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The Synthesis of ^{14}C Labelled Serotonin /2-(5'-Hydroxyindolyl-3')-ethylamine-[1-^{14}C]/

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By modification of published procedures on the 2-(indolyl)-3')-ethylamines syntheses it was possible to obtain 2-(5'-benzyloxy-indolyl-3')-acetonitrile-[1-^{14}C] (III) in a 82% yield, from equimolar amounts of 5-benzyloxygramine methosulphate (II) (1.1 m*M*) and sodium cyanide-^{14}C (1.0 m*M*, 1 mC). III was reduced with lithium aluminium hydride to the corresponding amine IV, catalitically debenzylated to serotonin V and isolated as creatinine sulphate complex VI in a 64% yield (calc'd on $Na^{14}CN$), having a specific activity of 1.71 μC/mg. (0.69 mC/m*M*).

A large amount of experimental work has been done with 2-(5'-hydroxy-indolyl-3')-ethylamine, (serotonin[1], enteramine[2]), a naturally occuring vaso-constrictor substance of serum and tissue fluids. Being a simple molecule it has been synthesized without great difficulty[3, 4]. However the role of serotonin in normal physiology as well as in mental and circulatory diseases is still obscure[5]. For the purpose of some metabolic studies (with this substance) we synthesized ^{14}C labelled serotonin (V).

In general, 2-(indolyl-3')-ethylamines are obtained in good yields by treatment of gramines or their quarternary salts with an aqueous solution of alkali cyanides yielding 2-(indolyl-3')-acetonitriles which by reduction give the corresponding amines[6-9]. All reactions of this type described in the literature use the alkali cyanide in a remarkable excess varying from three to twelve moles of alkali cyanide per one mole of gramine or its quarternary salt.

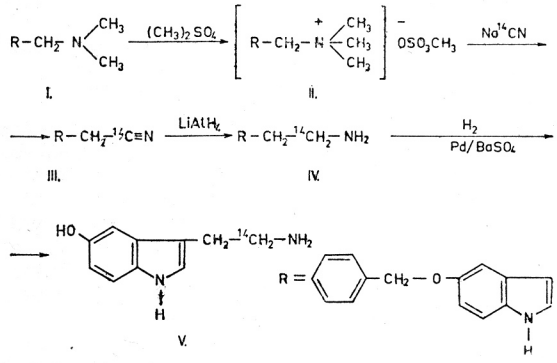
Except for this fact, sodium cyanide-^{14}C seemed to us to be a very convenient compound for introducing the radiocarbon in the serotonin molecule, first, for its availability, and second, because using it, only three radioactive reaction stages have to be run to obtain the labelled serotonin. While attempting to reduce the amount of alkali cyanide, we investigated the reaction between 5-benzyloxygramine methosulphate (II) and sodium cyanide.

Normally, when an excess of alkali cyanide is used, the reaction mixture remains alkaline during the whole process, in spite of the fact that the trimethylamine formed during the reaction escapes in the atmosphere. On the contrary, if equimolar amounts of reagents are used under the same conditions, the alkalinity of the reaction mixture decreases with the evolution of trimethylamine.

At *p*H lower than 8 a decomposition of the reaction product takes place, resulting in a poor yield of 2-(5'-benzyloxyindolyl-3')-acetonitrile (III), conta-

minated with reddish gummy products. If sodium hydroxide is added to the reaction mixture in order to keep it alkaline, the amount of III will also be very poor, probably owing to the reaction between II and alkali hydroxide.

However, we found that if the reaction of equimolar amounts of reagents is performed in a closed system under the slight pressure of the trimethylamine evolved and the mixture heated for a longer period than described in the literature, III is obtained in a 82% yield.



Thus obtained 2-(5′-benzyloxyindolyl-3′)-acetonitrile-[1-^{14}C] (III) was reduced with lithium aluminium hydride to the corresponding amine IV, and catalitically debenzylated to serotonin (V), which was isolated as creatinine sulphate complex (VI). The overall yield calculated on Na^{14}CN (1 m*M*, 1 mC) was 260 mg. (64%) of VI with a specific activity of 0.69 mC/m*M*.

EXPERIMENTAL

5-Benzyloxygramine, (5-benzyloxy-3-dimethylaminomethyl-indole) (I)

The starting material was 5-benzyloxy-indole-2-carboxylic acid obtained after the procedure given by Boehme[10]. The decarboxylation of the acid was performed after Stoll and al.,[8] and Mannich condensation of 5-benzyloxyindole after Ek and Witkop.[7]

5-Benzyloxygramine methosulphate, (5-benzyloxy-3-trimethylaminomethyl-indole methylsulphate) (II)

The compound was prepared according to the general procedure of Schöpf and Thesing[11]. A solution of 5-benzyloxygramine (I, 310 mg., 1,1 m*M*) in absolute tetrahydrofuran (peroxide free, 2.5 ml.), acidified with one drop of glacial acetic acid, was gradually dropped (20 minutes) in an ice cooled mixture of freshly distilled dimethyl sulphate (0.5 ml.), absolute tetrahydrofuran (0.5 ml.) and glacial acetic acid (one drop). The reaction mixture was stirred vigourously by a magnetic stirrer. After ten minutes, crystals of II began to separate. After standing overnight in the ice-box, the quarternary salt was collected as white crystals of indefinite melting point. Yield 98—100%.

