartic acid-^{3}H and the doubly labelled 3-indolylacetyl-carboxy-^{14}C-aspartic acid-^{3}H.

The literature dealing with the physiological effects and metabolism of 3-indolylacetic acid describes the occurrence of 3-indolylacetyl amino acid conjugates in plants [1, 3]. The details of the physiological roles of the individual conjugates have not been clarified. The problem of whether the bound 3-indolylacetic acid becomes free again can be investigated by means of an isotope technique. Labelling of the 3-indolylacetil amino acid conjugates on the amino acid permits study of the connection of the amino acid and protein metabolisms, while labelling on the 3-indolylacetic acid permits study of the turnover of 3-indolylacetic acid. Some labelled combinations of 3-indolylacetylaspartic acid were prepared, utilizing carboxy-^{14}C--3-indolylacetic acid and ^{3}H- and inactive aspartic acid.

Syntheses of a number of inactive 3-indolylacetyl amino acids have been dealt with by HUTZINGER *et al.* [2], who applied the methods for carboxyl activation of the amino acids, and made use of 3-indolylacetic acid active esters with N-hydroxysuccinimide.

MOLLAN *et al.* [4] describe the preparation of the compound in accordance with the known synthesis steps, by using 3-indolylacetic acid *p*-nitrophenyl ester. On the basis of inactive experiments we found that the latter method gives a substantially better yield in the active syntheses, and achieved the synthesis of 3-indolylacetyl-aspartic acid by coupling the *p*-nitrophenyl ester of 3-indolylacetic acid with L-aspartic acid.

The purity of the compounds formed were checked, among others, by radiochromatography; these compounds could be used to identify radiochromatographycally natural 3-indolylacetylaspartic acid.



Experimental

Carboxy-^{14}C-3-*indolylacetic acid* was synthesized by the method of STUTZ *et al.* [5] from 10.5 mmole gramine and 40 mmole 20 mCi K^{14}CN, *via* the reaction path indole, gramine, 3-indolylacetonitrile-^{14}C, 3-indolylacetic acid-1-^{14}C. Yield: 1225 mg, 70%. Molar activity: 0.5 mCi/mmole. Radioactive purity checked by chromatography and autoradiography. M.p.: 164—166°C.

Carboxy-^{14}C-L-*aspartic acid* was a New-England Nuclear preparation. Specific activity: 250 μCi/mmole.

^{3}H-L-*aspartic acid* was also a New-England Nuclear preparation. Specific activity: 1 mCi/mmole.

Inactive 3-*indolylacetic acid p-nitrophenyl ester.* 0.40 g (2.2 mmole) 3-indolylacetic acid and 0.32 g (2.2 mmole) *p*-nitrophenol were dissolved in 8.5 ml ethyl acetate and the mixture was cooled to 0—5°C. A solution of 0.46 g (2.2 mmole) N,N′-dicyclohexylcarbodiimide in 1.6 ml ethyl acetate was added at 0°C, and the mixture stirred for one hour at room temperature. The N,N′-dicyclohexylcarbamide was filtered off, and the solution concentrated to 1.6 ml, cooled and filtered again. Crystalline *p*-nitrophenyl ester was obtained by removing the residual solution. M.p.: 100—103°C. Recrystallization from an ethyl acetate—petroleum ether mixture gave pale-yellow needles. Yield: 0.85 g, 72%. M.p. 106—107°C.

3-*Indolylacetyl-carboxy-*^{14}C-*p-nitrophenyl ester* was prepared from 0.7 g 3-indolylacetyl-carboxy-^{14}C, 0.556 g (4 mmole) 3-*p*-nitrophenol and 0.832 g (4 mmole) N,N′-dicyclohexylcarbodiimide. Yield: 0.88 g, 75%. M.p. 106—107 °C.

3-*Indolylacetyl-*L-*aspartic acid.* 0.92 g (1.66 mmole) L-aspartic acid was dissolved in 25% aqueous tetramethylammoniumhydroxide solution (1.22 g, 3.33 mmole), and the mixture lyophilized. The salt was suspended in 8.3 ml dimethylsulfoxide. Dissolution was achieved after addition of 0.49 g (1.66 mmole) 3-indolylacetyl-*p*-nitrophenyl ester and stirring the mixture overnight. The dimethylsulfoxide was removed in vacuum, and the product taken up in 17 ml 5% $NaHCO_3$ solution and in 17 ml ether. The aqueous phase was extracted with 2×20 ml ether,

acidified with concentrated hydrochloric acid, and extracted with ether again. The aqueous phase was further acidified to pH 1, and extracted with 2×8.5 ml butanol. The butanol phase was washed with 8.5 ml 0.1*N* hydrochloric acid and with 10 ml water. Removal of the butanol in vacuum led to a pink glass, and recrystallization from water to colourless crystals. Yield: 0.31 g, 66%. M.p.: 189—190°C.

3-*Indolylacetyl-carboxy*-^{14}C-L-*aspartic acid.* The method used in the preparation of inactive 3-indolylacetyl-L-aspartic acid was used. A 25% aqueous solution of 0.365 g tetramethylammoniumhydroxide and 0.133 g (1 mmole) L-aspartic acid was lyophilized. The residue was dissolved in 6 ml dimethylsulfoxide and 0.296 g (1 mmole) 3-indolylacetyl-*p*-nitrophenyl ester was added. Yield after working up: 0.159 g, 60%. Activity: 1 mCi/mmole. M.p.: 188—190°C. Autoradiographically: one spot.

3-*Indolylacetyl*-L-*aspartic acid*-^{3}H. 1 mCi L-aspartic acid-^{3}H was diluted to an activity of 1 mCi/mmole with inactive L-aspartic acid. The synthesis was performed with mmole amounts. Yield: 0.145 g, 50%. Total activity: 450 μCi. Specific activity: about 1 mCi/mmole.

3-*Indolylacetyl-carboxy*-^{14}C-L-*aspartic acid*-^{3}H. 0.13 g (45%) 3-indolylacetyl--carboxy-^{14}C-L-aspartic acid-^{3}H was formed from 1 mmole L-aspartic acid, activity 1 mCi/mmole, and 1 mmole 3-indolylacetyl-*p*-nitrophenyl ester, activity 250 μCi/ /mmole. Total activity ^{14}C: 112 μCi; ^{3}H: 440 μCi. The activities were measured with a Nuclear Chicago liquid scintillation spectrometer.

References

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СИНТЕЗ ^{14}C И ^{3}H МЕЧЕННОЙ ИНДОЛ-3'-ИЛАЦЕТИЛ-АСПАРАГИНОВОЙ КИСЛОТЫ

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В работе описан синтез индол-3'-илацетил-карбоксил-^{14}C-аспарагиновой кислоты, индол-3'-илацетил-аспарагиновой-^{3}H кислоты и вдвойне меченной индол-3'-илацетил-карбоксил-^{14}C-аспарагиновой-^{3}H кислоты.