Detection of Regioisomeric Macrocyclic Tetraethers in the Lipids of *Methanobacterium thermoautotrophicum* and other Archaeal Organisms

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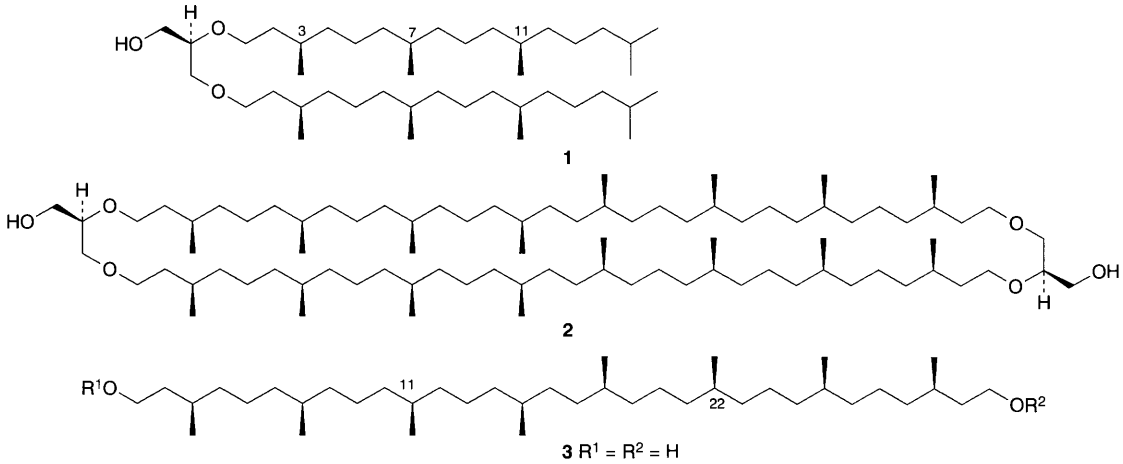
Chemical degradation shows that an apparently homogeneous preparation from the lipids of *Methanobacterium thermoautotrophicum* is in fact a mixture of the regioisomeric compounds **2** and **8** differing in the relative orientation of their glycerol units; a similar situation holds for the related diglycerol tetraethers of *Thermoplasma acidophilum* and *Sulfolobus solfataricus*.

One of the distinctive features of the archaea is the fact that their membrane core lipids consist of isoprenoid glycerol ethers instead of the fatty acid glycerol esters normally found in bacteria and eucarya.[1] Two main structural types have been identified, corresponding to the diphytanyl-glycerol diether **1**,† originally detected in *Halobacterium cutirubrum*,[3] and to the macrocyclic dibiphytanyl-diglycerol tetraether **2**,† first isolated from *Thermoplasma acidophilum*.[4] The unusual (*R*) configuration in the glycerol unit of **1** has been secured by comparison with synthetic material.[5] The (*R*) configuration of C-7 and C-11 in the phytanyl side chains follows from identification of degradation products with authentic material, whereas the configuration assigned to C-3 rests only on comparison of optical rotation.[6] The absolute configuration of the glycerol stereocentres of **2** was first taken to match the corresponding centre in **1** because of the similarity in the molecular rotations of the two compounds[7] and was later secured for the case of the diglycerol tetraethers from *Sulfolobus acidocaldarius* by appropriate incorporation experiments.[8] Proof for the configuration of the stereocentres in the biphytanyl units of **2** has been provided through a stereorational synthesis of the corresponding diol **3**.[9,10]‡ The antiparallel arrangement of the glycerol units indicated in **2** was first suggested arbitrarily by Langworthy;[4] later, NMR investigations by De Rosa *et al.*[11] on the related but more highly condensed tetraethers from *Caldariella acidophila* (now reclassified as *Sulfolobus solfataricus*[12]) were taken as a support for such an arrangement. This proposal has been tacitly extrapolated to all other tetraethers and has found unchallenged acceptance by other workers in the field. We now provide chemical evidence showing that in three different archaeal species the apparently homogeneous tetraether fractions consist of a nearly statistical mixture of regioisomers.

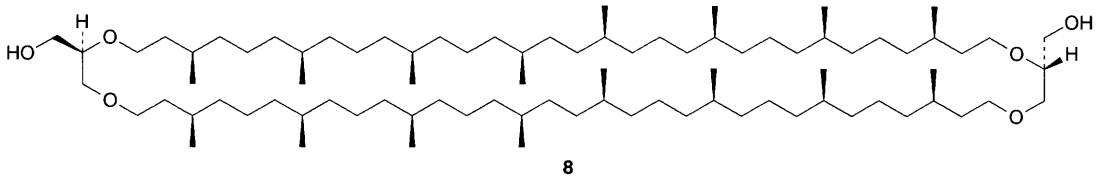
In the course of our studies on the biosynthesis of **2** in *Methanobacterium thermoautotrophicum* Marburg (DSM 2133) we were led to develop for this compound a degradation procedure that would preserve the regioheterotopicity of its head-to-head linked phytanyl moieties. A specimen of the tetraether isolated from lyophilized cells according to the standard procedure[13] appeared homogeneous by TLC and ^{13}C NMR spectroscopy and displayed an optical rotation $[\alpha]_D^{20}$ +8.8° (*c* 0.9, $CHCl_3$) comparable to literature values.[2,7] The desired chemical degradation was performed in two ways as shown in Scheme 1. In the first sequence (reactions i and ii) the ditosylate of the starting material was submitted to the Boord haloalkoxy elimination reaction.[14] This gave, after filtration through Sephadex LH 20, a mixture shown by TLC to contain three



