Establishment of *erythro* configuration of ceramides from beef brain and chicken liver

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SUMMARY The sphingosine moiety of ceramides isolated from beef brain and chicken liver has been characterized as having the *erythro* stereochemical configuration. This is in agreement with the configuration of sphingosine as found in cerebrosides and sphingomyelin.

KEY WORDS ceramides · *erythro* configuration · beef brain · chicken liver · tribenzoyl · triacetyl · dihydroceramide

FOR SOME TIME it has been assumed that the sphingosine moiety of all sphingolipids has the same stereochemical configuration as that found in the sphingosine moiety of cerebrosides. Carter and his co-workers (1, 2) have presented ample evidence that carbon atoms 2 and 3 of sphingosine, derived from cerebrosides, have the *erythro* configuration. Recently Shapiro and Flowers (3) have synthesized isomers of sphingomyelin and have shown that sphingomyelin isolated from beef spinal cord has the *erythro* configuration. In this laboratory,[1] we have characterized sphingomyelin isolated from beef brain, beef heart, and chicken liver and have confirmed the results of Shapiro and Flowers. Fujino and Zabin (4) have shown that dihydrosphingosine formed enzymatically in rat brain homogenates from palmityl CoA and serine has the same configuration as found in cerebrosides and sphingomyelin.

It is the purpose of this paper to report on the nature of ceramides that have been isolated from beef brain and chicken liver. This work arose from the finding that *threo* rather than *erythro* ceramides were required as acceptors of phosphoryl choline in the enzymatic synthesis of sphingomyelin (5). Sphingosine was characterized as the tribenzoyl dihydrosphingosine and triacetyl dihydrosphingosine derivatives.

[1] M. Sribney, unpublished results.

METHODS

Isolation of Ceramides

Beef brain and chicken liver, obtained from local slaughter houses, were first converted to their respective acetone powders by extraction with acetone at 0°. The ceramides were then isolated from these powders by extracting three times with 10 volumes of hot acetone. The crude extracts were taken to dryness in a flash evaporator, dissolved in chloroform, and chromatographed on a column of silicic acid–Celite 7:3. To every 7 g of silicic acid in the column 50 mg of crude lipid was applied. After thoroughly washing the column with chloroform, ceramides were eluted with chloroform–methanol 95:5 (v/v).

The still crude ceramide fraction was taken to dryness and rechromatographed in a similar manner using a Florisil column. The ceramide thus obtained was further purified by twice recrystallizing from acetone. Thin-layer chromatography using two different solvent systems (chloroform–acetic acid–methanol 94:10:2 and chloroform–methanol–acetone 4:1:1) revealed the presence of only one discrete compound. From 10 kg of beef brain, 500 mg of ceramide was obtained, while 320 mg was obtained from the same quantity of chicken liver.

Chicken Liver Ceramide:

Found	C	75.70	H	12.48	N	2.10

Beef Brain Ceramide:

Found	C	76.60	H	12.77	N	2.77

Calculated for *N*-stearoylsphingosine:

$C_{36}H_{71}O_3N$	C	76.39	H	12.64	N	2.47

Dihydroceramide from Ceramide

A portion of the ceramides (100 mg) was dissolved in absolute alcohol and hydrogenated by the method described by Carter et al. (1) using platinum oxide as catalyst.

Hydrolysis of Dihydroceramide

Dihydroceramides were converted to dihydrosphingosine by refluxing for 18 hr using 1 N KOH in 90% methanol. The hydrolysate was extracted with ether; the ether extracts were washed with water, dried over sodium sulfate, and evaporated to dryness. Yield of dihydrosphingosine varied from 85 to 95% of theory.

Preparation of Derivatives

Tribenzoyl dihydrosphingosine and triacetyl dihydrosphingosine were prepared as described by Carter and Fujino (2). Optical activities were determined at a concentration of 100 mg/10 ml chloroform.

The tribenzoyl derivative from beef brain ceramide had mp 146–148°, $[\alpha]_D^{23} = -29.9$.

Found	C 76.39	H 8.60	N 2.32

The tribenzoyl derivative from chicken liver ceramide had mp 144–145°, $[\alpha]_D^{23} = -29.4$.

Found	C 76.14	H 8.56	N 2.47
Calcd for $C_{39}H_{51}O_5N$:	C 76.31	H 8.37	N 2.28

The triacetyl derivative from beef brain ceramide had mp 101–103°, $[\alpha]_D^{23} = +18.1$.

Found	C 67.56	H 10.22	N 2.85

The triacetyl derivative from chicken liver ceramide had mp 101–103°, $[\alpha]_D^{23} = +18.0$.

Found	C 67.20	H 10.75	N 3.02
Calcd for $C_{24}H_{45}O_5N$:	C 67.41	H 10.60	N 3.27

DISCUSSION

Since it has been shown that *threo* rather than *erythro* ceramides are precursors of sphingomyelin, it was of interest to investigate the stereochemical configuration of ceramides as they occur in nature. Tipton (6) has isolated ceramides from beef lung and has shown that they probably exist in the *erythro* from, but the yield of derivative was too low to establish this fact unequivocally.

Carter and his co-workers (7) have shown that the *threo* isomer of dihydrosphingosine forms only the dibenzoyl derivative. We have been able to obtain only the tribenzoyl derivative, in high yield, of the sphingosine moiety of ceramides isolated from beef brain and chicken liver. The melting points and optical rotation values obtained for the tribenzoyl and triacetyl derivatives are substantially in agreement with those obtained by Carter and his co-workers (1, 2). We conclude that by far the greatest portion of the ceramides of beef brain and chicken liver have the *erythro* configuration. It is possible that some *threo* ceramides do exist in low concentration in these tissues, but that they were removed during the process of isolation and purification. It is also possible that any existing *threo* isomer might be isomerized to the *erythro* isomer during the hydrolysis of ceramides, but such isomerization would be minimal under the alkaline conditions employed.

These ceramides do not stimulate the incorporation of the phosphoryl choline moiety of CDP-choline into sphingomyelin, whereas the synthetic *threo* derivatives, with a fatty acid of comparable chain length in acyl linkage, are effective in forming sphingomyelin.[2] Although ceramides are enzymatically synthesized in rat brain and chicken liver (8, 9) they may be precursors not of sphingomyelin but of some other complex sphingolipid, or they may be metabolic end products in themselves. The possibility exists that sphingomyelin is biosynthetically formed by reactions analogous to that of cerebrosides (10), whereby sphingosine rather than ceramide is the immediate precursor. Experiments to resolve this problem are in progress.

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