NOTES

The Synthesis of Serotonin ^{14}C-labelled in the Ring (5-hydroxytryptamine-3-^{14}C)

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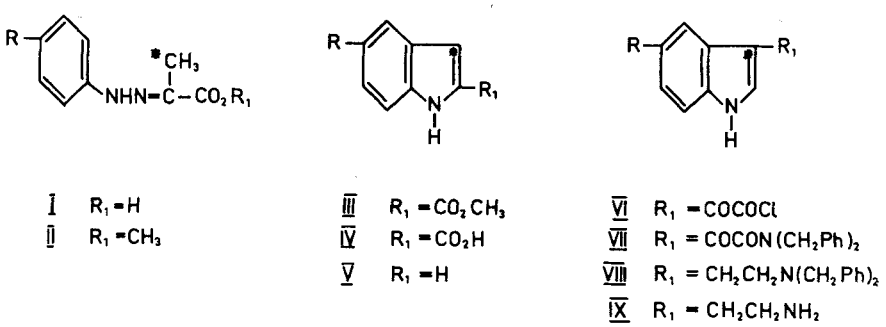
For metabolic studies in this Laboratory, 5-hydroxytryptamine (serotonin) labelled with ^{14}C in the pyrrole part of the indole ring was required. Previous attempts to reach this compound through Madelung cyclization of 3-benzyloxy--6-formylaminotoluene failed [1], although this way proved to be successful for the preparation of unsubstituted indole-2-^{14}C [2]. A number of methods leading to 5-hydroxytryptamine were then compared and the following synthetic pathway starting with pyruvic acid-3-^{14}C was elaborated on one-millimole scale :



I R$_1$ = H
II R$_1$ = CH$_3$

III R$_1$ = CO$_2$CH$_3$
IV R$_1$ = CO$_2$H
V R$_1$ = H

VI R$_1$ = COCOCl
VII R$_1$ = COCON(CH$_2$Ph)$_2$
VIII R$_1$ = CH$_2$CH$_2$N(CH$_2$Ph)$_2$
IX R$_1$ = CH$_2$CH$_2$NH$_2$

I–VIII R = PhCH$_2$O IX R = OH

SCHEME I.

The hitherto undescribed hydrazone I and its methyl ester II were prepared in high yields; the purity of the latter showed to be essential for a high yield in the next step. The indolization of II was smoothly performed following the procedure of Ash and Wragg [3] with slight modifications. The resulting indole ester III was then hydrolyzed without further purification into the acid IV; after one recrystallization, the radioactive yield of IV based on pyruvic acid-3-^{14}C was 39%. Decarboxylation of IV after Stoll *et al.* [4] resulted in pure 5-benzyloxyindole-3-^{14}C (V).

Introduction of the ethylamine side chain at the position 3 of the indole ring was achieved after Speeter and Anthony [5] through the formation of glyoxylyl chloride VI,

its conversion to the dibenzylamide VII, and the reduction of VII into the dibenzylamine VIII. The three-step procedure was performed without purification of intermediates and the crude dibenzylamine VIII was then subjected to fractionation on an Al_2O_3 column, whereupon the crystalline VIII was obtained as the hitherto undescribed free base in an overall 46% yield, calculated on 5-benzyloxyindole-3-^{14}C (V). Debenzylation of VIII and the isolation of 5-hydroxytryptamine-3-^{14}C (IX) in the form of creatinine sulfate complex was carried out in the same way as already described for serotonin ^{14}C-labelled in the side chain ([6]). After one recrystallization, the product was chemically and radiochemically pure; the overall radiochemical yield based on pyruvic acid-3-^{14}C was 10%.

Pyruvic acid-3-^{14}C p-*benzyloxyphenylhydrazone (I).*

To a solution of *p*-benzyloxyphenylhydrazine hydrochloride (275.8 mg, 1.1 millimole) in 35 ml of hot water, a solution of sodium pyruvate-3-^{14}C (Amersham, England, 0.5 mc, 0.077 millimoles), in 1 ml H_2O, followed by 0.065 ml of inactive pyruvic acid (0.923 millimoles) were added. After standing overnight at room temperature the yellow crystals were centrifuged off and washed with water; yield : 282.9 mg, 99.5%.

In a cold preparation the substance was recrystallized from 50% ethanol; m.p. 144-147° C. Anal. Calcd. for $C_{16}H_{16}N_2O_3$ (284.31) : C, 67.59; H, 5.67; N, 9.86. Found : C, 67.86; H, 5.43; N, 9.91.

