NOTES

SYNTHESIS OF (2R,S)- [2-^{2}H] AND [2,3-^{2}H] TRYPTOPHAN

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SUMMARY

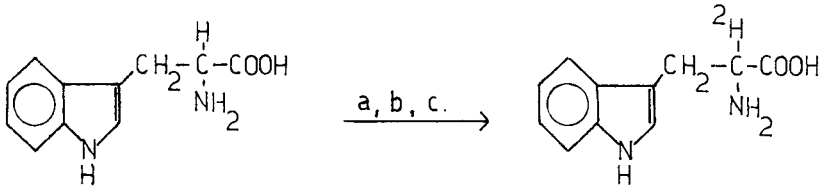
α-Deuterated tryptophan was prepared either by exchange of the α-hydrogen of tryptophan or by hydrolysis and decarboxylation of ethyl 2-formamido-2-carbethoxy-3-indole propionate, followed by treatment with CH_3COO^2H. α,β-Dideuterated tryptophan was in turn synthetized from ethyl-α-acetamido-indole-3-acrylate, by catalytic deuteration ($^2H_2$/Pd-C) and alkaline hydrolysis ($NaO^2H/^2H_2O$).

Key Words : α-Deutero tryptophan; α,β-dideutero tryptophan; deuterium labelling; exchange; ^{1}H-N.M.R.

For biochemical studies, specimen of the title mono and dideutero tryptophan were required. The preparation of α-deutero (2) was realized by two different routes. Racemization-exchange of the α-hydrogen, which was working satisfactorily for other α-amino acids[1] in our hands gave poor yields when applied to tryptophan (1). Refluxing (1) with excess acetic anhydride and ^2H_2O for a few minutes gave a mixture of products, which was treated with diazomethane. After purification on a silica gel column, N-acetyl tryptophan methylester could be recovered (10% yield) and then hydrolyzed with aqueous HBr (80%yield) to afford (2) (Scheme 1). A complete exchange of the α-hydrogen was observed by ^{1}H-N.M.R. and mass specrometric analysis. In the mass spectrum, the fragment at m/e 130, bearing the β-methylene, showed no incorporation of deuterium at the β-carbon. In conclusion, although yields are low, the above procedure offers some advantage such as complete exchange of hydrogen, easy purification of the intermediate N-acetyl

0362-4803/82/040611-07$01.00 Received July 27, 1981

tryptophan methylester and number of steps to be performed.

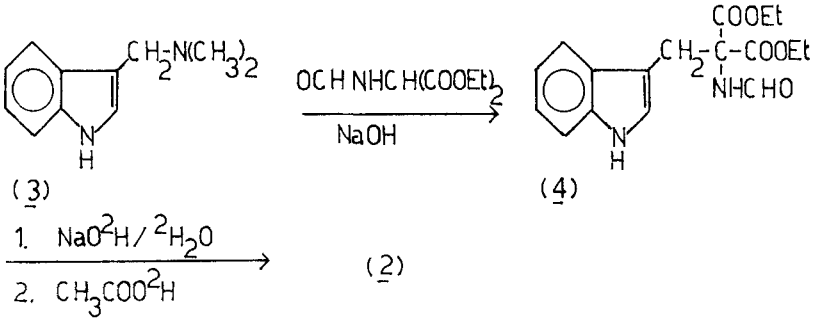


(1) (2)

a $Ac_2O/^2H_2O$; b. CH_2N_2; c. HBr/H_2O.

Scheme1

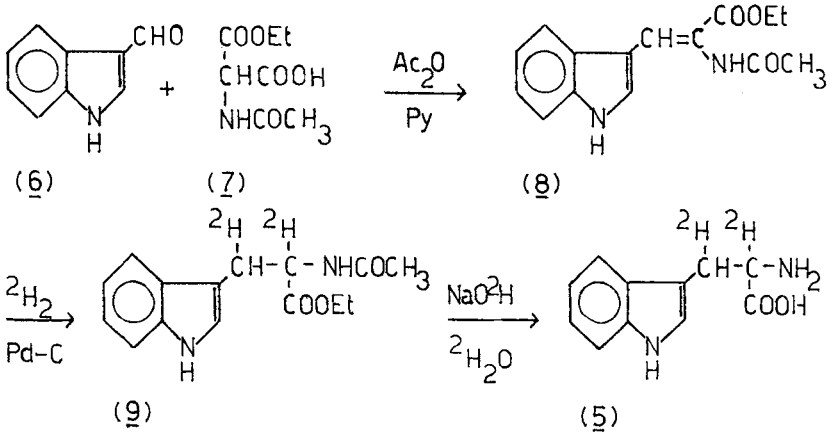
Better yields of (2) could be achieved starting from gramine (3)[2], preparing ethyl 2-formamido-2-carbethoxy-3-indole propionate (4) by the method of Weygand and Linden[3]. Subsequent hydrolysis and decarboxylation in $NaO^2H/^2H_2O$, treatment with CH_3COO^2H introduced a deuterium atom at the α-position (48% overall yields) (Scheme 2).