2-(5′-Benzyloxyindolyl-3′)-acetonitrile-[1-^{14}C] (III)

5-Benzyloxygramine methosulphate (II, 445 mg., 1.1 m*M*), dissolved in water (CO_2 free, 4 ml.) was placed in a pear-shaped two-necked 50 ml. flask. Sodium cyanide-^{14}C (Tracerlab, 1 mC, 1 m*M* stabilized with 0.75 m*M* natrium hydroxyde in excess) was dissolved in water (3 ml.), neutralized with *N*-sulphuric acid to *p*H 11,9

(the *p*H of 1 *m*M of pure natrium cyanide under the same conditions), and added to the solution of II. The vial was washed with water (3×1 ml.) and added to II. The stopcocks on the flask were fastened with metal springs and the flask immersed in a water bath (70—75°, 2.5 hrs.), with occasional shaking. After allowing the mixture to stand at room temperature (4 hrs.), the stopcocks were opened and the alkaline mixture extracted with anhydrous ether (5×15 ml.) by the aid of a spindle stirrer. The ether extracts were transferred in an Erlenmayer flask through a bent capillary tube, using a low vacuum suction and dried on sodium sulphate *sicc.* The solution was then transferred in the same manner to a round bottomed flask and ether evaporated by the stream of dry nitrogen.

A light-brown viscous oil (248 mg.) remained, which was chromatographed on an alumina column (Riedel de Haën, 10 g.). The column was eluted with benzene (100 ml.). After evaporation of the solvent by dry nitrogen, *2-(5'-benzyloxindolyl-3')-acetonitrile-[1-^{14}C]* (III) as an almost colourless oil (216 mg.) was obtained. Yield 82%, (calc'd on $Na^{14}CN$).

2-(5'-benzyloxyindolyl-3')-ethylamine-[1-^{14}C] (IV)

Reduction of 2-(5'-benzyloxyindolyl-3')-acetonitrile-[1-^{14}C] (III) was carried out with lithium aluminium hydride following in general the procedure given by Amundsen and Nelson[12] for the reduction of aliphatic nitriles to the corresponding amines.

In a three necked flask equipped with a magnetic stirrer, a dropping funnel and a reflux condenser, a solution of lithium aluminium hydride (0.3 g.) in absolute ether (50 ml.) was placed. 2-(5'-benzyloxyindolyl-3')-acetonitrile-[1-^{14}C] (III, 216 mg.), dissolved in absolute ether (3 ml.) was dropped (15 minutes) under vigorous stirring. The funnel was washed with ether (5×1 ml.) and the stirring of the mixture continued for 4 hrs. Water (0.5 ml.) and 10% sodium hydroxyde (0.5 ml.) were added, followed by crude sodium hydroxide (2 g.). Ethereal extracts were transferred as already described in a round bottomed flask and the granular residue washed several times with ether. After the evaporation of the solvent by a stream of dry nitrogen, *2-(5'-benzyloxyindolyl-3')-ethylamine-[1-^{14}C]* (IV) as a colourless very viscous oil (197 mg.) was obtained. Yield 74% (calc'd on $Na^{14}CN$) .

Serotonin creatinine sulphate (2-(5'-hydroxyindolyl-3')-ethylamine-[1-^{14}C] creatinine sulphate) (VI)

2-(5'-Benzyloxyindolyl-3')-ethylamine-[1-^{14}C] (IV, 197 mg., 0.74 m*M*), dissolved in methanol (15 ml.) was catalitically debenzylated at atmospheric pressure and at room temperature in the presence of 10% palladium-on-barium sulphate catalyst (200 mg.). After six hours 18.8 ml. of hydrogen were taken (theoretically 16.4 ml.). To the methanolic solution, obtained on removing the catalyst by centrifugation, one equivalent of *N* sulphuric acid (0.74 ml.) was added. After evaporation of the solvent by dry nitrogen, a heavy gum was obtained, which was dissolved in hot water (4 ml.). Creatinine (125 mg., 0.74 mM), followed by one equivalent of *N* sulphuric acid (0.74 ml.) was added, the mixture heated on a water-bath (50—60°), and acetone (20 ml.) added in portions under shaking. The glittering crystals of *serotonin creatinine sulphate* (VI) begun to separate. After standing overnight in the ice-box, they were centrifugated, washed with acetone, (3×5 ml.) and dried *in vacuo*. The yield of lightly pink crystals was 260 mg., (64% calc'd on $Na^{14}CN$). M. p. 210—213°. Specific activity 1.71 μC/mg. The crude product was purified by the solution in hot water, addition of a small amount of charcoal, filtration and precipitation with acetone. 240 mg. (59%) of analytically pure *serotonin creatinine sulphate* (VI) was obtained. M. p. 211—213°. Specific activity: 1.71 μC/mg, 0.69 mC/m*M*.