Methyl pyruvate-3-^{14}C p-*benzyloxyphenylhydrazone (II).*

I was directly esterified with an excess of diazomethane in ether and left to stand at 0° C for 5 hr. Ether was removed by a stream of nitrogen, and the residue crystallized from 80% methanol with addition of charcoal; yield : 264.8 mg, 89%.

In a cold preparation the substance had m.p. 152-154° C. Anal. Calcd. for $C_{17}H_{18}N_2O_3$ (298.33) : C, 68.44; H, 6.08; N, 9.39. Found : C, 68.44; H, 5.94; N, 9.69.

Methyl 5-benzyloxyindole-(3-^{14}C)-2-carboxylate (III).

The cyclization of II was performed in a 50 ml pear-shaped flask fitted with a mechanical stirrer, a reflux condenser fitted with a $CaCl_2$ tube, and a dropping funnel. Under vigorous stirring 4 ml of abs. methanol saturated with dry HCl was added in one portion; the mixture was heated to boiling and then stirred at room temperature for 2 hr. The clear red solution was transferred in a centrifuge tube, evaporated by a stream of nitrogen to about 2 ml and kept at 0° C overnight. The separated crystals of III were centrifuged off, and washed with 5 ml of cold methanol and 5 ml of hot water; yield : 182 mg, 74%.

The mother liquor and methanolic washing were combined and evaporated to dryness; by addition of unlabeled III to the residue, and subsequent recrystallization from methanol an additional crop of diluted III was recovered.

5-Benzyloxyindole-(3-^{14}C)-2-carboxylic acid (IV).

To III dissolved in 7 ml ethanol, 0.172 ml N KOH was added; the solution was mechanically stirred at reflux temperature for 2 hours, transferred on crushed ice in a centrifuge tube and acidified with 2 N HCl to pH 3 whereupon 171 mg, 100% of crude IV separated. It was centrifuged off and combined with 77.7 mg of IV prepared in a separate experiment from diluted III; after recrystallization from 50% ethanol 249 mg of lightly pink crystals with a spec. activity of 0.793 μC/mg were obtained; total radioactivity : 196.5 μC; radioactive yield based on pyruvic acid-3-^{14}C : 39%.

5-Benzyloxyindole-3-^{14}C (V).

A mixture of 290 mg IV (1 mmole, 196.5 μC), 0.5 ml chinaldin, and 5 mg Cu-powder was heated in a metal bath at 230-240° C under a stream of nitrogen for 4 hr. Chinaldin was distilled off at 0.008 mm (bath temp. 100° C) and the remaining brown residue extracted repeatedly with 10 ml portions of boiling heptane (b.p. 100-125° C). The combined extracts were concentrated by a stream of nitrogen to about 50 ml and kept at 0° C for 24 hr whereupon the separated crystals were centrifuged off; yield : 158.3 mg, 65%. In solvent A one radioactive, Ehrlich positive spot, R_f 0.90.

To the mother liquor, unlabeled V was added, and after crystallization the obtained material was combined with the first crop.

5-Benzyloxy-3-(2-N,N-dibenzylaminoethyl)-indole(3-^{14}C) (VIII).

To 210 mg (0.94 millimoles) of indole V, dissolved in 10 ml of abs. ether, a solution of 0.24 ml oxalyl chloride in 2 ml of abs. ether was added at 0° C under stirring and exclusion of moisture. The mixture was stirred at room temperature for additional 30 minutes, the precipitated chloride VI left to settle down, and the clear supernatant was removed by a bent capillary tube using a low vacuum suction; it was then three times resuspended in abs. ether, the washings being removed in the same way, and immediately used for the next step.

To the crude chloride VI, suspended in 10 ml of abs. ether a solution of 0.362 ml dibenzylamine in 5 ml abs. ether was added dropwise under stirring at room temperature; the mixture was then stirred for additional 4 hr. The ethereal layer was transferred through a tube with a sealed sintered disc into a centrifuge tube, and the residue repeatedly extracted with small portions of abs. tetrahydrofurane. The combined extracts were evaporated to dryness by a stream of nitrogen leaving the amide VII as a viscous yellow oil which was used without further purification in the next step.

