Tetrahedron 70 (2014) 7322–7335

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthetic epoxy-mycolic acids

Dakhil Z. Al Kremawi, Juma'a R. Al Dulayymi, Mark S. Baird *

School of Chemistry, Bangor University, Gwynedd LL57 2UW, UK

ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 12 June 2014
Accepted 20 June 2014
Available online 2 July 2014

Keywords:
Mycolic acid
Environmental
Mycobacteria
Epoxy

ABSTRACT

We report the synthesis of single enantiomers of epoxy-mycolic acids containing an α-methyl-*trans*-alkene or a *cis*-cyclopropane with structures that match those of major isomers of such molecules present in complex mixtures in Mycobacteria such as *Mycobacterium fortuitum* or *Mycobacterium smegmatis*

1. Introduction

Mycolic acids (MAs), **1** (Fig. 1), are major constituents of the cell envelope of *Mycobacterium tuberculosis* and other mycobacteria, some of which are pathogenic to animals and humans.[1–3] Their structure and biosynthesis has been reviewed,[4] and a number of structural and stereochemical relationships examined.[5] Their presence is thought to be linked to the resistance of these organisms to most current antibiotics and other chemotherapeutic agents.[6]

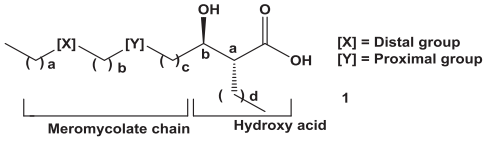
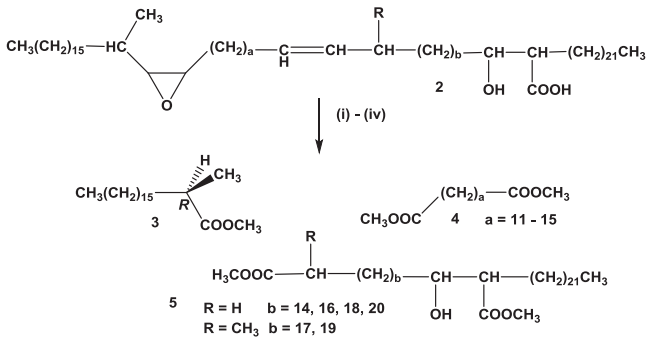


Fig. 1. The typical structure of a mycolic acid.

The two stereocentres in the α and β-positions relative to the carboxylic group have both been found to be in the *R*-configuration for all the mycolic acids examined, irrespective of the other functional groups.[7,8] In each case 'a–d' represent long alkyl chains, and generally each *Mycobacterium* contains a mixture of several homologues. In the common classes of MA, the proximal group is often a cyclopropane or an alkene and the distal group is a cyclopropane (α-MA), an α-methoxy-β-methyl fragment (methoxy-MA) or an α-keto-β-methyl fragment (keto-MA).[2,3] In 1981, Daffé et al. reported the identification of a new kind of mycolic acid in *Mycobacterium fortuitum* containing an α-methyl epoxy-group at the distal position and an alkene at the proximal position (**2**, R=H) (Scheme 1).[9] Minnikin et al. later described the presence of similar molecules in *M. fortuitum*, *Mycobacterium farcinogenes*, *Mycobacterium senegalense*, '*Mycobacterium peregrinum*', and *Mycobacterium smegmatis*;[10–12] in these cases the major isomers had a *cis*-alkene at the proximal position, but there was a minor component (ca. 30% by NMR spectroscopy) (**2**, R=Me) containing a proximal α-methyl-*trans*-alkene; the major isomer of the latter contained 78 carbons; the epoxy-MA had carbon skeletons similar to those in methoxy-, keto- or α-MA described earlier.[10]



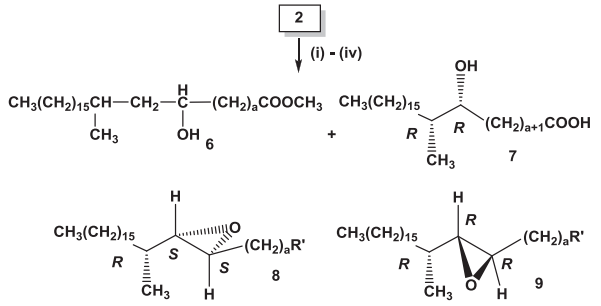
Scheme 1. (i) Acetolysis; (ii) saponification; (iii) oxidative cleavage; (iv) methylation.

