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CHEMICAL SYNTHESIS OF D,L-3-DEHYDROSPHINGANINE, ITS C_{14}-, C_{16}- AND C_{20}-HOMOLOGUES AND THE RESOLUTION INTO THE ENANTIOMERIC FORMS

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The chemical synthesis of D,L-3-dehydrosphinganine (3-keto sphinganine) and the resolution into its optical isomers via the mandelates is described. This procedure proved to be suitable for the homologous series. The products were characterized by elementary analysis, mass-spectroscopy, IR, NMR, gas chromatography and also by their mono-, di- and triacetyl derivatives respectively.

I. Introduction

It has been accepted for a number of years, that the biosynthesis of sphinganine and sphingenine proceeds according to the pathway suggested by Brady et al.[1,2]). These authors derived the conclusion from their experiments in vitro with a particulate fraction of rat brain, that palmitoyl-CoA is reduced to palmitaldehyde in the presence of NADPH. Palmitaldehyde then should condense with serine in a pyridoxal phosphate dependent reaction to sphinganine. A subsequent dehydrogenation of sphinganine catalyzed by a flavoprotein was believed to introduce the 4*trans*-double bond yielding 4t-sphingenine.

Chemical reasoning, however, makes a decarboxylation of the β-hydroxy acid, which would be the condensation product of palmitaldehyde and serine, very unlikely. On the other hand, a β-keto acid seemed to us the more reasonable intermediate for the decarboxylation of the serine carboxyl group. Therefore analogously to fatty acid biosynthesis our working hypothesis suggested the condensation of palmitoyl-CoA and serine to a 3-keto compound, which after the release of CO_2 would yield 3-dehydrosphinganine (for nomenclature see CBN[3])).

Stoffel et al.[4,5]) demonstrated in studies in vivo the key position of 3-dehydro-derivatives in the biosynthesis of long chain bases (C_{18} and C_{20}). [3-^{14}C]3-dehydrosphinganine and the C_{20} homologue were rapidly trans-

formed into 4t-sphingenine, sphinganine, eicosasphinganine and 4t-eicosasphingenine. The condensation of palmitoyl-CoA and serine to 3-dehydrosphinganine and its subsequent NADPH dependent reduction was achieved in experiments in vitro using the microsomal fraction of Hansenula cif.[6]) (for review see Stoffel[7])). The condensation product proved to be identical with synthetic 3-dehydrosphinganine. Simultaneously and independently Snell and collaborators[8,9]) derived from their experiments in vitro with Hansenula cif. the same pathway in sphinganine biosynthesis. Stoffel et al.[10]) studied the reduction of the 3-keto group of 3-dehydrosphinganine to sphinganine both with a yeast and liver microsomal enzyme preparation. The partial purification, properties and distribution of the enzyme which was named 3-dehydrosphinganine-NADP: oxidoreductase has been described. Also the stereospecificity of the reduction was studied. Only the B-hydrogen atom of NADPH is transferred to the carbonyl group forming D-erythro-dihydrosphingosine (sphinganine). When B-NADP^{3}H was used as coenzyme 93% of the radio-activity was present in sphinganine isolated as D-glutamate. This proved that only the D-isomer of the synthetic racemic 3-dehydrosphinganine was the substrate for the enzyme.

In this paper the synthesis of the racemic and the two enantiomeric forms of *3*-dehydrosphinganine will be described.

II. Results and discussion

A. N-acetyl-3-dehydrosphinganine

Gaver and Sweeley[11]) prepared N-acetyl-3-dehydrosphinganine and -sphingenine by chromic acid anhydride oxidation of N-acetyl-sphinganine and N-acetyl-sphingenine in 30–50% yield. N-acetyl-3-dehydrosphinganine had a melting point of 104–105 °C. We oxidized D,L-N-acetyl sphinganine according to the method of Snatzke[12]). The oxidation with chromic acid anhydride was carried out in dimethylformamide catalyzed by concentrated sulfuric acid. The reaction can be controlled by the acid concentration and the progress of the oxidation checked by thin-layer chromatography (solvent system: chloroform/methanol 15:1). For this purpose an aliquot was first distributed between water and ether. The yield of the pure reaction product amounts up to 45–50%. The melting point of racemic N-acetyl-3-dehydrosphinganine is 96.5–97.5 °C, whereas N-acetyl-3-dehydrosphinganine obtained by the same procedure from N-acetyl sphinganine prepared from natural 4t-sphingenine according to Carter et al.[13]) melted at 103–104 °C and had a specific rotation of 55°. IR- and NMR-spectra and the R_f-values in thin-layer chromatography of the racemic N-acetyl-3-dehydrosphinganine and the optically active compound were identical. Fig. 1 represents the

NMR-spectrum of racemic N-acetyl-3-dehydrosphinganine dissolved in a mixture of chloroform-dimethylsulfoxide which prohibits the exchange of the OH-proton by deuterium. The singlet of the OH-proton is overlapped by the multiplet of the isolated proton at 5.7 τ. The quartet at 6.3 τ and

