Chemistry and Physics of Lipids 14 (1975) 59–71

SYNTHESIS OF *threo*-4,5-DIHYDROXY DIASTEREOMERS OF SPHINGANINE*

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Received May 31, 1974, accepted September 23, 1974

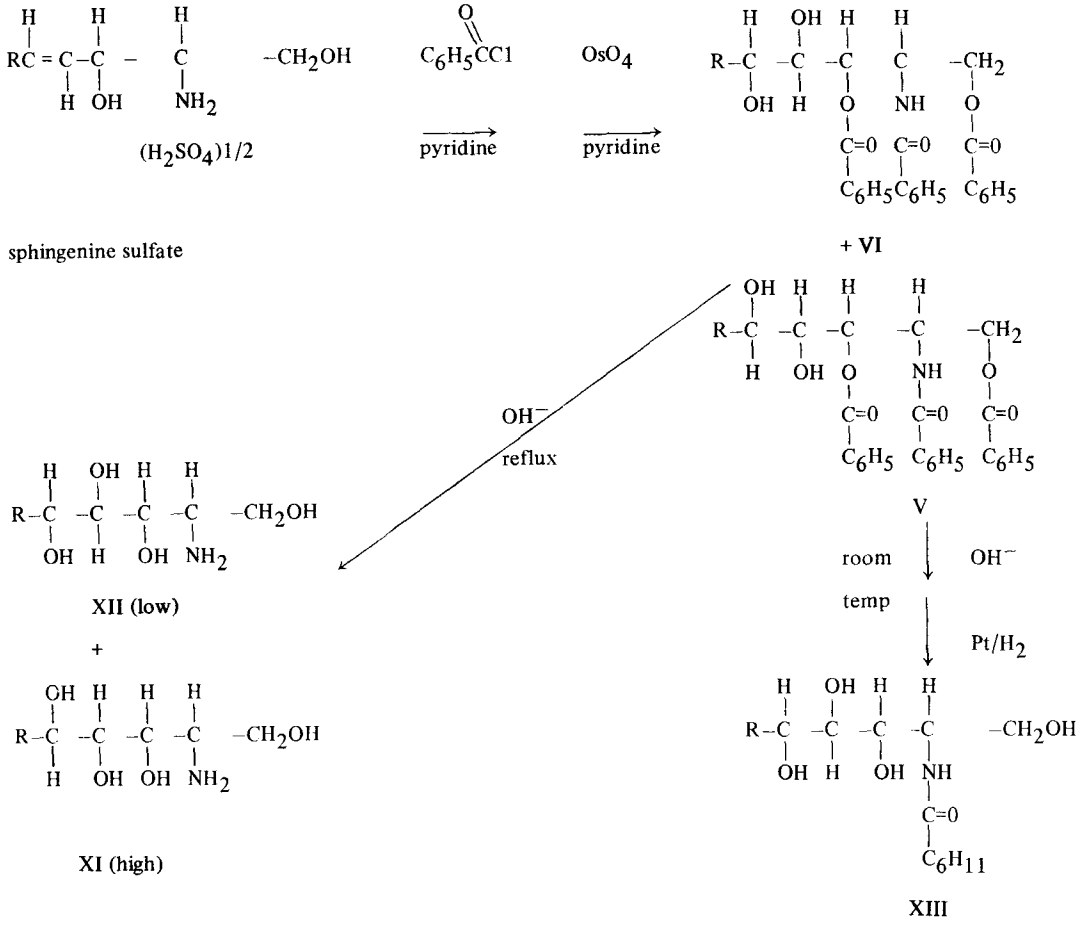
The *threo*-4,5-dihydroxy diastereomers of sphinganine were prepared by the following sequence of reactions: (a) benzoylation of sphingenine to the tribenzoyl derivative; (b) osmylation followed by resolution of the mixture of *threo*-4,5-dihydroxy tribenzoyl (DHTBS) diastereomers and (c) alkaline hydrolysis to yield the *threo*-4,5-dihydroxysphinganines (DHS). Carbon atoms 4 and 5 of the high and low melting *threo*-4,5-DHTBS diastereomers and the compounds derived from them were tentatively assigned 4R, 5S and 4S, 5R configurations, respectively.

