MYCOLIC ACIDS OF REPRESENTATIVE STRAINS OF *NOCARDIA* AND THE 'RHODOCHROUS' COMPLEX

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1. Introduction

Mycolic acids are long-chain fatty acids of high molecular weight containing a long alkyl branch in the 2-position and a hydroxyl group in the 3-position. Mycolic acids have been isolated and characterised from Mycobacteria, Nocardiae and Corynebacteria [1]. Characteristic lipids detected by thin-layer chromatography (TLC) in strains of Nocardiae and organisms of the closely related 'rhodochrous' complex [2] have recently been shown to be free mycolic acids [3]. It was noted [2] that the characteristic lipids of many 'rhodochrous' strains had a significantly lower R_F value on TLC than those from cultures of *Nocardia sensu stricto*. This report will demonstrate that the mycolic acids of a representative of the 'rhodochrous' complex are of significantly lower molecular weight than those of a typical *Nocardia* strain.

2. Materials and methods

Nocardia caviae NCTC 1934 and a representative of the 'rhodochrous' complex (*Nocardia calcarea* NCIB 8863) were grown in modified Sauton's medium, tested for purity and harvested as described previously [2]. A lipid extract was prepared from freeze-dried cells by soaking in ethanol–diethyl ether (1:1) overnight and removing the extracted cells by filtration; the extraction solvents were removed by evaporation under reduced pressure at low temperature (< 37°C). The partially-extracted cells were exhaustively extracted with chloroform and the residue treated with 2.5 N-methanolic KOH in benzene for 6 hr. The resulting mixture was acidified with 2 N HCl and extracted with diethyl ether. Mycolic acids were isolated from this extract and the original ethanol–diethyl extract by preparative TLC on Merck Silica Gel $PF_{254+366}$ using hexane–diethyl ether–acetic acid (80:20:1, by vol) as developing solvent. The purified acids were esterified with ethereal diazomethane and the methyl esters purified by TLC to yield samples of the total methyl mycolates from both ethanol–diethyl ether extracts and defatted cell hydrolysates of each strain. The mycolates were resolved into several components by argentation TLC on plates prepared from a slurry of silica gel (40 g) in 10% aqueous silver nitrate and developed in hexane–benzene (1:1, v/v).

3. Results and discussion

Argentation chromatography resolved the methyl mycolates from both *N. caviae* and the 'rhodchrous' strain (*N. calcarea*) into three major bands (N1, N2 and N3 and R1, R2 and R3 respectively, in order of decreasing mobility). Proton magnetic resonance spectra (Perkin–Elmer) R10 60 MHz, deuteriochloroform solutions) of component R1 had signals (τ6.30) due to methyl ester; the other components had additional signals (τ4.68) whose intensities were attribut-

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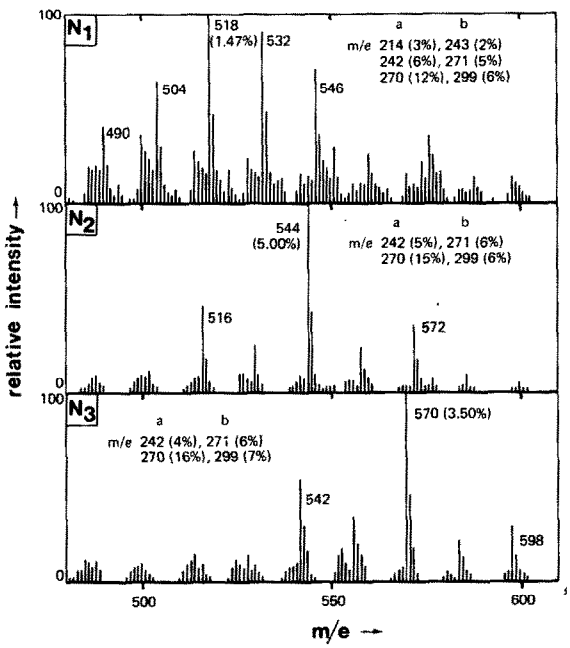
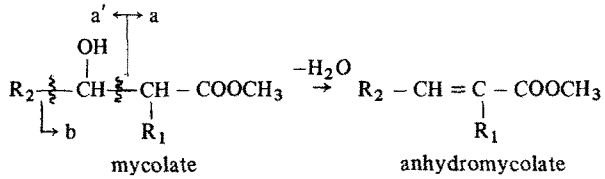


