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Absolute Stereostructure of a 2,3,7,13-Tetrahydroxyoctadecanoic Acid, the Framework of Taurolipid B Produced by a Fresh-water Protozoan, *Tetrahymena thermophila*

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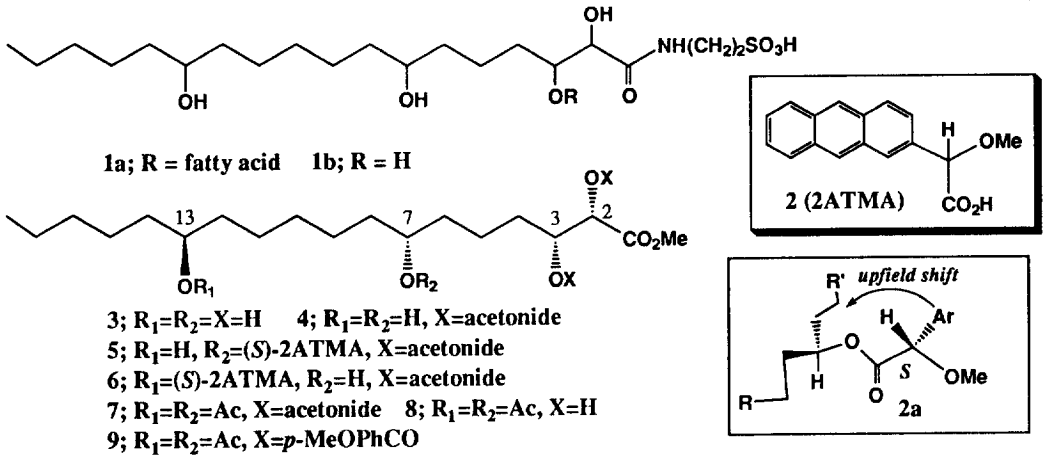
Abstract: The absolute configuration of the four asymmetric centers of 2-(2,3,7,13-tetrahydroxyoctadecanoylamino)ethanesulfonic acid, which is the framework of taurolipid B isolated from a fresh-water protozoan, *Tetrahymena thermophila*, as one of the major taurolipids, has been determined by NMR-spectroscopy with the use of a new chiral anisotropic reagent, 2ATMA, as well as CD spectroscopy.

A series of taurolipids[1] has been isolated from a fresh-water protozoan *Tetrahymena thermophila*, and their unique chemical features have been clarified as: *taurolipid A*; 2-(3-acyloxy-7,13-dihydroxyoctadecanoylamino)ethanesulfonic acid, *taurolipid B*[1b] (**1a**); 2-(3-acyloxy-2,7,13-trihydroxyoctadecanoylamino)-ethanesulfonic acid, *taurolipid C*; 2-(3-acyloxy-2,7,12,13-tertrahydroxyoctadecanoylamino)ethanesulfonic acid. Taurolipid B exhibits growth-inhibitory activity against HL-60. Mild hydrolysis of taurolipid B gives a tetrahydroxy compound (**1b**), which possesses four asymmetric centers. This paper describes determination of the absolute configuration of the four hydroxy groups of **1b** by using 2ATMA (**2**; 2-anthrylmethoxyacetic acid),[2] a newly developed chiral anisotropic reagent.

It has been firmly established that the conformation of the sterically unhindered MPA[3] (methoxyphenylacetic acid) and 2NMA[4] (2-naphthylmethoxyacetic acid) moieties is as shown in **2a**, in which the carbinyl proton, carbonyl oxygen, and methoxy groups are oriented in the same plane. We have recently demonstrated[5] that (i) the 2ATMA moiety also exists in the same conformation (**2a**) in acyclic and cyclic compounds, and (ii) the upfield shifts of the protons located on the same side of the anthryl group are both extraordinary and in a wide range. It may be, therefore, safely said that *the absolute configuration of simple long-chain secondary alcohols, in which no serious steric or dipole interaction from the other part of the molecule is present, can be deduced by analyzing the ^{1}H-NMR spectrum of either (R) or (S)-2ATMA ester, in other words, without calculating $\Delta\delta$ (δ_R - δ_S) values.*

Methanolysis of **1b** (12 *M* HCl/MeOH, reflux, 18 h) afforded tetrahydroxy methyl ester (**3**) in a good yield. The vicinal hydroxy groups at C-2 and 3 were protected by dimethylacetalization (acetone/$CuSO_4$/H^+) to give acetonide (**4**). This compound was treated with 1.0 eq (*S*)-2ATMA/EDC/DMAP/CH_2Cl_2, giving a mixture of mono-2ATMA esters (**5** and **6**) together with a small amount of di-2ATMA ester. In HOHAHA spectrum of **5**, 2-H and 3-H (δ 4.05) are correlated with the signal at δ 4.94 (CO-O-C<u>H</u>), thus suggesting that 7-OH is esterified and 13-OH is free. On the contrary, 2-H and 3-H (δ 4.10) are correlated with the signal at



δ 3.17 (HO-CH) in the ^{1}H NMR spectrum of **6**, confirming that 7-OH is free and 13-OH is esterified.