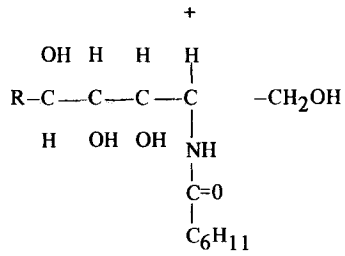
I. Introduction

In our studies on the synthesis of potential metabolic antagonists of long-chain bases and their complex lipids, the preparation of the four possible 4,5-dihydroxy diastereomers of sphingenine, D-erythro-1,3-dihydroxy-2-amino-*trans*-4-octadecene [1–3], was undertaken. Osmylation and epoxidation, both of which proceed by *cis* addition, should give in the former reaction two diastereomers with their hydroxyl groups *threo* to each other, whereas the two diastereomers obtained in the latter reaction would have their hydroxyl groups erythro due to the inversion which occurs upon scission of the oxirane ring. The synthesis of the *threo*-4,5-dihydroxy diastereomers as well as related compounds are herein described.

Various agents for blocking the functional groups of sphingenine and whose removal could be effected under mild conditions were examined. The N–9 fluorenylmethyloxycarbonyl [4] -1,3-diacetyl derivative of sphingenine was prepared but its further use was abandoned because of undesirable physical characteristics and purification difficulties. The N-carbobenzoxy-1,3-diacetyl derivative was satisfactory but complete separation of the *threo*-4,5-dihydroxy diastereomers could not be effected. In addition, yields of the free base were unsatisfactory after treatment of the pure or mixture of diastereomers under a variety of conditions ranging from hydro-

* This study was supported in part by Public Health Research Grant NB 06300-06 from the National Institute of Neurological Diseases and Stroke.


–CH2OH
C6H5CC1
OsO4
(H2SO4)1/2
pyridine
pyridine
sphingenine sulfate
+ VI
OH–
reflux
V
room temp
OH–
Pt/H2
XII (low)
+
XI (high)
XIII
R = CH3(CH2)12–



XIV

Fig. 1. Sphingenine sulfate was taken through the above sequence of reactions to yield the *threo*-4,5-dihydroxysphinganines, compounds XI and XII. The mixture of *threo*-4,5-dihydroxy tribenzoyl diastereomers, compounds V and VI, obtained after osmylation of tribenzoylsphingenine, was resolved at this stage prior to the formation of succeeding compounds. Assignment of configurations of carbon atoms 4 and 5 of compounds XI and XII are tentative. See text for details.

genolysis of the carbobenzoxy group in acidic ethanol followed by deacetylation with dilute alkali which is accompanied by $O \rightarrow N$ acyl migration or cleavage of the acetyl groups with catalytic amounts of sodium methoxide [5] and subsequent removal of the N-blocking group. In all cases, ninhydrin positive complex mixtures were obtained. Tribenzoyl sphingenine (TBS) proved to be the best intermediate (fig. 1); osmylation, followed by resolution of the mixture of *threo*-4,5-DHTBS diastereomers and alkaline hydrolysis under reflux yielded the *threo*-4,5-DHSs. Mild alkaline hydrolysis gave the *threo*-4,5-dihydroxy N-benzoylsphinganines which, upon hydrogenation over platinum, yielded crystalline *threo*-4,5-dihydroxy-N-cyclohexylcarbonyl derivatives (fig. 1, XIII, XIV). In all instances, complex mixtures, as revealed by tlc, were obtained after alkaline hydrolysis under either mild or reflux conditions; column chromatography was required for purification. Attempts to employ a milder method for removal of the N-benzoyl group by reduction with $LiAlH_4$ followed by hydrogenolysis over palladium were unsuccessful. Under a variety of conditions the benzamido or cyclohexamido group could not be reduced by $LiAlH_4$ to the amine which was attributed probably to intermediate oxazolidine formation with the adjacent primary or secondary hydroxyl group; the products were recovered unchanged. Shapiro et al. [6] reported earlier that N-benzoyl and other amides of 1-β-D-galactopyranosyl sphinganine could not be reduced by $LiAlH_4$. It is of interest that the *trans*-4,5-epoxides of TBS are smoothly reduced with this reagent to the N-benzyl-1,3,4-trihydroxy compounds [7]. Treatment of the *threo*-4,5-DHTBSs under reflux for 8 hr with 2N HCl in 90% methanol failed to yield the free amine, a procedure that is frequently used to liberate long-chain bases from naturally occurring complex lipids [8]; similar refractory behavior to acid hydrolysis was observed for TBS [9].