(3) (4) (2)

Scheme 2

The deuteration was complete as ascertained by ^{1}H-N.M.R. as well as mass spectrometric analysis of a crystallized sample of (2)[4]. The preparation of α,β-dideuterated tryptophan (5) was realized in the best way by condensation of indole-3-carboxaldehyde (6) with the mono ethylester of acetoamidomalonic acid[5] (7) and deuteration ($^2H_2$/Pd-C) of the formed indole-3-acrylate (8) (Scheme 3).



Scheme 3

A few experiments indicated incomplete deuteration at the β-position of (9) and we decided to minimize the exchange by treating (8) with ^2H_2O in dioxane before performing the catalytic deuteration. In this way, a 90% incorporation of deuterium on both α and β carbons was achieved. For the preparation of (5), the hydrolytic step was rather intriguing for the exchange of the label with the medium. Finally, we found that alkaline hydrolysis carried out with NaO^2H in ^2H_2O afforded (5) in good chemical yields and with retention of all the deuterium present in (9). The final purification of (5) was accomplished by ionic-exchange resin chromatography and the desired α,β-dideuterated tryptophan was obtained in overall 48% yields and 90% label present on each carbons, as clearly indicated by ^{1}H-N.M.R. and mass spectra.

EXPERIMENTAL

Deuterated material was purchased from Carlo Erba, Italy; ^{1}H-N.M.R. spectra were recorded on a Hitachi-Perkin Elmer R-24 spectrometer, using as internal standard TMS for spectra in $C^{2}HCl_3$ and DSS for spectra in $^{2}H_2O$. Elemental analyses were consistent with calculated values. Mass spectra of deuterated tryptophans were performed with a Varian XL-100 spectrometer. T.L.C. were carried out on silica gel HF 254 plates and the products visualized under U.V. light or by exposition at iodine vapours or spraying with ninhydrin.

N-Acetyl (2R,S)-[2-^{2}H] tryptophan methyl ester. (2R,S)-Tryptophan (0.408 g, 2 mmoles) was shaken with $^{2}H_2O$ (0.75 ml) to exchange the labile protons. The mixture was evaporated under vacuo below 70°C and the operation repeated twice. Immediately, acetic anhydride (4.35 ml) and $^{2}H_2O$ (0.5 ml) were added and the flask heated at 170° C (5 min) under nitrogen. After cooling at room temperature, $^{2}H_2O$ (0.4 ml) was added and the mixture evaporated. The residue was treated with ethyl acetate and a saturated sodium bicarbonate solution. After extractions , the water solution was additionally extracted with ethyl acetate and then acidified to pH 3 by addition of diluted HCl. Extraction with 3x10 ml of ethyl ether, drying on sodium sulphate and evaporation of the solvent, led to a residue (0.4 g), which was dissolved in the minimum amount of methanol and added with an excess of an ethereal solution of diazomethane. The reaction was followed by T.L.C. (chloroform-ethanol, 9:1) and the crude mixture evaporated to dryness. Purification on a silica gel column afforded the title compound in the fraction eluted with chloform-methanol, 7:3 (32 mg). ^{1}H-N.M.R. ($C^{2}HCl_3$), δ ppm, 3.3 (s, broad, 1H, β-CH^{2}H), 3.7 (s,3H,-COOCH$_3$), 6.1 (m,1H, indole -NH), 7.0 -7.7 (complex, 5H, indole), 8.4 (m,1H, -NH-Ac).

(2R,S)-[2-^{2}H] Tryptophan (3). A mixture of the above methyl ester (32 mg), 40% aqueous HBr (0.15 ml) and water (0.5 ml) was heated at 100°C (5h). After cooling at room temperature, the solution was brought to pH 5 and chilled,in order to precipitate

the α-deuterated tryptophan, which was rinsed and dried (20 mg); ^{1}H-N.M.R. (^2H_2O,^{2}HCl),δ(ppm) 2.5 (s, 2H, β-CH_2), 6.2-6.8 (5H, complex, indole). Unlabelled tryptophan in the same solvent showed the following resonances, (ppm) 2.5 (d, 2H, -CH_2β), 3.4 (t, 1H, -CH-COOH), 6.2-6.8 (5H, complex, indole); M.S. m/e 205 (M^+), 130.