Fig. 1. Partial mass spectra of the unsaturated components (N1, N2 and N3) of the methyl nocardomycolates from *N. caviae*. The most intense peak in each case is assigned a relative intensity of 100; the figures in parentheses shows the intensity of these peaks with respect to the common base peak *m/e* 55. Each spectrum includes intensities for the fragments arising by cleavage of types a and b (see scheme in text) expressed as a percentage of the base peak. High resolution mass measurements of peak at *m/e* 542 and 570 (N3) gave values *m/e* 542.5423 ($C_{38}H_{70}O$ = 542.5426) and 570.5727 ($C_{40}H_{74}O$ = 570.5739), respectively, confirming that these fragments represent aldehydes (cleavage type a', see text).

able to one (N1 and R2), two (N2 and R3) and three (N3) olefinic linkages. The mycolates from the ethanol–diethyl ether extracts were apparently identical to those from the corresponding defatted cell hydrolysates.

The components of the mycolic esters resolved by argentation chromatography were examined by mass spectrometry (A.E.I. MS9); partial spectra are shown in figs. 1 and 2. Methyl mycolates from nocardioform bacteria produce a characteristic fragmentation pattern on mass spectrometry [4] which is summarised in the following scheme:



Cleavage at a and a' involves net transfer of the hydroxyl proton to carbon 2 to produce an aldehyde and a straight-chain ester; a transformation also achieved by pyrolysis [1].

All mass spectra had peaks due to straight-chain esters (*m/e* 214, 242 and 270) and fragments due to cleavage of type b (*m/e* 243, 271 and 299); the intensities of these peaks, where present, are recorded in figs. 1 and 2. The spectra of the unsaturated mycolic esters from *N. caviae* (N1, N2 and N3) had a series of peaks corresponding to aldehydes (fig. 1) and anhydromycolates (*m/e* 700–900). The esters from *N. calcarea* had peaks in their spectra attributable to anhydromycolates (fig. 2); the unsaturated esters (R2 and R3) had peaks of low intensity (*m/e* 378, 406 and 434; 404, 432 and 460, respectively) corresponding to aldehydes. The spectrum of the saturated esters (R1) had small peaks attributable only to the dehydration products of aldehydes; this phenomenon has been encountered previously [4].

Interpretation of the above results shows that the mycolic acids from the 'rhodochrous' strain (*N. calcarea*) comprise three homologous series (main components $C_{38}H_{76}O_3$, $C_{40}H_{78}O_3$ and $C_{42}H_{80}O_3$) of overall size range C_{34} to C_{46}. The unsaturated mycolic acids from *N. caviae* form homologous series (main components $C_{50}H_{98}O_3$, $C_{52}H_{100}O_3$) ranging from C_{48} to C_{56}. It is apparent (figs. 1 and 2) that the more highly unsaturated components of the mycolic esters have higher molecular weights; a similar relationship is discernible in the results of a previous study [4]. This interrelation may be important in ensuring that the alkyl chains of the mycolic esters have the correct physical properties enabling them to associate together to produce a lipid-containing organelle of suitable fluidity in the cell envelope.

The discovery that the mycolic acids of a typical strain of the 'rhodochrous' complex have a lower molecular weight range than those of the major acids from *Nocardia caviae* is consistent with the relatively lower TLC mobility of the mycolic acids from some