Confirmation of the presence of hydroxyl groups on carbon atoms 4 and 5 of the DHTBSs was obtained by alkaline hydrolysis followed successively by periodate oxidation of the isolated long-chain bases, sodium borohydride reduction of the resultant aldehydes, formation of the trimethyl silyl (TMS) derivatives and analysis by gc which disclosed the presence of tetradecanol. The DHTBSs formed non-volatile TMS compounds. In addition, under the conditions employed the DHTBSs were not susceptible to periodate oxidation, perhaps due to ortho ester formation, because little or no tetradecanol was observed after treatment with sodium borohydride. However, periodate–permanganate oxidation [10] did yield, although not quantitatively, tetradecanoic acid. Infrared spectra showed that the *trans* double bond of TBS at 975 cm^{-1} was absent from the hydroxylated product.

In treatment of the DHTBSs with dilute alkali to produce the *threo*-4,5-dihydroxy N-benzoyl sphinganines, side products, compounds IX and X, which were insoluble in cold acetonitrile were formed. Since they gave the same elementary analysis as the *threo*-4,5-dihydroxy N-benzoylsphinganines, it was tentatively assumed that the compounds obtained had arisen by inversion of one or more of their hydroxyl groups. No evidence was found for dehydration or cyclization in any of the compounds isolated.

It was hoped that the configurations of the newly formed asymmetric centers at

carbon atoms 4 and 5 could be established by comparison of the direction of optical rotations of the *threo*-4,5-DHSs with the rotations of their corresponding carbohydrate analogues, 2-amino-2,6-dideoxy-D-gulitol and 2-amino-2,6-dideoxy-L-talitol. Unfortunately, no comparison of rotations was possible because these amino sugars were not available. Therefore, proof of configuration was sought by the following alternative procedure: (a) methylation of the high and low melting DHTBSs to the 4,5-dimethoxy derivatives; (b) alkaline hydrolysis; (c) periodate oxidation; and (d) sodium borohydride reduction. The desired comparison of the rotations of the resulting 1-hydroxy-2,3-dimethoxy hexadecanes with similar short or long chain triols was again not possible because of the unavailability of the latter compounds. Since the derived triols are deoxy analogues of D- and L-threitol, it was *tentatively* concluded from a comparison of specific rotations (table 1) that the triols obtained from the high and low melting DHTBSs corresponded to the D- and L-configurations, respectively (threitol [11, 12], D-[α] + 4.3; L-[α] − 4.4; triols, high [α] + 16.4, low [α] − 10.2). Therefore, the hydroxyl group on carbon atom 4 of the high and low melting diastereomers would be *erythro* and *threo,* respectively, to the functional groups on carbon atoms 2 and 3. The overall configurations for the high and low melters thus would be 2S, 3R, 4R, 5S and 2S, 3R, 4S, 5R, respectively (fig. 1, XI and XII).

It was thought from the present investigation that a stereospecific synthesis of 4,5-ditritio (^{3}H) sphinganine could be developed in order to study the stereochemical course of formation in brain of the *trans* double bond in sphingenine (13). It was hoped that mesylation of either *threo*-4,5-DHTBS diastereomer to the 4,5-dimesyl derivative followed by treatment with lithium aluminum tritide and debenzylation over palladium would yield 4,5-ditritiosphinganine with tritium of opposite configuration, due to inversion, to that of the leaving groups. However, this effort was abandoned when reaction of either *threo*-4,5-dimesyloxy TBS with $LiAlH_4$ failed to yield the desired N-benzylsphinganine but gave consistently instead a compound with about 2% sulfur, mp 107–108°C which was not further characterized.

In the preparation of the dimesyloxy derivative of the high melting DHTBS about 10 to 15% of tribenzoylsphinganine was isolated from the reaction mixture. Although sphinganine is normally present to the extent of a few percent in sphingenine isolated from natural sources, the tribenzoylsphinganine found was newly formed, probably by reductive elimination, because tlc disclosed that the compound was absent from both high and low melting DHTBSs. Further proof of identity of the tribenzoylsphinganine was obtained by mild hydrolysis to the benzamide and hydrogenation to the cyclohexamide.

Efforts were made to obtain the remaining pair of 4,5-dihydroxy diastereomers of sphinganine, in which both hydroxyl groups are either *erythro* or *threo* to the functional groups on carbon atoms 2 and 3, by acid-catalyzed opening [14] of the diastereomeric mixture of *trans*-4,5-epoxides of TBS [7]. Treatment with glacial acetic or methane sulfonic acids under a variety of conditions yielded a sirup consisting of a mixture of hydroxy–acyloxy diastereomers. Attempts to resolve this mixture after mild alkaline hydrolysis were unseccessful.