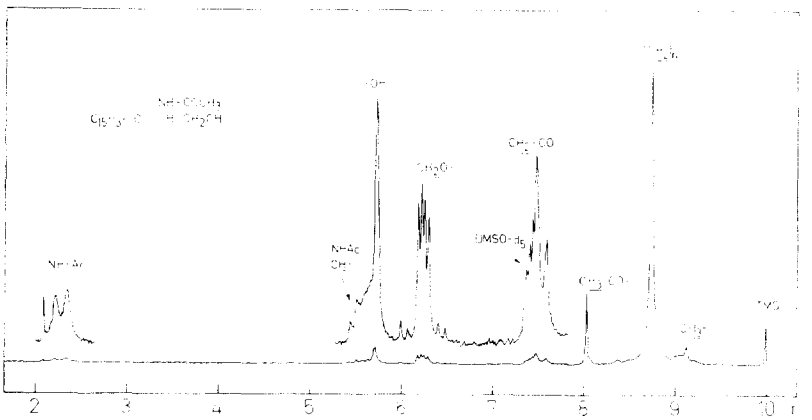


Fig. 1. NMR-spectrum of racemic N-acetyl-3-dehydrosphinganine.

the triplet at 7.5 τ correspond to the methylen groups ($\underline{CH_2}$–OH and $\underline{CH_2}$–C–O–). N-Acetyl-3-dehydrosphinganine and -sphingenine can be separated by gas-liquid chromatography as their O-trimethylsilyl derivatives. It was desirable to use more stable derivatives for analytical purposes. The diacetyl compounds of 3-dehydros-phinganine and -4-sphingenine proved to be very suitable for the gas-liquid chromatographic separation on 1% SE 30 at a column temperature of 230 °C. N,O-Diacetyl-3-dehydro-4-sphingenine had a retention time of 1.32 relative to N,O-diacetyl-3-dehydrosphinganine (fig. 2).

The diacetyl derivatives are easily prepared with acetanhydride in pyridine at room temperature in almost quantitative yield. Fig. 3 represents the mass-spectrum of racemic diacetyl-3-dehydrosphinganine. Besides the mass ion m/e 383 other prominent ions are present at m/e 322 (loss of acetic acid and one hydrogen atom), m/e 144 (loss of the acyl group $C_{15}H_{31}CO$). The elimination of ketene ($CH_2{=}C{=}O$) from the fragment m/e 322 and 144 forms two large mass-peaks at m/e 280 and 102. No fragment at m/e 239 corresponding to the acylium ion ($C_{15}H_{31}CO^+$) could be detected.

B. 3-dehydrosphinganine hydrochloride

Mendershausen and Sweeley[14]) described the preparation of 3-dehydro-sphinganine by hydrogenolysis of the N-carbobenzoxy derivative. No analytical data were reported since the authors described the product to

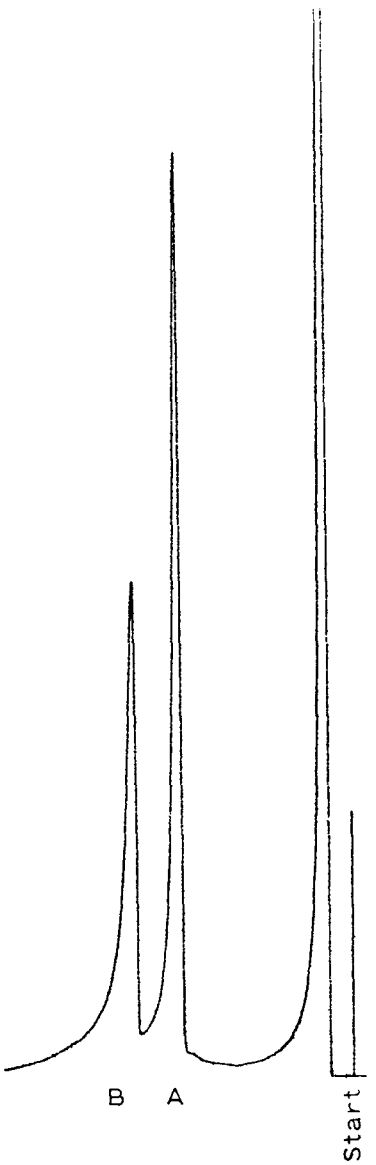


Fig. 2. Gas-liquid chromatography of N,O-diacetyl-3-dehydrosphinganine (A) and N,O-diacetyl-3-dehydro-4-sphingenine (B).

be very unstable, although the product was characterized by sodium borodeuteride reduction to [3-^{2}H] sphinganine, N-acetylation and combined gas chromatography-mass spectrometry of the di-O-trimethylsilyl derivative after the reduction of the N-acetyl compound.

