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# Structure-function relationships of mycolic acids in tuberculosis

**by**

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**List of Abbreviations**

| | |
|---|---|
| AmB | Amphotericin B |
| 5-BMF | 5-Bromomethyl fluorescein |
| $^{13}C$ NMR | Carbon 13 NMR |
| $CCl_4$ | Carbon tetrachloride |
| $CDCl_3$ | Deuterated chloroform |
| CHCA | Cyano-4-hydroxycinnamic acid |
| d | Doublet |
| dd | Double doublet |
| dt | Doublet of triplets |
| DMAC | Dimethylacetamide |
| ELISA | Enzyme-linked immunosorbent assay |
| g | Gram |
| GC | Gas chromatography |
| $^{1}H$ NMR | Proton NMR |
| $H_2$ | Hydrogen |
| $H_2O_2$ | Hydrogen peroxide |
| HIV | Human immunodeficiency virus |
| HMPA | Hexamethylphosphorotriamide |
| HPLC | High performance liquid chromatography |
| Hz | Hertz |
| IMS | Industrial methylated spirits |
| INH | Isoniazid |
| IR | Infra red |
| L | Liter |

| | |
|---|---|
| LDA | Lithium *N*,*N*,-di-isopropylamide |
| m | multiplet |
| M | Molar |
| MA | Mycolic acid |
| MALDI-TOF MS | Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry |
| MAs | Mycolic acids |
| MDR | Multi-drug resistant |
| MeOH | Methanol |
| mg | Milligram |
| ml | Milliliter |
| mmol | Millimol |
| MS | Mass spectrometry |
| *M. tb* | *Mycobacterium tuberculosis* |
| *M. tuberculosis* | *Mycobacterium tuberculosis* |
| NMR | Nuclear magnetic resonance |
| OAc | *O*-Acetyl |
| PCC | Piridinium chlorochromate |
| Pd on C | Palladium on charcoal |
| q | Quartet |
| Rf | Retardation factor |
| RT | Room temperature |
| s | Singlet |
| t | Triplet |
| TB | Tuberculosis |
| THF | Tetrahydrofuran |

| | |
|---|---|
| THP | Tetrahydropyran |
| TLC | Thin layer chromatography |
| TMDM | Trimethylsilyl diazomethane |
| WHO | World Health Organisation |
| XDR | Extreme drug resistant |

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# Summary

Title: Sturcture-function relationships of mycolic acids in tuberculosis
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Tuberculosis (TB) is the leading cause of death among HIV infected people. *Mycobacterium tuberculosis* (*M. tuberculosis*), the causative agent of TB, features a distinctive lipid-rich cell wall with mycolic acids (MA) the major component in the outer layer. Mycolic acids are α-alkyl β-hydroxy long chain fatty acids, which exist in a number of chemical subclasses depending on the presence of functional oxygenated and non-oxygenated groups in the meromycolate chain. In numerous studies the different MA subclasses have been shown to play different roles in antibody recognition, virulence and the ability to attract cholesterol. It was previously suggested that the oxygenated MA might be important for these properties. Except for the mycolic acid motif, little is known about the stereochemistry of the other chiral centres. The importance of the different functional groups, their position and stereochemistry, for immunological properties, are not yet clarified. This study set out to resolve the structure-activity relationships of mycolic acids from *M. tuberculosis* in terms of their antigenicity and the ability to attract cholesterol. To determine fine specificity of interaction of MA with antibodies, the subclasses of MA from *M. tuberculosis* were separated and the antigenicity of two was determined. $TB^+$ and $TB^-$ patient sera recognised natural MA, alpha-MA and methoxy-MA. It was confirmed that the carboxylic acid group played a fundamental role in its recognition. Interestingly, cord factor (trehalose-6,6'-dimycolate) was recognised specifically by $TB^+$ sera. This implies multiple epitopes in the MA structure, some of which are very specific for TB patients. A stereocontrolled diastereomer of *cis*-cyclopropane methoxy-MA was synthesized and along with other synthetic methoxy-, keto- and hydroxy-MAs, were tested for antibody recognition. One diastereomer, *SS*-*SR*-methoxy-MA, was recognised stronger by $TB^+$ serum than the other, it also is the one that closest approximates the signal strength of antibody binding to natural MA by $TB^+$ patient sera. While the others are not specifically recognised, this *SS*-*SR*-methoxy-MA may well represent one of the antigenically active

components that occurs in natural MA and that elicits specific antibodies in TB patients. This thesis reports a stereocontrolled chemical synthesis of biologically active mycolic acids and shows that a single component of the mycolic acid mixture can be sufficient to elicit an immunological response.