* Corresponding author. Tel.: +44 1248382374; fax: +44 1248370528; e-mail address: chs028@bangor.ac.uk (M.S. Baird).

http://dx.doi.org/10.1016/j.tet.2014.06.089
0040-4020/

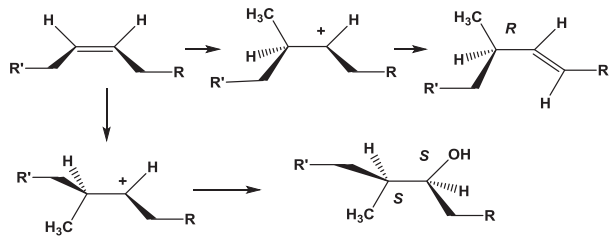
Epoxy-MAs are also present in *Mycobacterium chitae*,[13,14] '*Mycobacterium giae*',[13] *M. peregrinum*,[11–13] *Mycobacterium porcinum* and *M. senegalense*,[15] and in a mutant strain of *M. tuberculosis*.[16] In the final case, these included epoxy-MA with a *cis*-cyclopropane at the proximal position. Epoxy-mycolic acids have also been detected directly by MALDI-TOF mass spectrometry; the major isomers containing a *trans*-alkene at the proximal position are reported to be C_{78} and C_{80} molecules.[16–18] Studies using *M. smegmatis* have identified the function of the protein *MSMEG0913* in adding the methyl branch adjacent to both an alkene and a cyclopropane at the proximal position of epoxy-mycolates to produce the *trans*-homologues,[18] and examined other aspects of the biochemistry of such species.[19–22] The relative and absolute stereochemistry of epoxy-mycolates containing a proximal *cis*- or α-methyl-*trans*-alkene has been probed by two methods (Scheme 1).[23] Firstly, opening of the epoxide **2** by acetolysis, then saponification and oxidative cleavage of both the derived 1,2-diol and the alkene leads to three products, including (*R*)-ester **3**.

Secondly, reductive ring-opening of the epoxide **2** followed by oxidative cleavage of the proximal alkene, saponification and methylation led to the two acids **6** and **7** (Scheme 2). The latter was shown to have *R*,*R*-stereochemistry by comparison to a model compound. On this basis the authors assigned all the stereocentres in the epoxy-mycolic acid as *R*.[23] However, it seems clear in fact that the result actually suggests the epoxy fragment is *R*,*S*,*S* as in **8** rather than *R*,*R*,*R* as in **9**, the priorities in the epoxide being different from those in the ring-opened alcohol.



Scheme 2. (i) Reduction; (ii) oxidative cleavage; (iii) saponification; (iv) methylation.

What is arguably most interesting is that the methyl group adjacent to the epoxide is in the *R*-configuration, whereas that adjacent to the hydroxyl, methoxy or keto-groups at the distal position of the corresponding mycolic acids is of S-configuration. It is attractive to propose that the various types of mycolic acid are formed through a formal intermediate carbocation, formed by methylation of a *cis*-alkene by SAM (Scheme 3). Methylation from the *Re* or *Si* face of the alkene at the alkylated carbon could then lead to a divergence in stereochemistry.

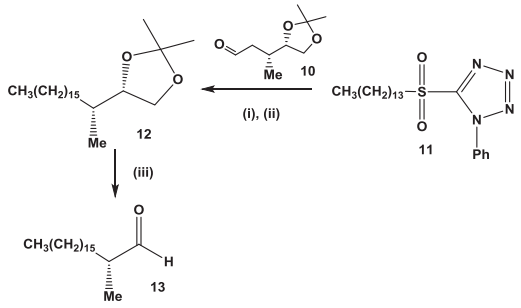


Scheme 3. Possible formal mechanism for production of *S*- and *R*-methyl branches by methylation of a *cis*-alkene by SAM.

