Studies on Sphingosines

9. C_{19}-Sphingosines, hitherto Unknown Sphingosines*

KARL-ANDERS KARLSSON and GÖRAN A. L. HOLM

Institute of Medical Biochemistry, University of Göteborg, Göteborg, Sweden

Sphingosines ** with 16, 17, 18, and 20 carbon atoms have so far been identified, see Table 1. The present communication concerns C_{19}-sphingosines of animal and plant tissues.

Cerebrin from corn (General Biochemicals, Ohio, lot number 47629) was degraded in hydrochloric acid-methanol.[14] The hydrolysate was partitioned according to Folch and the lower phase evaporated and chromatographed on silicic acid.[3] The sphingosine fraction was converted to the corresponding dinitrophenyl (DNP) derivatives.[3] These were separated on silicic acid into anhydro compounds (tetrahydrofuran derivatives [15]) (30 % diethyl ether in hexane, v/v) and natural structures (75 % diethyl ether in hexane, v/v). The last fraction was further separated on paper on a preparative scale as described earlier.[9] Of six fractions obtained three were further characterized. One was

* Communications 7 and 8 in this series are Refs. 1 and 2, respectively.

** Until an international nomenclature is agreed upon we will use the following semi-systematic designations. *Sphingosines* means the group of sphingosine (8 in Table 1), dihydrosphingosine (9 in Table 1), phytosphingosine (12 in Table 1), dehydrophytosphingosine (13 in Table 1), homologues of these and related amines. *Sphingosine* is the singular form of sphingosines as defined above. Individual sphingosines are given prefixes expressing chain length, number of hydroxyls, and unsaturation. The monoenic dihydroxy C_{18}-sphingosine, then, means both 8 and 11 in Table 1; the saturated trihydroxy C_{18}-sphingosine means phytosphingosine, *etc.*

Table 1.

Number of C-atoms	Structure	Reference
16	1. 1,3-dihydroxy-2-amino-4-*trans*-hexadecene [a]	3
16	2. 1,3-dihydroxy-2-amino-hexadecane	3
16	3. 1,3-dihydroxy-2-amino-hexadecadiene [b]	4
17	4. 1,3-dihydroxy-2-amino-4-*trans*-heptadecene	3
17	5. 1,3-dihydroxy-2-amino-heptadecane	3
17	6. 1,3-dihydroxy-2-amino-heptadecadiene [b]	4
17	7. 1,3,4-trihydroxy-2-amino-heptadecane	4
18	8. D-*erythro*-1,3-dihydroxy-2-amino-4-*trans*-octadecene	5
18	9. D-*erythro*-1,3-dihydroxy-2-amino-octadecane	5
18	10. 1,3-dihydroxy-2-amino-4,14-*trans*, *trans*-octadecadiene	3
18	11. 1,3-dihydroxy-2-amino-octadecene (double bond not in 4 position)	6
18	12. D-*ribo*-1,3,4-trihydroxy-2-amino-octadecane	7
18	13. D-*ribo*-1,3,4-trihydroxy-2-amino-8-*trans*-octadecene	7
19	14. 1,3,4-trihydroxy-2-amino-nonadecane	This paper
19	15. 1,3-dihydroxy-2-amino-nonadecene	This paper
20	16. D-*erythro*-1,3-dihydroxy-2-amino-4-*trans*-eicosene	8
20	17. 1,3-dihydroxy-2-amino-eicosane [c]	9
20	18. 1,3,4-trihydroxy-2-amino-eicosane	10

[a] Other workers [13] recently suggested the existence of this compound in a chromatographic mixture of sphingosines.