Figure 1 shows the parts of the ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of (*S*)-2ATMA esters **5** (a) and **6** (b). The remarkably shielded ($\Delta\delta' > 0.05$; $\Delta\delta' = \delta_{alcohol} - \delta_{2ATMA\ ester}$) signals are darkened in both spectra. The upfield signals of spectrum (a) were easily assigned to 8-H ~ 12-H [δ 1.36 (8-H), 0.82 (9-H), 0.90 (10-H), 0.72 (11-H), 0.93 (12-H)] by 2D spectra, and, because they are shielded by the anthryl group of (*S*)-2ATMA, the absolute configuration of 7-OH was determined to be *R* (see conformation **2a**). By a similar analysis of the spectrum (b) of **6**, *S*-configuration was assigned for 13-OH.

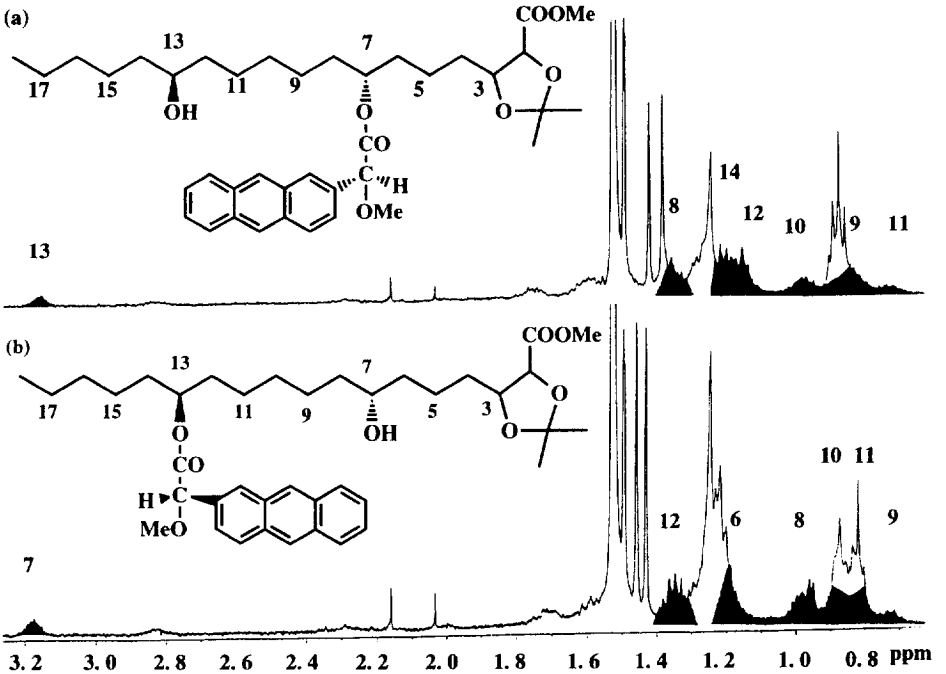
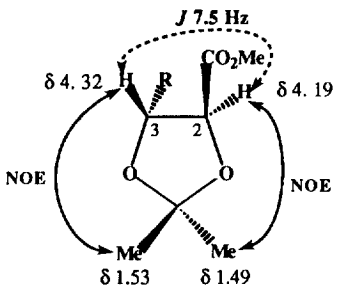


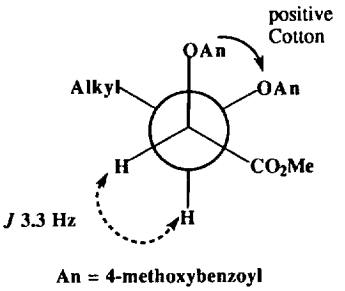
Figure 1. The parts of the ^{1}H-NMR (400 MHz, $CDCl_3$) spectra of **5** (a) and **6** (b).