α-Amino ketones are known to undergo easily condensation to dihydropyrazines with subsequent oxidation to the aromatic pyrazine derivatives. We therefore planned to produce a stable salt of 3-dehydrosphinganine during the acid hydrolysis. The most suitable conditions proved to be the hydrolysis of N-acetyl-3-dehydrosphinganine in a 5% dry hydrochloric acid in ether under refluxing for 8 hr. 3-Dehydrosphinganine hydrochloride

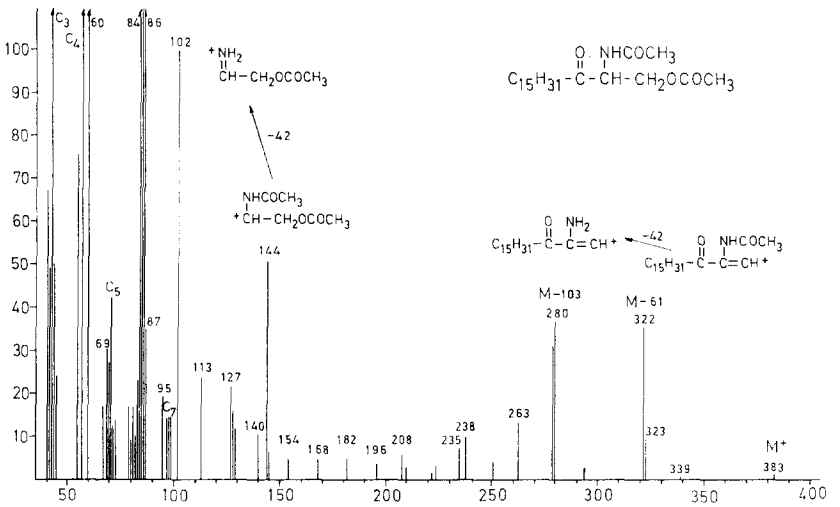


Fig. 3. Mass-spectrum of diacetyl-3-dehydrosphinganine.

crystallized out of the reaction mixture in almost pure form. The product exhibits a characteristic intensive yellow colour with ninhydrin e.g. on thin-layer chromatography. The analytical data of the purified product (see III) are very satisfactory. 3-Dehydrosphinganine hydrochloride is stable over months even when stored at room temperature. The IR-spectrum of the 3-oxo-compound with the intensive absorption at 1715 cm^{-1} is shown in fig. 4.

C. Resolution of diastereomers and enantiomers

The steric configuration of carbon atoms 2 and 3 of sphinganine and 4-sphingenine has been established as D-erythro configuration largely by Carter and his collaborators[15,16] and confirmed by Klenk et al.[17]). These results based on analytical procedures obtained their final proof by the synthesis of erythro D,L-sphinganine by Shapiro et al.[18]) and Jenny and Grob[20]), who synthesized the two diastereomers of sphinganine by a stereospecific synthesis. The racemic erythro- and threo-diastereomers

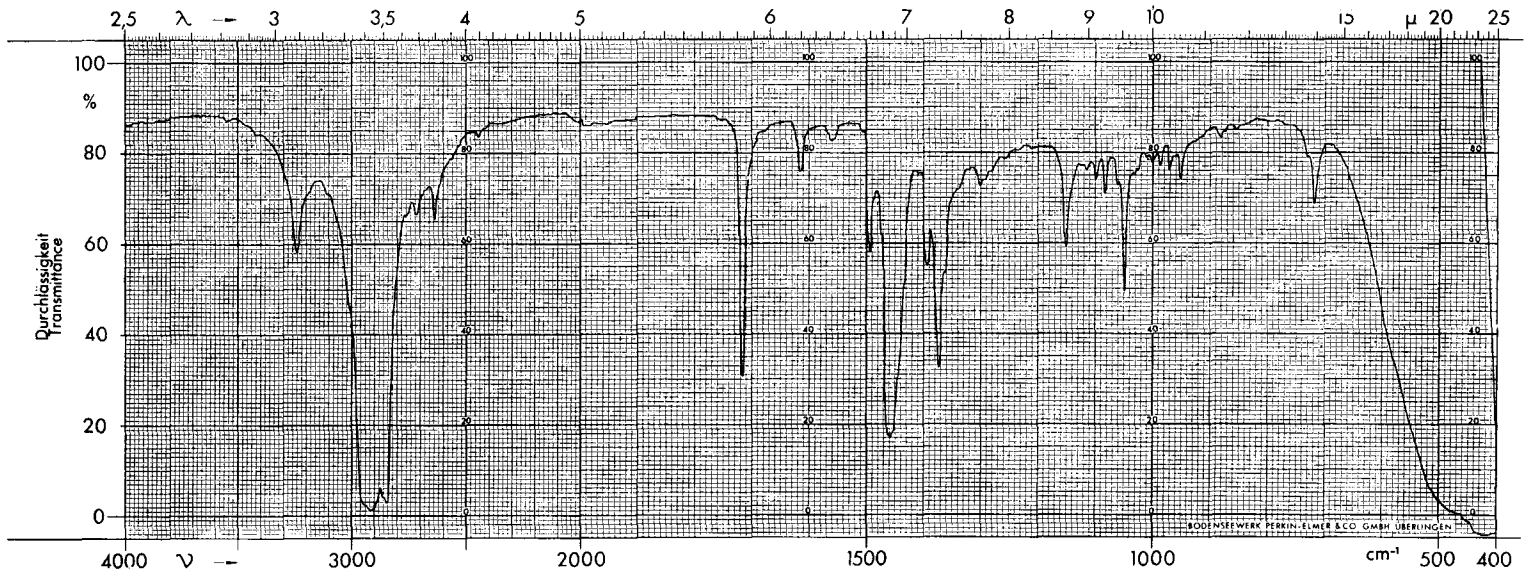


Fig. 4. IR-spectrum of D,L-3-dehydrosphinganine hydrochloride.