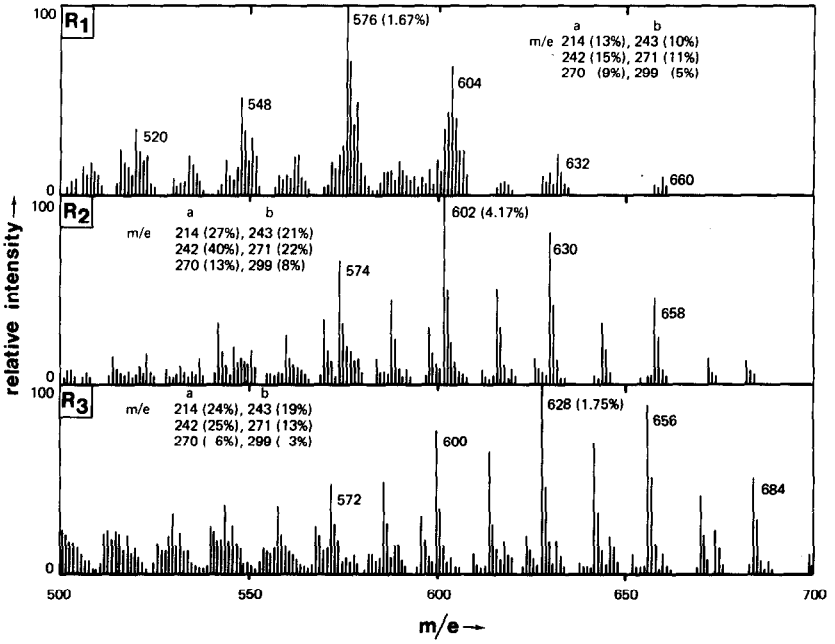


Fig. 2 Partial mass spectra of the methyl mycolates (R1, R2 and R3) of *N. calcarea*; for an explanation of other features see fig. 1. Mass measurements of the peaks at *m/e* 576, 604 (R1) and 602 (R2) gave values of *m/e* 576.5848 ($C_{39}H_{76}O_2$ = 576.5845), 604.6171 ($C_{41}H_{80}O_2$ = 604.6158) and 602.5995 ($C_{41}H_{78}O_2$ = 602.6002), respectively, confirming that these fragments represent anhydromycolates.

'rhodochrous' strains [2, 3]. Mycolic acids of certain 'rhodochrous' strains apparently fall into a similar molecular weight range as those of *N. calcarea* studied here; *Nocardia corallina* had acids in the range C_{38} to C_{46} [5] and similar acids (C_{38} to C_{46}) were found in a strain of *'Nocardia rhodochrous'* [6]. True nocardomycolic acids apparently have molecular weights centred somewhat above 50 carbon atoms [1, 4, 6] and corynomycolic acids have a range centred around 30 carbon atoms [1, 6, 7]. It has been noted previously [6] that the mycolic acids having around 40 carbon atoms are an anomalous group. The present results indicate that these intermediate mycolic acid types may be characteristic of organisms of the 'rhodochrous' complex which have been shown by numerical taxanomic [8–10] and other data [2] to be clearly distinct from strains of *Norcardia* and *Corynebacterium*. In this connection, the detailed relation of two strains of nocardioform bacteria, *N. farcinica* IM 1377 [4] and *N. erythropolis* [11], having mycolic acids in the molecular weight range C_{32} to C_{48} to strains of the 'rhodochrous' complex would repay investigation.

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References

[1] Etémadi, A.H. (1967) Bull. Soc. Chim. Biol. 49, 695–706.
[2] Mordarska, H., Mordarski, M. and Goodfellow, M. (1972) J. Gen. Microbiol. 71, 77–86.
[3] Goodfellow, M., Minnikin, D.E., Patel, P.V. and Mordarska, H. (1973) J. Gen. Microbiol. 74, 185–188.
[4] Maurice, M.T., Vacheron, M.J. and Michel, G. (1971) Chem. Phys. Lipids 7, 9–18.
[5] Batt, R.D., Hodges, R. and Robertson, J.G. (1971) Biochim. Biophys. Acta 239, 368–373.
[6] Ioneda, T. Lederer, E. and Rozanis, J. (1970) Chem. Phys. Lipids 4, 379–392.
[7] Yano, I. and Saito, K. (1972) FEBS Letters 23, 353–356.
[8] Goodfellow, M. (1971) J. Gen. Microbiol. 69, 33–80.
[9] Goodfellow, M., Fleming, A. and Sackin, M.J. (1972) Int. J. Syst. Bacteriol. 22, 81–98.
[10] Tsukamura, M. (1971) J. Gen. Microbiol. 66, 15–26.
[11] Yano, I., Saito, K., Furukawa, Y. and Kusunose, M. (1972) FEBS Letters 21, 215–219.