[b] Identified as homologues to 10 after lead tetraacetate oxidation of the DNP derivatives.[3]

[c] Proposed also by others [11,12] on basis of oxidation products of mixtures of sphingosines.

identified as a saturated trihydroxy C_{18}-sphingosine and one as a saturated trihydroxy C_{20}-sphingosine.[1] The third component had an R_F value suggesting a C_{19}-homologue of the other two. (Found: N 8.87. Calc. for the DNP derivative of a saturated trihydroxy C_{19}-sphingosine, $C_{25}H_{43}O_7N_3$: 8.44.) Gas chromatography after sodium periodate oxidation revealed a C_{16}-aldehyde consistent with the structure of a saturated trihydroxy C_{19}-sphingosine. Further information was obtained after gas chromatography of the trimethylsilyl ethers[16] of the free sphingosine fraction, see Fig. 1. The chromatogram shows six components. A (47 %) and C (50 %) were identified as saturated trihydroxy sphingosines with 18 and 20 carbon atoms, respectively. (1,3,4-trihydroxy-2-amino-octadecane, used as a reference, was a gift from Professor H. E. Carter.[7]) The retention time of B (3 %) is in agreement with a C_{19}-homologue. 1, 2, and 3 in Fig. 1 correspond in amounts to A, B, and C, respectively, and are probably tetrahydrofuran derivatives[15] formed during the acid degradation. The mass spectrometry of these components is under investigation.[16]

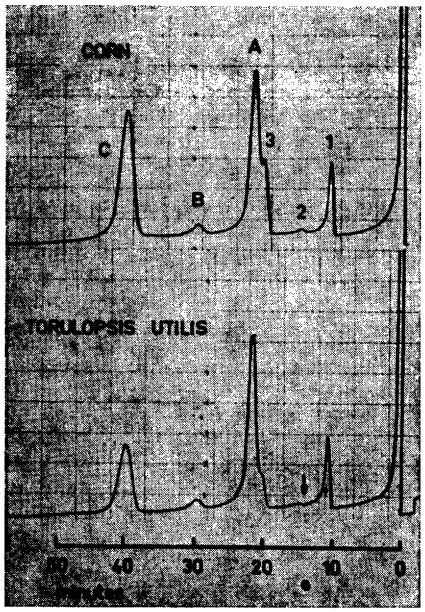

Fig. 1. Gas-liquid chromatograms showing trimethylsilyl ethers of total sphingosines of a cerebrin from corn and a cerebrin from the yeast *Torulopsis utilis*. A, B, and C correspond to saturated trihydroxy sphingosines with 18, 19, and 20 carbon atoms, repsectively. 1, 2, and 3 are probably their corresponding dehydration products. The arrow in the lower chromatogram indicates the retention time for the saturated dihydroxy C_{18}-sphingosine, not found to be present in the analyzed fractions.

Recently, the sphingosine composition of cerebrin from *Torulopsis utilis* was published.[17] After sodium periodate oxidation and gas chromatography aldehydes with 15 (82 %), 16 (3 %) and 17 (15 %) carbon atoms were established. On basis of this the probable presence of a saturated trihydroxy C_{18}-sphingosine, a saturated dihydroxy C_{18}-sphingosine, and a saturated trihydroxy C_{20}-sphingosine was concluded. However, the C_{16}-aldehyde could also derive from a saturated trihydroxy C_{19}-sphingosine. Furthermore, the absence of a corresponding saturated dihydroxy C_{20}-sphingosine made the presence of the dihydroxy C_{18}-sphingosine less likely. To test this we reinvestigated the sphingosine composition of cerebrin from *Torulopsis utilis*. After periodate oxidation C_{15}- (44 %), C_{16}- (7 %), and C_{17}-aldehydes (49 %) were identified. By concentrating the DNP sphingosine fraction no substance was found in the expected interval of a dihydroxy C_{18}-sphingosine. Instead the chromatogram in Fig. 1 indicates the presence of trihydroxy sphingosines with 18 (61 %), 19 (4 %), and 20 (35 %) carbon atoms.

The saturated trihydroxy C_{19}-sphingosine has also been isolated as a DNP derivative in small amounts from animal hair ceramides.

Evidence for the existence of a monoenic dihydroxy C_{19}-sphingosine in human and rat brain and human kidney glycolipids[18] was obtained after lead tetraacetate oxidation of a purified DNP sphingosine fraction.[3] After hydrogenation of the kidney glycolipids the saturated dihydroxy C_{19}-sphingosine was indicated both as a DNP derivative on paper chromatograms and as a trimethylsilyl ether on gas chromatograms. The mass spectrometry of the last mentioned component is under investigation.