were resolved by Grob and Jenny[19]) and Carter and Shapiro[21]). One enantiomeric form of each diastereomer was claimed to be identical with the long chain base isolated from natural sphingolipids. However many hydrolytic procedures lead to the rearrangement of the original long chain base. Jenny and Grob[19]) interpreted the formation of the threo diastereomeric form by an inversion of carbon atom 3 during the hydrolysis. The long chain base, which was first isolated by Seydel[22]), proved to be identical with the (−) enantiomeric form resolved from the racemic threo diastereomer. However, the absolute configuration of this enantiomer was not determined and no evidence given for an exclusive inversion of carbon atom 3 in the course of the hydrolysis. The hydrolysis procedure of cerebrosides described by Carter[23]) yields only the erythro diastereomer. Natural sphingenine proved to have the D-erythro configuration. The reaction sequence which we followed in the synthesis of the two enantiomeric 3-dehydrosphinganine hydrochlorides is presented in table 1.

Pure threo- and erythro-sphinganines were synthesized according to Grob et al.[24]) and Shapiro et al.[25]). These authors and more recently Eller et al.[26]) used D- and L-glutamic acid for the resolution of the antipodes of the two diastereomeric sphinganines. However, in our hands this method proved to be unsatisfactory and not reproducible. The low solubility of glutamic acid in ethanol and the decomposition of the glutamates at the melting point led us to use the mandelic acids for the resolution. The salts of the long chain bases are formed easily in hexane-ethanol. Mandelates have a lower solubility in the solvent mixture than the corresponding acid. They melt without decomposition and therefore permit to follow their purification. The bases can be recovered from the salt almost quantitatively.

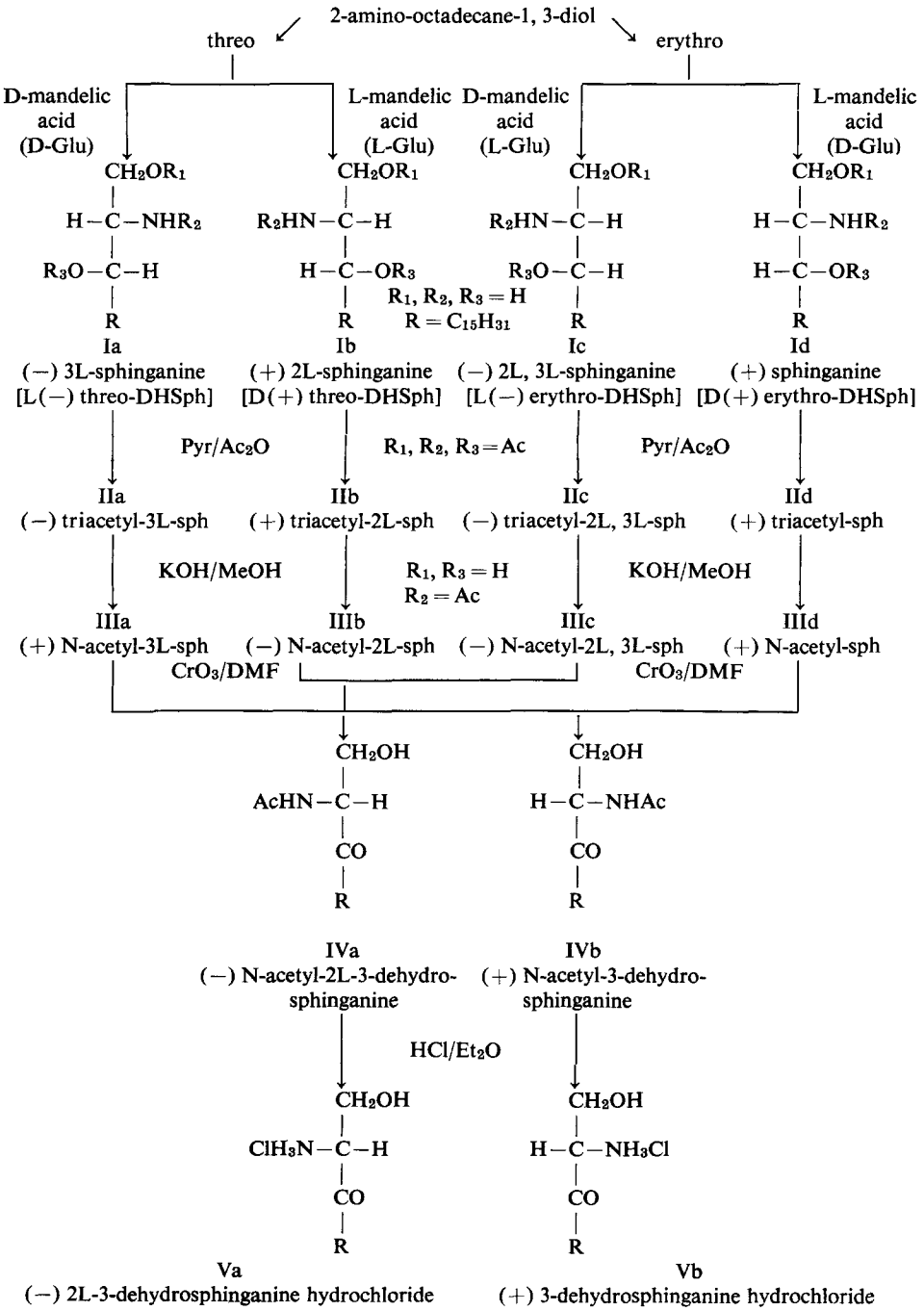
Table 2 summarizes the melting points and optical rotations of the free bases, their triacetyl and N-acetyl derivatives of the racemic diastereomers, their enantiomeric forms and of the long chain base isolated from natural sources. Most of our data agree well with those of other authors described so far. Melting points and optical rotation values are not reported for the synthetic enantiomeric erythro-sphinganines.