Table 1

	$[\alpha]_D^{21}$	
	High melter[a]	Low melter[a]
threo-4,5-Dihydroxytribenzoylsphinganine	−34.2	−28.0
threo-4,5-Dihydroxy N-benzoyl sphinganine	+ 2.8	+20.1
threo-4,5-Dihydroxysphinganine	− 3.0	+ 0.6
Compounds IX and X[a]	+16.6	+24.6
1-Hydroxy-2,3-dimethoxyhexadecane[b]	+16.4	−10.2

[a] Rotations (C, 1% in chloroform : methanol (1 : 1)) were performed on the high and low melting *threo*-4,5-dihydroxy tribenzoylsphinganines and on the products obtained therefrom.
[b] Since the specific rotations are not equally opposite, impurity may be present in one or both of the enantiomers. See text for details.

In a study of the opening of the oxirane ring, the *trans*-4,5-epoxides of TBS were reacted with benzylamine to give the corresponding deesterified hydroxy benzylamino derivatives. Debenzylation followed successively by N-benzoylation and periodate oxidation which yielded 2-benzamidopentadecanal established that the oxirane ring was attacked at carbon atom 5 and that the initial compounds formed were a diastereomeric mixture of 4-hydroxy-5-benzylamino-TBSs.

II. Experimental

A. Materials and methods

Osmium tetroxide was purchased from Ventron. Bovine spinal cord sphingolipids were a gift from Dr. H.E. Carter. Bovine brain sphingolipids were isolated as described previously [9]. Normal alcohols and fatty acids as standards for glc were obtained from Applied Science and Supelco. Diazald, 9-fluorenylmethoxycarbonyl chloride and m-chloroperbenzoic acid were purchased from Aldrich.

Elementary analyses were done by Schwartzkopf Microanalytical Laboratory. Infrared spectra were obtained on a Perkin-Elmer 567 Infrared Spectrophotometer on KBr discs and chloroform solutions; the polyhydroxy compounds, insoluble in chloroform, gave opaque discs at concentrations of 0.5 mg sample/400 mg KBr and fair spectra. Mass spectroscopic analyses were performed by Mr. V. Saltamach, Columbia University. Ascending tlc on silica gel G with solvent systems chloroform–methanol–H_2O (100–42–6), chloroform–methanol–conc. NH_4OH (65–35–5) and petroleum ether (Skellysolve B)–ether–glacial acetic acid (60–40–4) were used to examine the purity of compounds, as well as the monitoring of fractions obtained by cc. Components were detected after separation by iodine vapor or Rhodamine G under ultraviolet light. Glc was done on a Perkin-Elmer 881 equipped with flame ionization detector. A 6.0 ft × 0.25 in i.d. glass column packed with 3 percent SE-30

on gas chrom Q was used for the determination of long-chain alcohols and fatty acids; the nitrogen flow rate was 15 ml/min and the injector and detector temperatures were 240°C; column temperatures were 165°C. Alcohols were treated with a silylating reagent [15] and determined as TMS derivatives. Fatty acids were converted to methyl esters with diazomethane prepared from Diazald prior to gc.

B. Isolation of sphingenine

Sphingenine was isolated from bovine brain sphingolipids by a modification involving fewer steps of a previously described procedure [9]. A solution of 2 liters of methanol containing 180 ml conc. HCl and 100 g of crude sphingolipids was refluxed in a 6 liter Erlenmeyer flask, equipped with a cold finger and previously flushed for 15 min with nitrogen, by immersion in a 72–74°C water bath. At the end of 5 hr a stream of nitrogen was passed over the surface of the reaction mixture while cooling to room temperature. Fatty acids and their esters were removed with 5 X 600 ml portions of petroleum ether (Skellysolve B) (discarded). After chilling the lower phase in an ice bath, approximately 500 ml of cold aqueous 4 N KOH were added followed by 100 ml of 1 N $NaHCO_3$ to pH 7.0 – 8.0. The free bases were removed by addition of 3 X 400 ml portions of chloroform without mixing and 2 X 400 ml portions with mixing of the phases. The combined chloroform extracts were washed successively with 200 ml of water and with 300 ml of water : methanol (1 : 1). The chloroform solution was concentrated to a sirup (not to dryness) below 50°C on a water pump and the dark brown sirup was dried to a gel over phosphorus pentoxide. After dissolving the residue in 100 ml of ethanol, the bases were precipitated by dropwise addition of freshly prepared 1 N ethanolic sulfuric acid to pH 2.5 – 3.0 with the Beckman Zeromatic II pH meter equipped with temperature compensator and a Futura pH combination electrode for routine and non-aqueous titrations. The precipitate, removed from the chilled solution, was suspended in 200 ml of hot methanol. The cooled solution was filtered with suction and the precipitate was successively washed with cold methanol and acetone; yield of crude sphingenine sulfate containing less than 3% of O-methyl compounds, 12–15 g. Attempts to improve the yield and quality of base by scaling up the periodic acid oxidation–sodium borohydride reduction procedure of Carter et al. [16] from the original 1.0 g of sphingolipids to 100 g were unsuccessful.

