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A New Ceramide from the Ascomycete *Tuber indicum*

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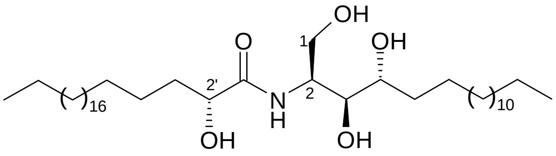
Abstract: A new C-$_{18}$ phytosphingosine derivative (**1**) was isolated from the fruiting bodies of *Tuber indicum*. Its structure was established as (2*S*, 3*S*, 4*R*, 2′*R*)-2-N-(2′-hydroxytricosanoyl)-octadecan-1, 3, 4-triol by spectral and chemical methods.

Keywords: *Tuber indicum* Cooke *et* Massee, ascomycete, ceramide.

In the course of our study on fungi-derived bioactive metabolites in Yunnan province, the chemical constituents of the truffle *T. indicum* (Tuberaceae) have been investigated. The present report deals with the structural elucidation of a new ceramide **1**, isolated from the $CHCl_3$-MeOH (1:1) extract of the fruiting bodies of this fungus.

Figure 1 The structure of new ceramide **1**



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Compound **1**, white amphorous powder, $[\alpha]_D^{27}$ +9.6 (c 0.25, pyridine). Its molecular formula was determined as $C_{41}H_{83}NO_5$ by high resolution EI-MS (669.6214 $[M]^+$, calcd. 669.6271). Its IR spectrum revealed the absorptions of hydroxyls and an amide at 3340~3220 cm^{-1}, a secondary amide at 1548, 1619 cm^{-1}, and long aliphatic chains at 722 cm^{-1}. The ^1H NMR spectrum of **1** showed the presence of two terminal methyls at δ 0.86 (br t, 6H, J = 6.8 Hz), and methylenes at δ 1.25-1.40 (br s), an amide proton at δ 8.58 (d, 1H, J = 8.8 Hz). The ^{13}C NMR (DEPT) spectrum of **1** further furnished 1×C, 4×CH, $1\times CH_2$, $2\times CH_3$ (**Table 1**), in which a quaternary carbon at δ 175.45 (CONH), four methines at δ 53.12 (CHNH), 73.16 (CHOH), 72.55 (CHOH), 76.90 (CHOH), and a methylene at δ 62.13 (CH_2OH) were given, respectively. **1** contained five characteristic signals of geminal protons to hydroxyls at δ 4.29 (m), 4.35 (dd, J = 6.5, 4.1 Hz), 4.63 (dd,

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J = 7.6, 4.1 Hz), 4.42 (dd, J = 10.7, 5.2 Hz) and 4.53 (dd, J = 10.7, 4.5 Hz), and a sixth signal at δ 5.12 (m) as a methine proton vicinal to the nitrogen atom. These data revealed that **1** should be a phytosphingosine-type ceramide containing 2-hydroxy fatty acid[1]. Methanolysis of **1** afforded a methyl 2′-hydroxytri- cosanoate identified by GC/MS[2,3]. The occurrence of this fatty acyl moiety in **1** was also supported by the significant fragment ion peaks at *m/z* 370 $[CH_3(CH_2)_{20}CH(OH)\ CONH_2+H]^+$ and 357$[M-CH_3(CH_2)_{20}-OH]^+$ in the EI-MS. The remaining part is there- fore a C_{18}-phytosphingosine unit containing three hydroxyls and an amino group. Treatment of methanolysis residue of **1** with Ac_2O/pyridine provided a tetraacetylphyto-sphingosine and its ^1H NMR spectrum was found to be identical to that of the known counterpart[1, 2].

The relative stereochemistry of **1** at C-2, C-3, C-4, and C-2′ was proposed as 2*S*, 3*S*, 4*R*, 2′*R*, since the chemical shifts and coupling constants of H-1, H-2, H-3, H-4 and H-2′ in **1** were in good agreement with those of the synthetic ceramide[4]. The comparison of the optical rotations of **1** with the synthetic ceramide ($[\alpha]_D$ +9.1° pyridine, c =1.0) suggested that **1** has the same configuration at asymmetric centers 2, 3, 4, 2′ as that of the synthetic one. Thus, from all of the above evidence the structure of **1** was characterized as (2*S*, 3*S*, 4*R*, 2′*R*)- 2-N-(2′-hydroxytricosanoyl)-octadecan-1, 3, 4-triol (**Figure 1**).

Table 1 The NMR spectral data for compound **1** in pyridine-$d_5{}^{\delta}$ (δppm)

·C/H	^{13}C	^1H (*J* Hz)	C/H	^{13}C	^1H (*J* Hz)
1	62.13 (t)	4.53 (dd, 10.7, 4.5) 4.42 (dd, 10.7, 5.2)	1′	175.45 (s)	
2	53.12 (d)	5.12 (m)	2′	72.55 (d)	4.63 (dd, 7.6, 4.1)
3	76.90 (d)	4.35 (dd, 6.5, 4.1)	3′	35.76 (t)	2.23, 2.04 (m)
4	73.16 (d)	4.29 (m)	4′	25.85 (t)	1.77 (m)
5	34.24 (t)	1.93 (m)	$(CH_2)_{18}$	29.64 ~30.37 (t)	1.25 ~1.40
6	26.66 (t)		23′	14.26 (q)	0.86 (t, 6.8)
$(CH_2)_9$	29.64~30.37 (t)	1.25 ~1.40	NH		8.58 (d, 8.8)
16~17	32.15, 22.96 (t)				
18	14.26 (q)	0.86 (t, 6.8)			

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