The data of table 2 reveal a number of examples, in which the racemate has a higher melting point than the corresponding enantiomers, e.g. N- and triacetyl threo-sphinganines. Furthermore the melting point of the enantiomers of the higher melting racemic diastereomer increases. The melting point of the enantiomers derived from the lower melting racemic diastereomer however decreases.

The analysis of the crystal lattices of these compounds and more examples with this regularity can probably explain these findings. Each pair of enantiomers leads, respectively, to the same oxo-derivative with a remarkably

TABLE 1

Synthesis of the two enantiomeric 3-oxo-2-amino-octadecane-1-ol hydrochlorides (3-dehydrosphinganine- and 2L-3-dehydrosphinganine-hydrochloride)



TABLE

deriviates	physical constants	rac.	threo-2-amino-octadecane-1, 3-diol L a)	D b)
D(−) mandelates	m.p.	–	107–108.5 °C	–
L(+) mandelates	m.p.	–	–	107–108.5 °C
I. free bases	m.p.	[99.5–100.5 °C][16, 20]	107–108 °C [109 °C][19, 22]	106–107 °C [109 °C][19]
	α	–	−13°[22] [−14.1°][19]	−13° [+13.5°][19]
II. triacetyl-	m.p.	[67–68 °C][24]	45.5–46.5 °C	4.5.–46.5 °C
	α	–	−8.2°	−8.0°
III. N-acetyl-	m.p.	[102–103 °C][20]	96–97 °C	96–97 °C
	α	–	−6.2°	−6.5°

	2-amino-3-oxo-octadecane-1-ol		
derivatives	physical constants	L	D
IV. N-acetyl	m.p.	105–106 °C	105–106 °C
	α	−57°	+57°
V. hydrochlorides	m.p.	89–92 °C	90–92 °C
	α	−26.5°	+28°

high optical rotation value, as shown in tables 1 and 2. The (−) threo form and (+) sphinganine (D-erythro form) prepared either by resolution of the racemic erythro diastereomer or from natural sources result in the same 3-oxo derivative. This is another conclusive evidence that C-2 of both compounds has the same configuration. Also the inversion mechanism proposed by Grob et al., which occurs at C-3 via an oxazoline intermediate is fully supported by this work. The two enantiomeric 3-dehydrosphinganine hydrochlorides show an chromatographic behaviour identical with that of the racemate.

2

erythro-2-amino-octadecane-1,3-diol

rac.	L c)	D d)	D n)
–	148.5–149.5°C	–	–
–	–	148.5–149.5°C	–
[85–86°C][18])	78.5–79°C	78.5–79°C	[102–104°C][22])
	−6.0°	+6.0°	[+0.6°][22])
[91–92°C][18])	96–97°C [98–100°C][21])	96–97°C [98–100°C][21])	[101°C][13]) [98°C][28])
	−20.5° [−19.35°][21])	+21° [+19.2°][21])	+18°
[120–121°C][16])	124–125°C	124–125°C	[125–126°C][19])
	−8.0°	+7.7°	+11.0°

2-amino-3-oxo-octadecane-1-ol

rac.	L c)	D d)	D n)
96.5–97.5°C	105–106°C	105.5–106°C	102.5–103°C [104–105°C][11])
–	−58°	+59°	+55.5°
107–108°C	–	–	88–91°C
–	–	–	+26°

Our studies of the chain length specificity of the condensation reaction and of the reduction to the homologous sphinganines required the 3-oxo-derivatives of the C_{14}-, C_{16}- and C_{20}-long chain bases. The essentially corresponding 2-amino-1,3-dihydroyalkanes were first synthesized according to the procedures of Shapiro et al.[18]) and the 3-dehydro-derivative following the experimental conditions described for 3-dehydrosphinganine. Again these aminoketones were isolated as their stabile, crystalline hydrochlorides.