In the ^{1}H NMR spectrum of acetonide (**4**), 2-H appears at δ 4.19 as a doublet (J = 7.5 Hz). This coupling constant agrees well with J_{trans} (4, 5-H) of 2,2,4,5-tetramethyl-1,3-dioxolane.[6] The *trans* relationship of 2-H and 3-H of **4** was further confirmed by (i) the downfield chemical shift of 3-H (δ 4.32), deshielded by the vicinal methoxycarbonyl group, and (ii) the presence of NOEs between 2-H and upfield methyl (δ 1.49), and 3-H and downfield methyl (δ 1.53) (see **4a**). These findings firmly established the *trans* relation of 2-H and 3-H, and thus the *threo* relationship of the 2,3-dihydroxy group of **3**.

Acetylation of dihydroxyacetonide (**4**) afforded diacetate (**7**), which was treated with aqueous acetic acid to give diol (**8**). The glycol was allowed to react with 4-methoxybenzoyl chloride in pyridine, producing



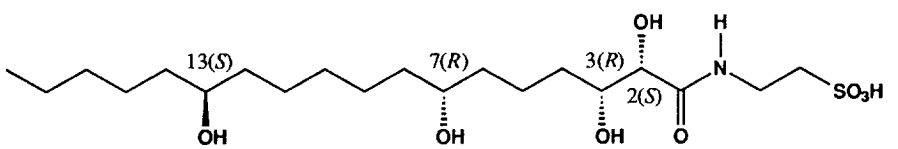
4a



9a

dianisoate (**9**). The coupling constant between 2-H and 3-H was 3.3 Hz, indicating the *gauche* conformation of these two protons.[7] Moreover, the CD spectrum shows a positive Davydov-split Cotton effect [λ_{ext} 263 nm (Δε +8.01), λ_{ext} 242 nm (Δε -1.39) (MeOH)], which established the 2(*S*) and 3(*R*) configurations (see **9a**) of the glycol moiety.[8,9]

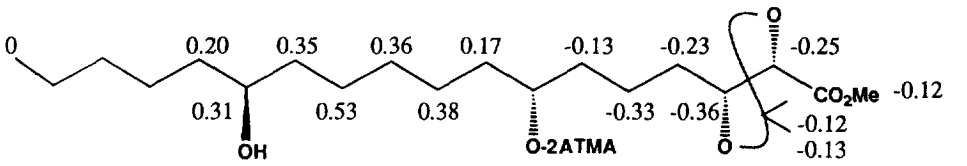
All these experiments lead to 2(*S*), 3(*R*), 7(*R*), 13(*S*) configuration of the tetrahydoxystearyl amide (**1b**).



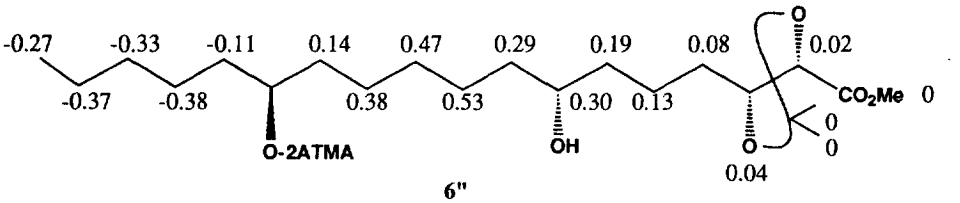
1b

It should be emphasized that only one enantiomer of 2ATMA, (*S*)-2ATMA in this case, was necessary for the absolute configuration of 7 and 13-hydroxy groups. The same absolute configuration, of course, must be deduced when (*R*)-2ATMA is applied. In fact, the same conclusion was obtained by analysis of 7-[(*R*)-2ATMA]-oxy [**5'**; (*R*) instead of (*S*)] and 13-[(*R*)-2ATMA]-oxy [**6'**; (*R*) instead of (*S*)] acetonides: In their ^{1}H NMR spectra, 2-H ~ 6-H are remarkably (Δδ'> 0.05) shielded [δ 3.81 (2-H), 3.68 (3-H), 1.43 (4-H), 1.05 (5-H), 1.46 (6-H)] in the case of **5'**, and 14-H ~ 18-H [δ 1.38 (14-H), 0.89 (15-H), 0.91 (16-H), 0.87 (17-H), 0.56 (18-H)] showed marked upfield shifts in the case of **6'**, supporting 7(*R*) and 13(*S*) configurations, respectively.

The $\Delta\delta$ values [$\Delta\delta = \delta_{(R)\text{-2ATMA}} - \delta_{(S)\text{-2ATMA}}$] obtained for **5** and **6** are depicted in structures **5"** and **6"**. The systematic arrangement of positive and negative $\Delta\delta$ values is further evidence of the absolute configuration of 7 and 13-OH as well as the "ideal conformation" of the 2ATMA moiety as shown in **2a**.



5"



6"

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