C. N-9-Fluorenylmethoxycarbonylsphingenine (I)

To 7.0 g of crude sphingenine sulfate in 100 ml of ether, 20 ml of p-dioxane and 50 ml of 10% Na_2CO_3, surrounded by an ice bath, was added 5.2 g of 9-fluorenylmethoxycarbonyl chloride in 100 ml of ether. After magnetic stirring for 1 hr at room temperature, 100 ml of ethyl acetate was added. The organic layer was washed several times with water and concentrated to dryness. The residue in 100 ml of methanol was treated with Norite A, heated to boiling, filtered and concentrated. The

dried wax, dissolved in chloroform, was applied to a 40 g silicic acid column, 2.5 × 50 cm, which was developed with 125 ml of chloroform. After concentration of the eluate, the residue was crystallized from 200 ml of petroleum ether. Yield 4.7 g; mp 75–78°C.

Anal. Calcd for $C_{33}H_{47}O_4N$ (521.4): C, 75.95; H, 9.08. Fd: C, 76.15; H, 9.19.

D. N-9-Fluorenylmethoxycarbonyl-1,3-diacetylsphingenine

To 4.0 g of compound I in 75 ml of pyridine, surrounded by an ice bath, was added 4 ml of acetic anhydride. After standing overnight at 5°C, the reaction mixture was poured into 200 ml of ice-H_2O and the product was removed with 2 × 200 ml portions of ethyl acetate : ether (1 : 1). After concentration of the water washed organic layer, the residue was crystallized from petroleum ether. Yield 2.6 g; mp 64–66°C.

Anal. Calcd for $C_{37}H_{51}O_6N$ (605.4): C, 73.33; H, 8.49. Fd: C, 73.51; H, 8.60.

E. N-Carbobenzoxy-1,3-diacetylsphingenine (II)

To 8.7 g of N-carbobenzoxysphingenine [17] in 100 ml of chilled pyridine was added 5 ml of acetic anhydride. After standing overnight at 5°C, the reaction mixture was poured into ice-water. The flocculent precipitate was washed with cold water on the filter, dried over phosphorus pentoxide and crystallized from 200 ml petroleum ether. Yield 6.95; mp 73–75°C.

Anal. Calcd for $C_{30}H_{47}O_6N$ (516.3): C, 69.58; H, 9.15. Fd: C, 69.75; H, 9.27.

F. Diastereomeric threo-4,5-dihydroxy-N-carbobenzoxy-1,3-diacetylsphinganines (III)

Four 1.0 g vials of osmium tetroxide were washed with acetone, dried, scored and the halves of each vial were added to 50 ml of pyridine containing 7.40 g of compound II. After 48 hr at room temperatures with intermittent swirling of the reaction mixture, 10.8 g of $NaHSO_3$ in 180 ml of water and 120 ml of pyridine were added. The mixture was stirred for 4 hr followed by the addition of 100 ml of ice-water and 300 ml of ethyl acetate. The upper phase was washed with water until colorless; the wax obtained after removal of solvent was dried and crystallized from acetonitrile. Yield 0.65 g; mp 126–127°C.

Anal. Calcd for $C_{30}H_{49}O_8N$ (551.4): C, 65.28; H, 8.95. Fd: C, 65.48; H, 9.01.

A sirup still consisting of both diastereomers and which slowly deposited crystals, mp 126–127°C, while standing at room temperature was obtained after concentration of the acetonitrile filtrate. Yield 5.9 g.

Anal. Calcd for $C_{30}H_{49}O_8N$ (551.4): C, 65.28; H, 8.95. Fd: C, 65.32; H, 9.02.

G. Tribenzoylsphingenine (IV)

To a suspension of 10.5 g of crude sphingenine sulfate in 100 ml of dry pyridine, surrounded by an ice bath, was added over several minutes 17 ml of benzoyl chloride. The remainder of the procedure was the same as described previously [9]. Yield 7.9 g; mp 121–123°C.

H. Diastereomeric threo-4,5-dihydroxy tribenzoylsphinganines

To 9.4 g of compound IV in 100 ml of pyridine was added four 1.0 g vials of osmium tetroxide. The remaining procedure was the same as that described for preparation of compounds III.