We have reported the synthesis of an α-mycolic acid,[24,25] of methoxymycolic acids with either absolute stereochemistry at the *cis*-cyclopropane or α-methyl-β-methoxy fragment,[26] and of keto mycolic acids.[27,28] We have also reported the synthesis of methoxy and keto mycolic acids containing an α-methyl-*trans*-cyclopropane unit,[29,30] of *cis*-alkene mycolic acids,[31] and of hydroxy- and keto mycolic acids containing an (*R*)-α-methyl-*trans*-alkene unit.[32] We now report in full the synthesis of two stereoisomeric epoxy-mycolic acids containing an (*R*)-α-methyl-*trans*-alkene at the proximal position; these have the chain lengths reported for one mycolic acid in *M. fortuitum*,[4,5] and the total carbon number is consistent with those observed for one component of a mixture from *M. smegmatis* by MALDI mass spectrometry.[17,18] This has been reported briefly earlier.[33] We also report the synthesis of an epoxy-mycolic acid containing a *cis*-cyclopropane.

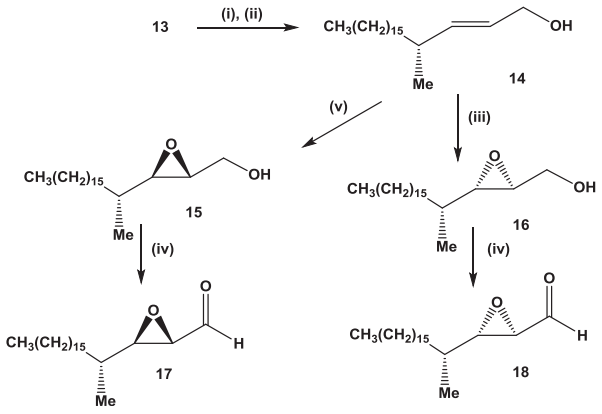
2. Results and discussion

The aldehyde **13** was first prepared from the known aldehyde **10**,[28,34,35] with sulfone **11** (see Supplementary data) and base in a modified Julia-Kocienski reaction, followed by hydrogenation to give **12**. Deprotection and oxidative cleavage of the acetal **12** led to **13** (Scheme 4).



Scheme 4. (i) **10,** $LiN(SiMe_3)_2$ (LiBSA), THF, −10 °C (67%); (ii) H_2, Pd/C, ethanol, (92%); (iii) periodic acid, dry ether (77%).

A Wittig reaction and then reduction with DIBAL led to the *trans*-alcohol **14** (Scheme 5). Asymmetric epoxidation using Sharpless conditions provided the two diastereoisomers **15** and **16**.[36,37] The two epoxides showed $[\alpha]_D^{22}$ of +18.1 and −21.2, respectively. The literature value for (2*R*,3*R*)-dodecyloxiranyl-methanol is −25.5.[38]



Scheme 5. (i) $Ph_3P{=}CHCOOMe$, toluene (65%); (ii) DIBAL, CH_2Cl_2 (95%); (iii) L-(+)-diethyl tartrate, $Ti(Oi\text{-}Pr)_4$, *t*-BuOOH, CH_2Cl_2, −20 °C (75%); (iv) PCC, CH_2Cl_2 (59%, **18**), (60%, **17**); (v) D-(−)-diethyl tartrate, $Ti(Oi\text{-}Pr)_4$, *t*-BuOOH, CH_2Cl_2, −20 °C (67%).

Oxidation of each alcohol led to the corresponding aldehyde. The aldehyde **18** was chain extended by reaction with the sulfone **19** (see Supplementary data) and base, followed by saturation of the derived alkene using di-imide. The derived bromide **20**, $[\alpha]_D^{22}$ −13.1 (*c* 1.2, $CHCl_3$) ($[M]_D$ −76) {molecular rotation, $[M]_D$ = (molecular weight×specific rotation)/100}, was then converted into the corresponding sulfone **21**. In the same manner, diastereoisomer **17** was converted into **22** (Scheme 6). The molecular rotations of the two