Studies in the Sphingolipids Series. V.* Synthesis of Racemic Dihydrosphingosine Derivatives Starting with DL-Serine**

M. Proštenik and N. Ž. Stanaćev

Department of Chemistry, Medical Faculty, University of Zagreb
and
Department of Biochemistry, Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

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Derivatives of racemic dihydrosphingosine were obtained by condensation of dibenzyl sodiotetradecylmalonate [II] with DL-α-phthaloylamino-β-ethoxypropionic acid chloride [III]. Following new compounds are described: 1. 1-ethoxy-2-phthaloylamino-3-octadecanone [V], m. p. 40—40.5°; 2. 1-ethoxy-2-(N-isoindolinyl-)3-hydroxyoctadecane [VI], m. p. 59—60°; 3. 1-ethoxy-2-phthaloylamino-3-hydroxyoctadecane [VII], m. p. 80—81°. It has been shown in this manner that DL-serine can also be used as starting material in the in vitro formation of the C_{18}-chain of sphingosine bases. A convenient preparation of DL-α-phthaloylamino-β-ethoxypropionic acid and of its chloride [III] is given.

It has been known that the oxidative degradation of sphingomyelin leads *inter alia* to the formation of L-serine.[1] The opinion has also been expressed that L-serine might serve as a precursor in the biosynthesis of sphingosine.[2] The confirmation of this opinion has been given by means of the bioassays with labeled compounds. After administration of carboxyl-labeled acetate[3] methyl-labeled formate and acetate,[4] and labeled serine[5] to rats, tracer containing derivatives of sphingosine could be isolated and detected from the brain and carcass of experimental animals. In such a way it was found that carbon atom 3 and 2 and nitrogen of serine are utilized for carbon atoms 1 and 2 and nitrogen of sphingosine. The remainder of the carbon chain is probably formed from acetate.

It should be noted that a number of syntheses of both racemic forms of dihydrosphingosine and of its derivatives have been published in the course of the past eight years.[6–17]. The racemic base was also resolved into the optically active forms.[10, 15] Moreover a short communication about the synthesis of sphingosine itself appeared recently.[18] However, none of these syntheses made use of serine as a starting material for the polar moiety.

Recently it was shown in our laboratories that α-amino acids could be condensed via the α-phthaloylamino acid chlorides with dibenzyl or ditetrahydropyranyl sodioalkylmalonates into the α-phthaloylamino ketones.[19] The method represents an extension of the Bowman ketone synthesis.[20, 21] When the optically active natural amino acids are used, the opposite i. e. D-confi-

* Paper IV, see reference.[22]

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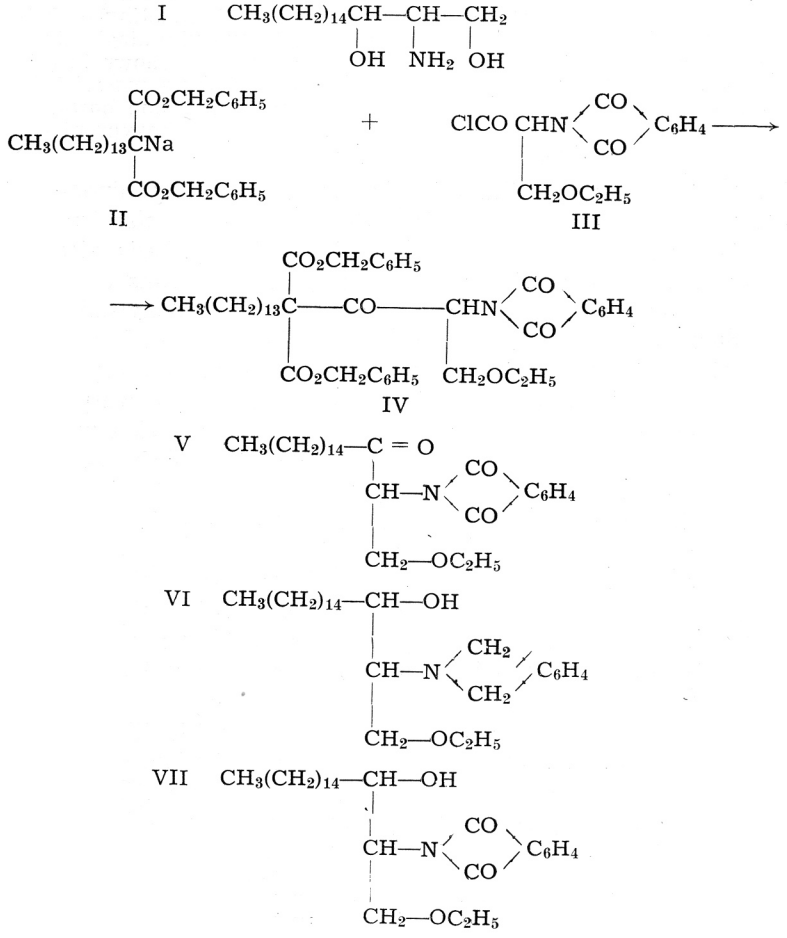
guration around the amino carbon atom should be assigned to the resulting compound. Prošteník, Munk-Weinert and Sunko succeded in this manner to give a synthetic chemical proof for the D-configuration of sphingosine starting with L-alanine.[22]