Ethyl 2-formamido-2-carbethoxy-3-indole propionate (4). This compound was prepared according to Ref.3. Elemental analysis was in accord with the structure and ^{1}H-N.M.R. spectrum was as follows: δ (ppm) 1.2 (t, 6H, -CH_2-CH_3), 3.7 (s, 2H, β-CH_2), 4.1 (q, 2H, -CH_2-CH_3), 7.0-7.6 (complex, 6H, indole), 8.1 (s, 1H, -CHO), 8.5 (s, 1H, -NH-CHO).

Hydrolysis of (4) to (2). The prepared malonic ester (0.512 g, 1.54 mmoles) was refluxed with 10% NaO^2H in ^2H_2O (3.2 ml) for 6 h. At the end of this time, CH_3COO^2H (0.6 ml, 10.5 mmoles) was added and the solution refluxed (2 h). After cooling at room temperature, the solution was crystallized by addition of benzene and pure α-deutero tryptophan obtained (150 mg, 47% yield). ^{1}H-N.M.R. and M.S. spectra were in accord with ≐ 95% incorporation of deuterium and were as described previously.

Mono ethyl acetamidomalonate (7). Title compound was prepared by an improved procedure with respect to the one described in the literature[6]. To a suspension of diethyl acetamidomalonate (8.7 g, 40 mmoles) in ethanol (30 ml), a solution of K_2CO_3 (8.3 g, 60 mmoles) in water (30 ml) was added. After 3 h at room temperature, the solution was concentrated at room temperature and extracted with diethyl ether (3x30 ml). In this way side products were removed and acidification to pH 2-3 by addition of 2N HCl led to the formation of a precipitate, essentially pure (7) (4.54 g, 60% yields). The chemico-physical properties of the compound were as described[6].

Ethyl-α-acetamido-indole-3-acrylate (8). To a stirred solution of indole-3-carboxaldehyde (6) (2.05 g, 14 mmoles) in anhydrous pyridine (12 ml),the ester (7) (2.66 g, 14 mmoles) and acetic

anhydride (4 ml) were added at room temperature. After 22 h, crushed ice was added (20 g) and stirring continued for 2 h. Water was added (44 ml) and extraction performed with ethyl acetate (3x30 ml). The organic layer was washed with water, dried (Na_2SC_4) and evaporated to dryness. After a column chromatography on silica gel the title compound was obtained by elution with chloform - ethanol, 9:1 (1.76 g, 65% yield); ^{1}H-N.M.R. in C^2HCl_3, δ (ppm), 1.3 (t, 3H, $-CH_2-CH_3$), 2.15 (s, 3H, $-NHCOCH_3$), 3.35 (s, 1H, -CH=), 4.25 (q, 2H, $-CH_2-CH_3$), 7.1-7.9 (complex, 5H, indole), 9.1 (s, 1H, amidic -NH).

[2,3-^{2}H] -Tryptophan (5). The above acrylate (8) (600 mg, 2.2 mmoles) was dissolved in anhydrous tetrahydrofuran (5 ml) and a few drops of ^2H_2O added. The solution was brought to dryness in vacuo and the operation repeated three times. The residue was dissolved in anhydrous tetrahydrofuran (5 ml) and deuterated with $^2H_2$ on 10 % Pd-C (60 mg). The obtained N-acetyl tryptophan ethyl ester (9) exhibited a ^{1}H-N.M.R. spectrum in accord with the presence of 0.9 atoms of deuterium at the α- and β-positions. The above (9) (500 mg) was treated with a 40% solution of NaO^2H in ^2H_2O (0.9 ml) and with ^2H_2O (2 ml) and refluxed under stirring (8 h). After cooling at room temperature, the pH was brought to 9-10 with 1N HCl and the obtained (5) was purified on a Dowex 2x8 columv in acetate form (elution with 2M acetic acid). 315 mg of pure (5) were obtained; ^{1}H-N.M.R. in ^2H_2O and ^{2}HCl, δ (ppm) 3.2 (m, 1H, β-CH_2-), 4.15 (m, 0.1 H, $-CH-NH_3^+$), 7.0-7.6 (complex, 5H, indole); M.S., 206 (M^+), 131.

Acknowledgement : We thank C.N.R. (Rome) for financial support and Dr. Rosangela Casati for participation to some experimental part of the work.

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