I. Resolution of diastereomers. High melter (V)

The dried mixture of diastereomers obtained after concentration of the organic phase was dissolved in 50 ml of hot ethanol; the precipitate from the centrifuged chilled solution was resuspended in 25 ml of ethanol at room temperature. After centrifugation the precipitate was successively rewashed with ethanol and twice with cold petroleum ether. The precipitate dissolved in chloroform was applied to a 20 g silicic acid column and developed with 150 ml of chloroform. After concentration of the eluate to dryness the residue was crystallized successively from 40 ml each of ethanol and acetonitrile. Yield 5.6 g; mp 123–124°C. The infrared band at 975 cm^{-1} (*trans* double bond) was absent.

Anal. Calcd for $C_{39}H_{51}O_7N$ (645.4): C, 72.51; H, 7.97. Active H, 0.45. Fd; C, 72.36; H, 7.99; Active H, 0.41.

J. Low Melter (VI)

The combined ethanol and petroleum ether supernates from compound V were concentrated; the dried residue was chromatographed on silicic acid in the same manner as was described for compound V. The product from the chloroform eluate was crystallized with chilling from 20 ml of acetonitrile. Yield 2.3 g; mp 117–118°C. The infrared spectrum was identical to that of compound V.

Anal. Calcd for $C_{39}H_{51}O_7N$ (645.4): C, 72.51; H; 7.97; Active H, 0.45. Fd: C, 72.34, H, 7.97; Active H, 0.44.

K. Analysis of cleavage products

Compound V or VI, 2 mg, in 3 ml of 2 N KOH in 90% methanol contained in a 12 ml teflon lined screw cap centrifuge tube was held 8 hr in a heating block at 75°C. After addition of an equal volume of water, the base was removed with ethyl acetate : ether (1 : 1). The solvent was evaporated under a stream of nitrogen and

the residue was subjected to periodic acid oxidation–sodium borohydride reduction [8]. Tetradecanol was identified as the TMS derivative by gc.

L. Diastereomeric threo-4,5-dihydroxy-N-benzoyl sphinganines (high VIII, low VII)

Compound V or VI, 1.0 g in 12.0 ml of ethanol, 1.5 ml of H_2O and 0.75 ml of 5N NaOH was gently warmed until the reaction mixture cleared. After standing overnight at room temperature, the slight precipitate was removed by centrifugation and discarded. The supernate was poured into 60 ml of 1 N NaOH and the product was recovered with ethyl acetate : ether (1 : 1). After washing with water and evaporation of solvent, the dried residue was crystallized from acetonitrile, mp 121–139°C. The crude product in warm chloroform was applied to an 8.0 g silicic acid column. The product was maintained in solution during entry into the column by means of a heating tape. After passage of 100 ml of chloroform, the compound was eluted from the column with 100 ml of 2% methanol in chloroform. The eluate was concentrated and the residue was crystallized from ethanol or acetonitrile. Yield from compound V 316 mg; mp 137–138°C; tlc, 1 component. Yield from compound VI 305 mg; mp 137–138°C; tlc, 1 component.

Anal. Calcd for $C_{25}H_{43}O_5N$ (437.3): C, 68.60; H, 9.91. Compound VII, Fd; C, 68.69; H, 10.01. Compound VIII, Fd: C, 68.58; H, 9.89.

The acetonitrile filtrate from each diastereomer was chilled. Yield of compound IX from compound VII 185 mg; mp 89–90°C; tlc, 1 component. Yield of compound X from compound VII 193 mg; mp 88–89°C; tlc, 1 component. The infrared spectra were similar to those from compounds VII and VIII.

Anal. Calcd for $C_{25}H_{43}O_5N$ (437.3): C, 68.60; H, 9.91. Compound IX, Fd: C, 68.74; H, 9.88. Compound X, Fd: C, 68.76; H, 9.98.

M. Diastereomeric threo-4,5-dihydroxy N-cyclohexylcarbonylsphinganines (high XIV, low XIII)

Either diastereomer VII or VIII, 200 mg, in 25 ml of ethanol containing 50 mg of platinum oxide was hydrogenated at 45°C for 5 hr. The catalyst was washed with hot ethanol after filtration of the hot solution and the combined filtrate and washings were concentrated to dryness. The residue was crystallized from ethanol. Yield of XII from compound VII 130 mg; mp 169–170°C; tlc, 1 component. Yield of XIV from compound VIII 119 mg; mp 170–171°C; tlc, 1 component; mass spectrum (high, 30 eV, 170°C) *m/e* (rel. intensity) 170 (100), 183 (100), 200 (100), 213 (100), 230 (100) 243 (100), 412 (42) and 425 (21).