It seemed to us that the in vitro synthesis of racemic dihydrosphingosine or of its derivatives starting with DL-serine might be of certain interest. The present communication describes the extension of the Bowman ketone synthesis to the DL-α-phthaloylamino-β-ethoxypropionic acid chloride [III]. The latter was brought into reaction with the dibenzyl sodiotetradecylmalonate [II]. By working up the reaction mixture we obtained the DL-1-ethoxy-2-phthaloylamino-3-octadecanone [V], m. p. 40—40.5°, which was easily converted into DL-1-ethoxy-2-(N-isoindolinyl-)-3-hydroxyoctadecanes [VI] when reduced with lithium aluminum hydride. Fractional crystallization of [VI] from ethanol gave a compound which melted sharply at 59—60°, representing probably only one purified racemic form. The reduction of the ketone [V] with sodium



borohydride gave a compound which melted at 80—81° and was identified as 1-ethoxy-2-phthaloylamino-3-hydroxyoctadecane [VII]. The yield of ketone [V] was rather low and might be probably increased by the application of the optically active serine instead of the racemic one. Compounds [V], [VI], and [VII] can be considered as derivatives of racemic dihydrosphingosine.

We did not try to prepare racemic dihydrosphingosine itself by this route. Our intention was just to show, that DL-serine could also be used as a starting material in the in vitro formation of the C_{18}-chain of sphingosine bases.

EXPERIMENTAL

The melting points are uncorrected. All compounds described are racemic.

α-Phthaloylamino-β-ethoxypropionic acid

A. Finely ground α-amino-β-ethoxypropionic acid (1.42 g., 10.6 mMoles) (prepared essentially according to Rinderknecht and Niemann[23]) and phthalic anhydride (1.58 g., 10.6 mMoles) were suspended in toluene (50 ml.) and refluxed with stirring for 2 hr. using the water separator. At the end of the reaction, a clear yellow solution resulted which was evaporated to one third of its volume. On cooling, 2.18 g. (78% yield) colorless prisms, m. p. 135° (recorded m. p. 136—138° [23]) were obtained.

B. Ethyl α-bromo-β-ethoxypropionate (285.2 g.) was hydrolyzed as described previously,[24] the crude acid added to 3 l. of 25% ammonia water and allowed to stand for a week at room temperature. The slightly yellow solution was evaporated in vacuo to dryness yielding 282 g. of yellow product which was suspended in 1200 ml. of toluene. Phthalic anhydride (187.5 g.) was added and the suspension refluxed for 2 hr. using the water separator. After the ammonium bromide was filtered off, the hot brown-colored filtrate was decolorized with charcoal. On cooling the resulting crystals were recrystallized from a mixture of ethanol-water (600 ml.) yielding 199 g. of a product melting at 135°. The over-all yield was 60%, calculated on ethyl α-bromo-β-ethoxypropionate.

α-Phthaloylamino-β-ethoxypropionic acid chloride [III]

The acid (19 g., 72.2 mMoles) and thionyl chloride (50 ml.) were refluxed for 1 hr., the excess of thionyl chloride evaporated in vacuo and finally the last traces were removed by azeotropic distillation with two 30 ml. portions of absolute benzene. The brown, crystalline residue was recrystallized from petroleum ether (70—80°), yielding 18.6 g. (91.5%) colorless needles, m. p. 70—71° (recorded m. p. 70—72° [23]).