Details of this work will be published later. The authors are indebted to Zellstoff-Fabrik Waldhof, Mannheim, Germany, for the generous gift of the cerebrin from *Torulopsis utilis*.

1. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 2397.
2. Karlsson, K.-A. *Sphingosine Composition of Sphingomyelins after Different Acid Degradation Procedures*, Lecture at The 9th International Conference on the Biochemistry of Lipids at Nordwijk, Holland, September 5th–10th, 1965.
3. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 2395.
4. Karlsson, K.-A. *To be published.*
5. Carter, H.E. and Fujino, Y. *J. Biol. Chem.* **221** (1956) 879.
6. Carter, H.E., Hendry, R.A., Nojima, S., Stanaćev, N.Ž. and Ohno, K. *J. Biol. Chem.* **236** (1961) 1912.
7. Carter, H.E. and Hendrickson, H.S. *Biochemistry* **2** (1963) 389.
8. Majhofer-Oreščanin, M. and Prostenik, M. *Croat. Chem. Acta* **33** (1961) 219.
9. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 565.
10. Prostenik, M. and Stanaćev, N.Ž. *Chem. Ber.* **91** (1958) 961.
11. Sambasivarao, K. and McCluer, R.H. *J. Lipid Res.* **5** (1964) 103.
12. Stanaćev, N.Ž. and Chargaff, E. *Biochim. Biophys. Acta* **98** (1965) 168.
13. Gaver, R.C. and Sweeley, C.C. *J. Am. Oil Chem. Soc.* **42** (1965) 294.
14. Sweeley, C.C. and Moscatelli, E.A. *J. Lipid Res.* **1** (1959) 40.
15. O'Connell, P.W. and Tsien, S.H. *Arch. Biochem. Biophys.* **80** (1959) 289.
16. Karlsson, K.-A. *Acta Chem. Scand.* **19** (1965) 2425.
17. Stanaćev, N.Ž. and Kates, M. *Can. J. Biochem.* **41** (1963) 1330.
18. Mårtensson, E. *Acta Chem. Scand.* **17** (1963) 2356.

Received November 10, 1965.

Studies on Sphingosines

10. Use of Trimethylsilyl Ethers for the Gas Chromatography and Mass Spectrometry of Sphingosines*

KARL-ANDERS KARLSSON

Institute of Medical Biochemistry, University of Göteborg, Göteborg, Sweden

At present about 20 sphingosines are known,[1] differing in chain length, number of hydroxyls, and unsaturation (for nomenclature, see Ref. 1). To study the metabolism of these substances methods for the microscale identification and estimation of the different components must be available. The recent development of instruments which combine a gas chromatograph and a mass spectrometer [2-4] has afforded excellent opportunities for both structural analysis and estimation of isotopes in biosynthetic studies.[5] The present communication describes the application of this type of analysis to trimethylsilyl ethers of saturated dihydroxy sphingosines.

Use of trimethylsilyl ethers for gas chromatography of hydroxy compounds has appeared frequently in the literature the last few years, *e.g.* Refs. 6, 7. We have found these derivatives helpful also for sphingosine analysis [8] and recently Gaver and Sweeley used the same derivatives for the gas chromatography of sphingolipid hydrolysates.[9] However, when acid has to be used to free the sphingosines (which is the case for all lipids except ceramides) there is a problem of interference of by-products of trihydroxy sphingosines (tetrahydrofuran derivatives [10]) and of allylic group containing sphingosines (nucleophilic substitution,[11,8] isomerization,[12] and dehydration [12] products). This is illustrated in Fig. 1. For plant tissues, where no allylic sphingosines have yet been found, the problem is limited to one product of

* Communication 9 in this series is Ref. 1. The results of the present communication were presented at *The 9th International Conference on the Biochemistry of Lipids* at Nordwijk, Holland, September 5th–10th, 1965.