One dimensional paper-chromatography gave a single spot with *p*-dimethylaminobenzaldehyde (2% in 2 *N* hydrochloric acid in 80% ethanol) in isopropanol: ammonia : water (10:1:1) (Rf=0.54)[13], as well as in 20% potassium chloride (Rf=0.38)[14].

A continuous record of radioactivity along the same strips was performed on a paper chromatogram scanner, and the radioactive counts drawn on a pen recorder. A single peak of activity was found as shown in Fig. 1.

Anal. 6.725 mg. subst.: 10.283 mg. CO_2, 3.522 mg. H_2O
$C_{14}H_{23}N_5O_7S$ (405.38) calc'd.: C 41.47; H 5.72%
found: C 41.73; H 5.86%

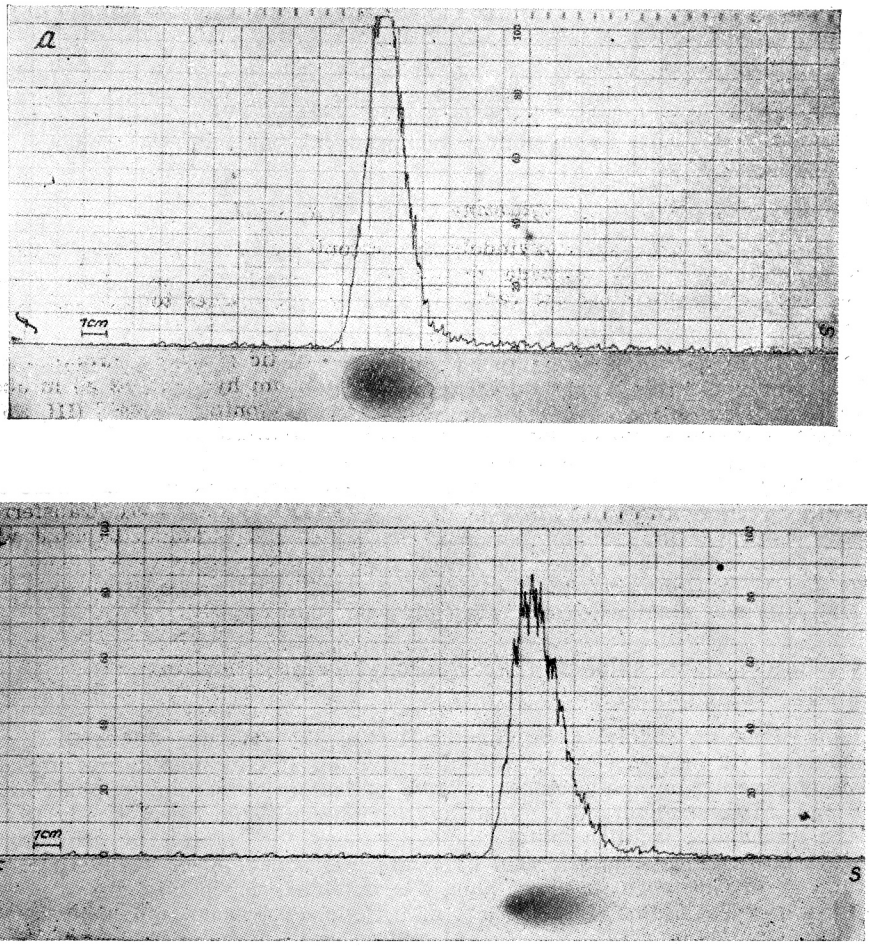


Fig. 1. Automatic record of radioactivity on chromatographic paper strips. a) isopropanol: ammonia: water, b) 20% KCl; s=start, f=front; abscissa: cm ordinate: counts per second.

Determination of radioactivity

The measurements were made using a thin mica window G-M counter. The samples of »infinite thickness« were mounted on 1 cm^2 polythene discs and compared with the ^{14}C Amersham standard of the same size, after corrections for background and selfabsorption were made.

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IZVOD

Sinteza ^{14}C markiranog serotonina (2-(5′-hidroksiindolil-3′)-etilamina-[1-^{14}C])

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Mijenjajući uvjete dosad opisanih sinteza 2-(indolil-3′)-etilamina uspjelo je iz ekvimolarnih količina 5-benziloksigramin metilsulfata (II) i $Na^{14}CN$ dobiti 2-(5′-benziloksiindolil-3′)-acetonitril-[1-^{14}C] (III) s iskorištenjem od 82%. III reduciran sa $LiAlH_4$ dao je 2-(5′benziloksiindolil-3′)-etilamin-[1-^{14}C] (IV). Katalitičkom debenzilacijom IV, dobiven je 2-(5′-hidroksiindolil-3′)-etilamin-[1-^{14}C] (serotonin) (V), koji je izoliran kao serotonin kreatinin sulfat (VI) s 64%-tnim iskorištenjem (računano na $Na^{14}CN$). Specifična aktivnost VI : 1.71 μC/mg, (0.69 mC/m*M*).

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