Anal. Calcd for $C_{25}H_{49}O_5N$ (443.4): C, 67.66; H, 11.13. High, Fd: C, 67.83; H, 11.07. Low, Fd: C, 67.71; H, 11.15.

Compound IX, 150 mg, was hydrogenated in the same manner as was used for compound VII. The product was crystallized from acetonitrile. Yield 104 mg; mp 97–100°C.

Anal. Calcd for $C_{25}H_{49}O_5N$ (443.4): C, 67.66; H, 11.13. Fd: 67.81; H, 11.36.

N. Diastereomeric threo-4,5-dihydroxysphinganines (high XI, low XII)

Compound V or VI, 1.5 g, in 45 ml of methanol and 6 ml of 5 N NaOH was refluxed 4 hr. After cooling to room temperature and addition of an equal volume of water, the product was removed with ethyl acetate : ether (1 : 1). The organic layer was washed with water, concentrated and the dried residue dissolved in chloroform was loaded onto an 8.0 g silicic acid column which was successively developed with 100 ml portions of 8-, 20- and 40% solutions of methanol in chloroform. The 20 and 40% eluates (8% was discarded) were combined, concentrated and the residue was crystallized by dissolving in 3 ml of hot ethanol followed by the addition of 30 ml of hot acetonitrile. Yield of XI (fig. 1) from compound V 315 mg; mp 126–128°C; ninhydrin positive; TLC, 1 major component with trace of minor component. Yield of XII from compound VI 280 mg; mp 126–128°C; ninhydrin positive; TLC, 1 major component with trace of minor component.

Anal. Calcd for $C_{18}H_{39}O_4N$ (333.3): C, 64.80; H, 11.79, High, Fd: C, 64.71; H, 11.73. Low, Fd: C, 64.63; H, 11.81.

O. Diastereomeric 4,5-dimesyloxy tribenzoylsphinganines (high, low)

To 2.6 g of compound V or VI in 60 ml of pyridine, surrounded by an ice bath, was added 1.2 g of mesyl chloride. After standing overnight at 5°C, the reaction mixture was poured into ice-H_2O. The product was removed with ethyl acetate: ether, washed with water and concentrated. The dried residue (wax) from compound V was dissolved in hot acetonitrile; the precipitate from the centrifuged chilled solution was recrystallized from the same solvent. Yield of tribenzoylsphinganine 303 mg; mp 142–143°C; mass spectrum (75 eV, 120°C) *m/e* (rel. intensity) 268 (100), 357 (100), 369 (100), 370 (100), 371 (26) and 386 (45).

Anal. Calcd for $C_{39}H_{51}O_5N$ (613.4); C, 76,31; H, 8.38. Fd: C, 76.08; H, 8.44.

Tribenzoylsphinganine, 120 mg, subjected to mild alkaline hydrolysis in the manner as used for the preparation of compound VII gave N-benzoylsphinganine. Yield 52 mg; mp 118–119°C. Hydrogenation of this compound yielded N-cyclohexamidosphinganine, mp 128–129°.

Anal. Calcd for $C_{25}H_{49}O_3N$ (411.4): C, 72.92; H, 12.00. Fd: 72.79; H, 11.97.

The acetonitrile supernates from compound V were combined and concentrated. The dried sirup and the sirup from compound VI, each was dissolved in benzene and applied to 20 g silicic acid columns which were packed from chloroform and washed with 1 column volume of benzene. The columns were developed with 200 ml of benzene (discarded) and 200 ml of benzene : chloroform (1 : 1) which upon concentration gave in each case a light yellow sirup. Yield from compound V 1.70 g; yield from compound VI 1.56 g.

Anal. Calcd for $C_{41}H_{55}NO_{11}S_2$ (801.3): C, 61.38; H, 6.92; S, 7.99. High, Fd: C, 61.23; H, 6.94; S, 7.86. Low, Fd: C, 61.69; H, 6.92; S, 8.00.