The crude amide VII dissolved in 20 ml of tetrahydrofurane was reduced with 320 mg $LiAlH_4$ at reflux temperature under mechanical stirring for 3 hr. The complex was destroyed by addition of tetrahydrofurane-water (1 : 1) and

water, and the mixture filtered through a column of Kieselguhr (BDH, 1.2 × 16 cm). Th column was washed with 50 ml $CHCl_3$, and the combined effluents evaporated *in vacuo*. The remaining brown oil (358 mg) was dissolved in 2 ml of benzene and subjected to chromatography on a Al_2O_3 column (Merck, neutral, 1 × 35 cm) packed with petroleumether-benzene (1 : 1). The column was eluted successively with 75 ml petroleum-ether-benzene (1 : 1) and 125 ml benzene, 25 ml fractions being collected. The amine VIII emerged with benzene; by this way 82 mg (fract. Nr 5-8) of pure, and 164 mg of still impure VIII were obtained. The latter was subjected to a second column chromatography, whereupon additional 111 mg of pure VIII resulted. Total yield : 194 mg of a crystalline solid (46%, based on 5-benzyloxyindole-3-^{14}C), showing in solvent A one radioactive, Ehrlich positive spot, R_f 0.93.

In a cold preparation the substance was recrystallized from petroleum-ether; colorless crystals, m.p. 96-98° C. Anal. Calcd. for $C_{31}H_{30}N_2O$ (446.57) : C, 83.37; H, 6.78; N, 6.27. Found : C, 83,58; H, 6.70; N, 6.44.

5-Hydroxytryptamine-3-^{14}C creatinine sulfate (IX).

The benzylated amine VIII (194 mg, 0.442 millimoles) was catalytically debenzylated in methanol with 590 mg of 10% Pd/$BaSO_4$ until the consumption of hydrogen was complete (48 hr). After centrifugation, the catalyst was repeatedly washed with methanol, to the combined supernatants one equivalent of N H_2SO_4 was added and the solvent evaporated to dryness by a stream of nitrogen. The remaining gum, dissolved in 4 ml of hot water was treated under shaking at 50° C with 50 mg creatinine and 0.442 ml. N H_2SO_4 followed by 40 ml acetone, whereupon 127.7 mg (71.5%) of IX precipitated. It was redissolved in 4 ml water and precipitated with acetone; 121 mg of shiny crystals showing one radioactive, Ehrlich positive spot in solvent A, R_f 0.55 were obtained. Specific activity : 0.446 μC/mg, total activity : 50.09 μC, radioactive yield based on pyruvic acid-3-^{14}C : 10%.

The radioactivity measurements of IV and IX creatinine sulfate were done on diluted recrystallized samples. One-dimensional paper chromatograms in isopropanol : ammonia : water (10 : 1 : 1) (solvent A) were scanned with an automatic GM scanner and sprayed with Ehrlich reagent.

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D. KEGLEVIĆ AND L. STANČIĆ

Tracer Laboratory, Institute «Ruder Bošković,
P.O.B. 171, Zagreb, Yugoslavia

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Trans-N-deoxyribosylase activity in dairy cultures of Lactobacillus helveticus

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An excellent communication dealing with the enzymatic preparation of deoxyribonucleosides labelled with ^{14}C in the base, using a crude enzyme extract of *Lactobacillus helveticus* has been published in this Journal [1] quite recently by Cardinaud and Viswanthan. The most important result was a high degree of conversion of pyrimidine bases to the corresponding deoxyribosides.

In connection with this work we wish to present some observations from the same field made in our Laboratory [2]. It was the purpose of our work to isolate the enzyme trans-N-deoxyribosylase from a *Lactobacillus helveticus* culture grown just in sterile milk without any additives, to test the enzymatic activity of the isolated enzyme and its suitability for rapid enzymatic synthesis of deoxyribonucleosides labelled with radiocarbon ^{14}C.

The culture of *Lactobacillus helveticus*, var. pragensis BMSM — 5/8, strain TX was inoculated into sterile full-fat milk (fat content 3.6-3.8%), so that a 0.5-1.0% solution of this culture was obtained. After inoculation the culture was incubated for 14-16 hours at 40° C (all these procedures were carried out in the Institute for Dairy Cultures in Prague). About 100 ml of the grown suspension was centrifuged at 4,000 r.p.m. and 0° C for 20 minutes. The clear supernatant and the fat layer were removed, the centrifuged cells were suspended in 100 ml of 0.05 M citrate buffer of pH 6 and again centrifuged. The wet weight of cells was 55.0 grams. The washed cells were suspended in 120 ml of 0.1 M Tris-buffer of pH 7.2 and disrupted in the MSE ultrasonic desintegrator (input 60 Watts, frequency 20.10^3 cycles/sec) at 0° C for 5 minutes, then the suspension was centrifuged, the cell debris removed and the enzyme present in the supernatant fluid was purified according to the procedure of Roush and Betz [3]. In 7.4 ml of the final enzyme solution, 31.1 mg of protein was found by the method of Lowry *et al.* [4]. The enzyme was then frozen and stored at −15° C.