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Studies in the Sphingolipids Series. XI.***Synthesis of 1-¹⁴C-hydroxy-2-aminoöctadecane
(DL-1-¹⁴C-Sphingine)***D. Keglević, A. Kisić, N. Krvavica-Feretić, and M. Proštenik**Tracer Laboratory and Department of Biochemistry, Institute »Ruđer Bošković«,
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The preparation of a ¹⁴C-labeled sphingine is described. Radioactive carbon was introduced by the treatment of heptadecanoic acid chloride with ¹⁴C-diazomethane, prepared from ¹⁴C-methyl iodide (1 mC). The obtained ketone was converted *via* 1-¹⁴C-acetoxy-octadecan-2-one and 1-¹⁴C-hydroxyoctadecan-2-one oxime into 1-¹⁴C-hydroxy-2-aminoöctadecane (spec. activity: 33.48 μ C/mM). The over-all radiochemical yield based on ¹⁴C-methyl iodide was 12,15%.

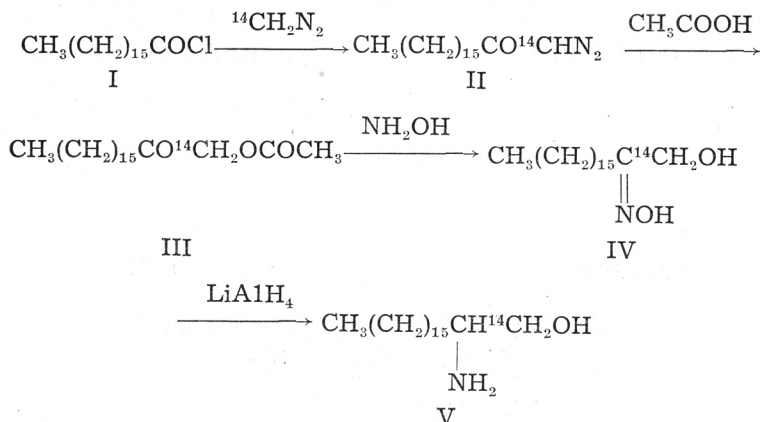
Little is known about the metabolic pathways of sphingolipids, sphingosine and related bases. In order to facilitate the biochemical studies of the latter — carried out in this department — we prepared a sample of sphingine — 1-hydroxy-2-aminoöctadecane (V) — labeled with ¹⁴C.

The present report deals with the synthesis of the radioactive base. The inactive base was described repeatedly as a degradation product of sphingosine¹⁻⁶. The synthesis of both optical antipodes was carried out a few years ago by Sunko and Proštenik⁷. To our knowledge no attempt has been made to incorporate the isotopic tracer atoms *in vitro* into the molecule of sphingosine bases.

We prepared labeled sphingine following essentially the data reported for the inactive compound⁷. This was accomplished by starting with ¹⁴C-methyl iodide through ¹⁴C-diazomethane and heptadecanoic acid chloride (I) according to the scheme I—V.

The over-all yield of the labeled sphingine (V) based on I was 68.6%, which is considerably higher than the reported yield for the inactive base⁷. The difference in specific activities between the calculated value of ¹⁴C-methyl iodide (50 μ C/mM) and the measured value of the first reaction product — ¹⁴C-methyl phthalimide (39.3 μ C/mM) — is probably caused by some decomposition of the ¹⁴C-methyl iodide, which was stored for about one year in this laboratory. The radiochemical yield based on ¹⁴C-methyl iodide was 12.15%.

* Paper X: M. Proštenik and N. Ž. Stanaćev, *Chem. Ber.* **91** (1958) 961.