P. Degradation of compounds V and VI to 1-hydroxy-2,3-dimethoxyhexadecanes (high, low)

Compound V or VI, 1.0 g, in 25 ml of dimethylformamide, 4 ml of CH_3I and 4.0 g Ag_2O was magnetically stirred in the dark for 8 hr at room temperature. After centrifugation of the reaction mixture, the precipitate was washed with centrifugation 3 X 25 ml portions of chloroform. Ether, 250 ml, was added to the combined supernate and washings which were successively filtered, washed with 25 ml of 5% KCN, 2 X 50 ml portions of water, 25 ml of 5% $Na_2S_2O_3$ and 3 X 75 ml portions of water. After drying over Na_2SO_4, the solution was concentrated to a clear colorless sirup which was dissolved in 45 ml of methanol containing 6 ml of 5 N NaOH. An equal volume of water was added to the solution after refluxing for 4 hr. The combined extracts, after treatment with 3 X 100 ml portions of ethyl acetate : ether (1 : 1), were washed with water, dried over Na_2SO_4 and concentrated. The clear colorless sirup in 20 ml of methanol and 2 ml of water containing 900 mg of periodic acid was treated, after standing overnight and the addition of 4 ml of water, with 4 X 25 ml portions of petroleum ether. The combined extracts were washed with water, filtered and concentrated; the residue was dissolved in 15 ml of methanol to which was added 200 mg of $NaBH_4$ in 2 ml of 0.1 N NaOH. After standing overnight and the addition of 1 N HCl to about pH 6.0, the reaction mixture was treated with 3 X 25 ml portions of ethyl acetate. The combined extracts were washed successively with water, 5% $Na_2S_2O_3$, water, dried over Na_2SO_4 and concentrated. Yield (high, sirup) from compound V, 99.3 mg; yield (low, sirup) from compound VI, 100.6 mg.

Anal. Calcd for $C_{18}H_{38}O_3$ (302.3): C, 71.45; H, 12.67; OCH_3, 20.52. High, Fd; C, 70.51; H, 12.77; OCH_3, 19.96. Low, Fd: C, 72.69; H, 12.49; OCH_3, 19.81.

Q. Diastereomeric 4-hydroxy-5-benzylamino-2-benzamidosphinganines (XV)

Diastereomeric *trans*-4,5-epoxides of TBS, 4.0 g, prepared as previously described with perbenzoic [7] or m-chloroperbenzoic acids, in 25 ml each of dimethylformide and benzylamine were heated at 110°C for 48 hr. The reaction mixture was concentrated under reduced pressure at 90°C and the solid residue was crystallized successively from 30 ml of 95% ethanol and 2 X 15 ml of ethanol. Yield 788 mg; mp 165–166°C.

Anal. Calcd for $C_{32}H_{50}N_2O_4$ (526.4): C, 72.94; H, 9.57; N, 5.31. Fd: C, 73.15; H, 9.41; N. 5.49.

R. Diastereomeric-2,5-dibenzamido-4-hydroxy sphinganines (XVI)

Compound XV, 700 mg, in 100 ml of ethanol: ethyl acetate (1 : 1) containing 125 mg of Pd on carbon, was hydrogenated at room temperature for 5 hr under slightly greater than atmospheric pressure with magnetic stirring. After filtration of

the warmed reaction mixture and concentration, the residue was crystallized from 75 ml of acetonitrile. Yield 450 mg; mp 126–138°C; ninhydrin positive. The crude product, 200 mg, in 8 ml of pyridine and 0.3 ml of benzoyl chloride, after 2 hr was poured into 25 ml of ice-H_2O. Ethyl acetate : ether (1 : 1), 100 ml, was added and, after washing with water, the solvent was removed; the residue in 10 ml of methanol was treated with Norite A, filtered and concentrated to one half its initial volume. Yield 121 mg; mp 170–171°C, ninhydrin negative.

Anal. Calcd for $C_{32}H_{48}N_2O_5$ (540.4): C, 71.06; H, 8.95. Fd: C, 71.62; H, 8.72.

Compound XVI, 75 mg, in 9 ml of ethanol and 1.5 ml of water containing 90 mg of periodic acid was gently warmed until clear. After 4 hr, 5 ml of 5% $Na_2S_2O_3$ were added and the reaction mixture was treated with 2 × 20 ml portions of ethyl acetate. The combined extracts were washed with water, concentrated and the residue was stirred successively with 1.0 ml and 0.5 ml of water with centrifugation each time. The turbid supernates were discarded and the 2,4-dinitrophenylhydrazone was prepared from the precipitate which was crystallized from 95% ethanol. Yield of 2,4-dinitrophenylhydrazone of 2-benzamidopentadecanal, 8.0 mg; mp 116–117°C.

Anal. Calcd for $C_{28}H_{39}N_5O_5$ (525.3): C, 63.96; H, 7.48. Fd: C, 63.99; H, 7.51.

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