III. Experimental

A. Methods

Melting points are uncorrected.

Infrared spectra were measured with the Perkin Elmer IR-spectrophotometer model 257 or 125.

NMR spectra were recorded with a Varian A-60 model and mass spectra with a mass spectrometer Varian-MAT, model CH5. The ion source was 100 μA and temperature 120 °C. An electron energy of 70 eV was applied.

Gas chromatographic analyses of the diacetyl-3-dehydro- and of TMS derivatives of long chain bases were carried out with a Perkin Elmer F20 Fraktometer using a 1% SE30 column at a temperature of 230 °C. The purity of all compounds was checked by thin-layer chromatography on plates coated with silicic acid (Kieselgel G, Merck).

Depending on the products the following solvent systems were used:

Long chain bases: chloroform-methanol-2N NH_4OH 40:10:1[27]).

Peracetylated derivatives of long chain bases: chloroform-methanol 15:1.

N-acetyl derivatives of long chain bases: chloroform-methanol 8:1.

The chromatoplates were developed by charring with 5% CrO_3 in 50% sulphuric acid or with ninhydrin reagent.

Optical rotations were measured in a Lichtelektrisches Präzisionspolarimeter 0.005°, Carl Zeiss.

Micro-analyses were carried out by Mikroanalytisches Laboratorium Ilse Beetz, Kronach.

B. Materials

Racemic erythro-2-amino-octadecane-1,3-diol was synthesized according to the procedure of Shapiro and Sheradsky[25]). Separation of the two diastereomeric bases via the dichloroacetyl derivatives was carried out only for the synthesis of the enantiomeric 3-dehydrosphinganines. Threo-2-amino-octadecane-1,3-diol was prepared as described by Grob et al.[24]), but in a modified manner. Palmitaldehyde was condensed with nitroethanol in the presence of potassium carbonate in analogy to the procedure of Grob and Gadient[29]). The reduction of the threo-2-nitro-octadecane-1,3-diol was carried out with aluminium amalgam as described for the acetylenic compound by these authors.

C. Resolution of racemic erythro- and threo-2-amino-octadecane-1,3-diols

DL-threo-2-Amino-octadecane-1,3-diol (1 g, 3.33 mmoles) and D(−)mandelic acid (506 mg, 3.33 mmoles) were dissolved in a mixture of 20 ml hexane and 1 ml 95% ethanol. After standing at room temperature for a

few minutes 860 mg 3L-sphinganine (L-threo-2-amino-octadecane-1,3-diol-) D-mandelate crystallized from the solution. The product was recrystallized four times from a mixture of hexane-ethanol 10:1 and had then a sharp melting point of 107–108.5 °C. (Bright Leaflets) yield: 345 mg (0.76 mmoles) =46% of the theory.

The mother liquids were evaporated to dryness, dissolved in 50 ml of methanol, and 5 ml 1 N NaOH were added dropwise under shaking. The precipitation was complete after the addition of 50 ml of water. The precipitate was filtered off, washed with water and dried over P_2O_5. The pure 2L-sphinganine (D-threo-2-amino-octadecane-1,3-diol-) L-mandelate was obtained after adding of the equivalent amount of L(+)mandelic acid to a solution of the base received from the mother liquid and after some recrystallizations of the mandelate; colourless leaflets, m.p. 107–108.5 °C.

The resolution of the erythro-diastereomer was carried out in the same way except that a solvent mixture of hexane-ethanol 4:1 was used and that more crystallizations were necessary. 1 g of erythro-2-amino-octadecane-1,3-diol yielded:

2L,3L-sphinganine-(L-erythro-2-amino-octadecane-1,3-diol-) D-mandelate (175 mg; 0.385 mmoles) 23% of theory,

Sphinganine-(D-erythro-2-amino-octadecane-1,3-diol-) L-mandelate (120 mg; 0.264 mmoles) 16% of theory,

melting points and appearance of the two salts were identical, m.p. 148.5–149.5 °C.

The enantiomeric bases were liberated as described above and crystallized from the solution in pure form with sharp melting points.

(Ia) (−) 3L-sphinganine (L(−)threo-2-amino-octadecane-1,3-diol) yield 97% of theory; m.p. 107–108 °C (lit. 109 °C[19]), $[\alpha]_{546}^{28} - 13.0° \pm 0.2$ in C-M 10:1 (lit. $-14.1°$[19]).

(Ib) (+)2L-sphinganine (D(+)threo-2-amino-octadecane-1,3-diol) yield 95% of theory; m.p. 106–107 °C (lit. 109 °C[19]), $[\alpha]_{546}^{28} + 13.0° \pm 0.2$ in C-M 10:1 (lit. $+13.5°$[19]).