EXPERIMENTAL

¹⁴C-Diazomethane

The starting material was ¹⁴C-methyl iodide (1 mC, 70 mg.) purchased from the Radiochemical Centre Amersham. It was diluted on vacuum line with inactive methyl iodide to yield 2.84 g. (1.25 ml., 20 mM) and converted *via* ¹⁴C-methyl-phthalimide (39.3 μC/mM) into ¹⁴C-methylamine hydrochloride according to the procedure of Cox and Warne⁸. However, the hydrolysis of ¹⁴C-methylphthalimide by boiling in conc. hydrochloric acid did not yield any results after 4 hrs. — the time quoted by Cox and Warne, — and in the present experiment 35–40 hrs. of boiling was necessary to obtain a quantitative yield of ¹⁴C-methylamine hydrochloride. The obtained ¹⁴C-methylamine hydrochloride was converted by the method of Stoll et al.⁹ *via* *p*-toluene sulphonyl-nitroso-¹⁴C-methylamide (specific radioactivity obtained: 38.87 μC/mM) into ¹⁴C-diazomethane. The overall yield in inactive runs based on methyl iodide, was found to be 64–66% (12.8–13.2 mM).

1-¹⁴C-Diazo-octadecan-2-one (II)

The trial experiments showed that the lowest amount of diazomethane, which still gave a quantitative yield of II, was 2.5 mM per 1 mM of heptadecanoic acid chloride (I). From ¹⁴C-diazomethane (13.2 mM in 150 ml. of dry ether) and 1.55 g. (5.36 mM) I, 1.70 g. of crude II was obtained. M. p. 60.5–63.5°C; specific activity: 32.69 μC/mM; total activity: 188.7 μC. The crude product was used without further purifications.

1-¹⁴C-Acetoxy-octadecan-2-one (III)

1.70 g. ¹⁴C-diazoketone (II) yielded 1.61 g. (92.0%) of III (m. p. 76–78°C) with a specific activity of 32.94 μC/mM.

1-¹⁴C-Hydroxy-octadecan-2-one oxime (IV)

From 1.61 g. (4.93 mM) of III a quantitative yield of crude IV (1.49 g.) was obtained. It was used for further preparation without a radioassay being made.

1-¹⁴C-Hydroxy-2-aminoöctadecane (V)

The crude IV yielded 1-¹⁴C-hydroxy-2-aminoöctadecane, which after one crystallization from petroleum-ether (b. p. 70–100°C) gave white crystals (1.05 g.; 74.7%; m. p. 69–73°C) specific activity: 32.97 μC/mM. Radiochemical yield based on ¹⁴C-methyl iodide: 12.15%. The product was recrystallized three times more from the same solvent until the specific activity 33.48 μC/mM and m. p. 74.5–75.5°C remained unchanged.

Paper chromatograms run in three solvent systems confirmed the purity of V giving a single spot with ninhydrin. *R_f* values: 0.86 (butanol-acetic acid-water, 4:1:5);

0.84 (isopropanol-ammonia-water, 10:1:1); 0.81 (octan-2-ol-lutidin-acetic acid, 8:1:1). In addition, the obtained chromatograms were submitted to a scanning analysis which revealed only one radioactive spot.

Radioactive measurements

The prepared compounds were counted on 1 sq cm polythene discs at infinite thickness using a thin-end-window G-M counter (Tracerlab INC, TGC-2). The specific radioactivities were obtained by comparing corrected sample counts with a *poly*-¹⁴C-methyl methacrylate standard containing 10 μ C/g., furnished by the Radiochemical Centre Amersham.

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IZVOD

Studije u redu sfingolipoida. IX.

Sinteza 1-¹⁴C-hidroksi-2-aminooktadekana (DL-1-¹⁴C-sfingina)

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Opisana je sinteza ¹⁴C-markiranog sfingina. Kao polazna radioaktivna supstanca uzet je ¹⁴C-metiljodid (1 mC), koji je preveden u ¹⁴C-diazometan; njime je onda djelovano na klorid heptadekanske kiseline. Dobiveni keton preveden je u 1-¹⁴C-acetoksi oktadekan-2-on, zatim u odgovarajući oksim, te nakon redukcije s LiAlH₄ u 1-¹⁴C-hidroksi-2-amino oktadekan (sfingin). Dobivena spec. aktivnost: 33.48 μ C/mM. Ukupno radiokemijsko iskorištenje, bazirano na ¹⁴C-metil jodidu iznosi 12.15%.

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