1-Ethoxy-2-phthaloylamino-3-octadecanone [V]

The ketone was prepared according to the procedure described earlier.[19] Thus, starting with 4.38 g. of powdered sodium, 91.45 g. of dibenzyl tetradecylmalonate (the preparation of which will be described at a later date) and 53.6 g. of III, 138 g. of brown oil was obtained. The oil was dissolved in 500 ml. of 96% ethanol and debenzylated hydrogenolytically with three 3 g. portions of 10% palladium on barium sulphate catalyst at room temperature and at atmospheric pressure. The yellow filtrate was decarboxylated by boiling it for 4 hrs. The oily residue (94.7 g.) was crystallized from 800 ml. of petroleum ether (80—90°). The separated crystals — a mixture of tetradecylmalonic acid and α-phthaloylamino-β-ethoxypropionic acid — were discarded and the residue (52. g.) dissolved in 200 ml. of benzene and chromatographed on 520 g. of activated alumina (Riedel de Haën). The benzene fractions were collected yielding 9.11 g. (10.5%) of the slightly yellow-colored, crude, oily ketone, which was used in the subsequent reactions without further purification. For analysis the substance was crystallized from absolute ethanol. Colorless leaflets, m. p. 40—40.5°.

Anal. 8.165 mg. subst.: 21.99 mg. CO_2, 6.80 mg. H_2O
7.655 mg. subst.: 0,221 ml. N_2 (26°, 761 mm)
$C_{28}H_{43}O_4N$ (457.63) calc'd: C 73.48; H 9.47; N 3.06%
found: C 73.50; H 9.32; N 3.30%

1-Ethoxy-2-(N-isoindolinyl-)-3-hydroxyoctadecane [VI]

A solution of 200 mg. of [V] in 30 ml. of absolute ether was added dropwise to a suspension of 500 mg. of lithium aluminum hydride in 20 ml. of absolute ether. The mixture was then refluxed for 10 hrs. After addition of a slightly more than the theoretical amount of water, the ether solution was separated by decantation and the solid residue washed with ether. The combined ether extracts gave after evaporation of the solvent 240 mg. of a violet-colored oil, which was crystallized from 2 ml. of 96% ethanol. Thereby 70 mg. (37% yield) colorless prisms, m. p. 59—60°, were obtained. After six crystallizations from ethanol and acetonitrile the melting point was unchanged.

Anal. 8.865 mg. subst.: 25.32 mg. CO_2, 9.34 mg. H_2O
7.300 mg. subst.: 0.211 ml. N_2 (21.2°, 745 mm)
$C_{28}H_{49}O_2N$ (431.68) calc'd: C 77.90; H 11.44; N 3.24%
found: C 77.94; H 11.79; N 3.29%

1-Ethoxy-2-phthaloylamino-3-hydroxyoctadecane [VII]

To a solution of 2 g. of [V] (oily, crude ketone) in 10 ml. of dioxane and 36 ml. of methanol, 500 mg. of sodium borohydride was added. The reaction mixture was left to stand at room temperature for 24 hr. and then poured into 50 ml. of water. The resulting emulsion was extracted with ether, the extracts dried with sodium sulphate and evaporated to dryness leaving 2 g. of an oily residue which was dissolved in benzene and chromatographed on 30 g. of activated alumine (Riedel de Haën). Only ether fractions were crystalline. Recrystallizations from 96% ethanol gave 55 mg. colorless prisms m. p. 80—81°.

Anal. 9.205 mg. subst.: 24.63 mg. CO_2, 8.58 mg. H_2O
7.905 mg. subst.: 0.214 ml. N_2 (21.5°, 746 mm)
$C_{28}H_{45}O_4N$ (459.65) calc'd: C 73.16; H 9.87; N 3.05%
found: C 73.02; H 10.43; N 3.08%

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IZVOD

Studije u redu sfingolipoida V.
Sinteza derivata racemičkog dihidrosfingosina polazeći od DL-serina

M. Prošteník i N. Ž. Stanaćev

Neki derivati racemičkog dihidrosfingosina [I] priređeni su kondenzacijom dibenzilnog estera tetradecilmalonske kiseline [II] s kloridom DL-α-ftaloilamino-β-etoksi-propionske kiseline [III]. Pored dobivanja α-ftaloilamino-β-etoksi-propionske kiseline i njezina klorida opisane su i ove tvari: 1. 1-Etoksi-2-ftaloilamino-3-oktadekanon [V], t. t. 40—40.5°; 2. 1-etoksi-2-(N-isoindolinil-)-3-hidroksi-oktadekan [VI], t. t. 59—60°; 3. 1-etoksi-2-ftaloilamino-3-hidroksi-oktadekan [VII], t. t. 80—81°. Na taj je način pokazano, da i DL-serin može poslužiti pri in vitro sintezi C_{18} lanca sfingosinskih baza.

MEDICINSKI FAKULTET
INSTITUT ZA PRIMIJENJENU KEMIJU
I
INSTITUT »RUĐER BOŠKOVIĆ«
BIOKEMIJSKI ODJEL
ZAGREB

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