The threo enantiomers crystallized in colourless leaflets.

(Ic) (−)2L,3L-sphinganine (L(−)erythro-2-amino-octadecane-1,3-diol) yield 98% of theory; m.p. 78.5–79 °C, $[\alpha]_{546}^{28} - 6.0° \pm 0.5$ in C-M 10:1.

(Id) (+)sphinganine (D(+)erythro-2-amino-octadecane-1,3-diol) yield 95% of theory; m.p. 78.5–79 °C, $[\alpha]_{546}^{28} + 6.0° \pm 0.5$ in C-M 10:1.

The erythro-enantiomers cristallized in felted needles.

D. 2-Acetamido-octadecane-1,3-diol

Crude 2-amino-octadecane-1,3-diol (7 g, 2.32 mmoles) obtained after reduction of ethyl 2-amino-3-oxo-octadecanoate hydrochloride with $LiAlH_4$ was

dissolved in a mixture of 150 ml abs. pyridine and 30 ml acetanhydride and left at room temperature for 8 h. The solvent was removed by flash evaporation below 40°C. The crude triacetyl derivative was dried over P_2O_5 and KOH in a desiccator yielding 9.95 g = 2.32 mmoles. For saponification the product was dissolved in 400 ml methanol without a further purification and a solution of KOH (520 mg; 9.3 mmoles) in 40 ml water was added. After standing at room temperature over night the mixture was acidified with acetic acid and concentrated under vacuum. The product was precipitated by the addition of water (100 ml), filtered off, washed with water and dried over P_2O_5. Yield 7.9 g (23 mmoles) 99% of theory.

The enantiomeric N-acetyl derivatives were prepared in the same manner except that the intermediate triacetyl compounds were purified by crystallization.

(IIa) (−)triacetyl-3L-sphinganine (L(−)threo-2-acetamido-2,3-diacetoxy-octadecane). Yield after two crystallizations from pentane 80% of theory; m.p. 45.5–46.5° C, $[\alpha]_{546}^{28} -8.2 \pm 0.2$.

(IIb) (+)triacetyl-2L-sphinganine (D(+)threo-2-acetamido-2,3-diacetoxy-octadecane). Yield after two crystallizations from pentane 77% of theory; m.p. 45.5–46.5°, $[\alpha]_{546}^{28} +8.0 \pm 0.2$.

(IIc) (−)triacetyl-2L,3L-sphinganine (L(−)erythro-2-acetamido-1,3-diacetoxy-octadecane). Yield after two crystallizations from hexane-ethanol (10:1) 79% of theory; m.p. 96–97°C (lit. 98–100°C[21]), $[\alpha]_{546}^{28} -20.5° \pm 0.5$ (lit. −19.35°[21]).

(IId) (+)triacetyl-sphinganine (D(+)erythro-2-acetamido-1,3-diacetoxy-octadecane). Yield after two crystallizations from hexane-ethanol (10:1) 86% of theory; m.p. 96–97°C (lit. 98–100°C[21]), $[\alpha]_{546}^{28} +21.0° \pm 0.5$ (lit. +19.2°[21]).

(IIIa) (+)N-acetyl-3L-sphinganine (L(+)threo-2-acetamido-octadecane-1,3-diol). Yield after one crystallization from hexane-ethanol (10:1) 95% of theory; m.p. 96–97°C, $[\alpha]_{546}^{28} +6.2° \pm 0.2$.

(IIIb) (+)N-acetyl-2L-sphinganine (D(−)threo-2-acetamido-octadecane-1,3-diol). Yield after one crystallization from hexane-ethanol (10:1) 93% of theory; m.p. 96–97°C, $[\alpha]_{546}^{28} -6.5° \pm 0.2$.

(IIIc) (−)N-acetyl-2L,3L-sphinganine (L(−)erythro-2-acetamido-octadecane-1,3-diol). Yield after one crystallization from hexane-ethanol (10:1) 91% of theory; m.p. 124–125°C, $[\alpha]_{546}^{28} -8.0° \pm 0.2$.

(IIId) (+)N-acetyl-sphinganine (D(+)erythro-2-acetamido-octadecane-1,3-diol). Yield after one crystallization from hexane-ethanol (10:1) 93% of theory; m.p. 124–125°C, $[\alpha]_{546}^{28} +7.7° \pm 0.2$.

E. 2-Acetamido-3-oxo-octadecane-1-ol

2-Acetamido-octadecane-1,3-diol (7.9 g; 23 mmoles) was dissolved in 400 ml dimethyl formamide. After the addition of 5 g of chromic anhydride and 80 drops of concentrated sulphuric acid the mixture was allowed to react for 4 hr. The progress of the reaction was controlled by thin-layer chromatography as described in III. The reaction mixture was poured into water and extracted three times with ether. After washing with saturated NaCl-solution the ethereal extracts were dried over sodium sulphate and evaporated to dryness. The crude product was purified according to the procedure of Gaver and Sweeley[11]) and crystallized from hexane-ethanol (20:1) yielding 3.74 g (11 mmoles) 48% of theory; m.p. 96–97 °C; m.p. after two crystallizations 96.5–97.5 °C.