1

2

3 $R^1 = R^2 = H$
4 $R^1 = H$, $R^2 = CH_2CH{=}CH_2$
5 $R^1 = R^2 = CH_2CH{=}CH_2$
6 $R^1 = H$, $R^2 = CH_2COMe$
7 $R^1 = R^2 = CH_2COMe$



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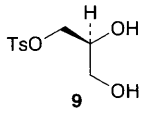

9

compounds. Futher purification by silicagel chromatography yielded, next to the expected monoallylether **4**§ as the main product, two additional compounds, which were identified as the diol **3** and its diallylether **5**. In the second sequence (reactions iii, iv and v) the ditosylate from reaction i was converted *via* the diiodide into a bisenol ether which upon hydrolysis yielded, in addition to the diol **3**, its mono- and bis-derivatives **6** and **7**. Since compounds **4**–**7** are recovered unchanged when submitted to the reaction conditions which lead to their formation, the results of the two degradations demonstrate that the apparently homogeneous starting material was in fact a mixture of the regioisomeric macrocyclic tetraethers **2** and **8**. An identical value of 45 : 55 for the ratio of the two isomers can be estimated from the relative yields of the products obtained in the two independent degradations. To verify the absolute configuration expected for the glycerol subunits of **2**, the mixture of ditosylates from **2** and **8** was treated with boron trichloride in methylene chloride[15] to give a sample of 1-tosylglycerol, **9**, with $[\alpha]_D^{20}$ +9.4 (*c* 3, MeOH), shown by comparison with literature data[16] to be enantiomerically pure and to possess the (*S*) configuration. This result confirms independently the (*R*) configuration of the glycerol subunits of **2** and proves the same absolute configuration for the corresponding units of **8**.¶ For convenience, we suggest to retain the caldarchaeol denomination[2] for tetraethers with an antiparallel arrangement of the glycerol units as in **2** and to adopt the name isocaldarchaeol for tetraethers of type **8**.

To check on the generality of our finding we have carried out similar degradations on diglycerol tetraether preparations from the Hveragerdi strain of *M. thermoautotrophicum* as well as on the related compounds from *T. acidophilum*[4] and *S. solfataricus* P1.[17] The results showed that the starting materials were in each case a nearly statistical mixture of the caldarchaeol–isocaldarchaeol type compounds exemplified by **2** and **8**. In biosynthetic terms the nearly statistical occurrence of the two structural types suggests that the unknown process responsible for the head-to-head condensation of the diterpene precursors is largely insensitive to the relative orientation of the polar glycerol heads.

We thank Dr P. Galliker for developing and making available to us a 2 l fermenter appropriate for incubation experiments. We are indebted to Professor Dr T. Leisinger (ETH Zürich) and his coworkers for technical help in the fermentation of large batches of *M. thermoautotrophicum*, to Professor Dr R. Bachofen (Universität Zürich) for providing frozen cells of *M. thermoautotrophicum* and to Professor Dr W. Zillig (Max Planck Institut für Biochemie, Martinsried, Germany) for a generous gift of batches of *S. solfataricus* and *T. acidophilum*. Grateful acknowledgement is made to Sandoz, Basel, for financial support. O. G. acknowledges a Kekulé-Stipendium (Stiftung Stipendien-Fonds des Verbandes der chemischen Industrie, Frankfurt/Main) and a G. Rosenkranz fellowship.

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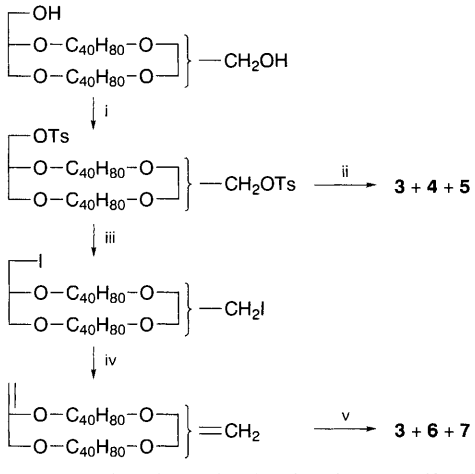

Scheme 1 *Reagents and conditions*: i, 1.5 equiv. toluene-*p*-sulfonyl chloride in pyridine, 3 d, room temp., 90%; ii, 3.25 equiv. NaI, 7.5 equiv. Zn powder in DME, 1 d, 90 °C, 65%; iii, 3 equiv. NaI in DME, 3 d, 90 °C, 97%; iv, 3 equiv. KOBu^t in Me_2SO, 1 h, room temp., 93%; v, HCl (50%), 20 min, 95%

Footnotes

† Nishihara *et al.*[2] have proposed the general name 'archaeol' for the glycerol diethers and 'caldarchaeol' for the diglycerol tetraethers.

‡ In the original paper[9] wrong descriptors have been used for the designation of the C-11 and C-22 stereocentres. This has been corrected,[10] but unfortunately the correction has been widely ignored by subsequent authors.

§ All new compounds mentioned in this paper gave satisfactory IR, MS, ^{1}H NMR and ^{13}C NMR data.

¶ Note that the same absolute configuration requires different descriptors in **9** *vs.* **2** and **8** as a consequence of a change in the priority of the substituents.

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