Calc. for $C_{20}H_{39}NO_3$ (MW 341.54)	%C 70.34	H 11.51	N 4.10
Found	%C 70.70	H 11.85	N 4.22

The enantiomeric 2-acetamido-3-oxo-octadecane-1-ols were prepared by oxidation of the enantiomeric threo-and erythro-2-acetamido-octadecane-1,3-diols.

(IVa) (−)N-acetyl-2L-3-dehydrosphinganine (31 mg; 0.091 mmole), yield 45% of theory calculated for (−)N-acetyl-2L-sphinganine; m.p. 105–106 °C; $[\alpha]_{546}^{28} - 57° \pm 0.5$.

(IVb) (+)N-acetyl-3-dehydrosphinganine (56 mg; 0.159 mmole), yield 46% of theory calculated for (+)N-acetyl-3L-sphinganine; m.p. 105–106°; $[\alpha]_{546}^{28} + 57° \pm 0.5$.

(IVc) (−)N-acetyl-2L-3-dehydrosphinganine (14 mg; 0.041 mmole), yield 40% of theory calculated for (−)N-acetyl-2L,3L-sphinganine; m.p. 105–106 °C; $[\alpha]_{546}^{28} - 58° \pm 0.5$.

(IVd) (+)N-acetyl-3-dehydrosphinganine (14 mg; 0.041 mmole), yield 40% of theory calculated for (+)N-acetyl-sphinganine; m.p. 105–106 °C; $[\alpha]_{546}^{28} + 59° \pm 0.5$.

(IVn) (+)N-acetyl-3-dehydrosphinganine (75 mg; 0.22 mmole), yield 44% of theory calculated for (+)N-acetyl-sphinganine derived from natural 4t-sphingenine; m.p. 102.5–103 °C (lit. 104–105 °C[11])); $[\alpha]_{546}^{28} + 55.5° \pm 0.5$. Infrared spectrum: 3420 cm^{-1} (O–H), 3360 cm^{-1} (amide N–H), 1710 cm^{-1} (ketone C=O), 1610 cm^{-1} (amide C=O), 1525 cm^{-1} (amide N–H deform.), 1380 cm^{-1} (O–H deform.).

F. 2-Amino-3-oxo-octadecane-1-ol hydrochloride

2-Acetamido-octadecane-1-ol (1 g; 2.93 mmoles) was added to a mixture of 50 ml 5% ethereal hydrochloric acid and 60 μl water and refluxed at an

oil bath temperature of 50 °C under stirring and exclusion of moisture. The process of the cleavage was controlled by thin-layer chromatography (solvent system: chloroform-methanol 15:1). The reaction mixture was allowed to stand over night and then centrifuged with the exclusion of moisture. The supernatant was decanted and the precipitate washed three times with absolute ether and dried under vacuum. Yield 530 mg; m.p. 105–106 °C. For analytical purposes the product was dissolved in methanol and precipitated with O.1 N aqueous hydrochloric acid. Yield 485 mg (1.44 mmole) 49% of theory; m.p. 107–108 °C.

Calc. for $C_{18}H_{38}NO_2Cl$ (MW = 335.967) %C 64.35 H 11.40 N 4.17 Cl 10,55
Found %C 65.18 H 11.16 N 4.27 Cl 10,84

The enantiomeric 3-dehydrosphinganine hydrochlorides were prepared by hydrolysis of the enantiomeric 2-acetamido-octadecane-1-ols.

(Va) (−)2L-3-dehydrosphinganine hydrochloride (12.5 mg; 37 μmoles), yield 42% of theory calculated for (−)N-acetyl-2L-3-dehydrosphinganine; m.p. 89–92 °C; $[\alpha]_{546}^{28} -26.5° \pm 0.5$.

(Vb) (+)3-dehydrosphinganine hydrochloride (12.5 mg; 37 μmoles), yield 42% of theory calculated for (+)N-acetyl-3-dehydrosphinganine; m.p. 90–92 °C; $[\alpha]_{546}^{28} +28° \pm 0.5$.

(Vn) (+)3-dehydrosphinganine hydrochloride (9 mg; 27 μmoles), yield 30% of theory calculated for (+)N-acetyl-3-dehydrosphinganine derived from natural 4-sphingenine; m.p. 88–91 °C; $[\alpha]_{546}^{28} +26° \pm 0.5$.

G. 2-Acetamido-3-oxo-1-acetoxy-octadecane

2-Acetamido-3-oxo-octadecane-1-ol (100 mg; 0.293 mmole) was dissolved in a mixture of 5 ml absolute pyridine and 1 ml acetanhydride and left at room temperature for 10 hr. The solvent was removed by evaporating under vacuum at a water bath temperature of 40 °C and dried over P_2O_5 and KOH. The crude product (112 mg) was recrystallized twice from hexane. Yield 98 mg (0.255 mmole) 87% of theory, m.p. 95.5–96 °C.

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