

ISOLATION AND IDENTIFICATION OF A NEW METABOLITE OF MICROBIAL  
CONVERSION OF UPGRADED NEUTRAL FRACTION OF POLISH TALL OIL BY  
MEANS OF FOUR STRAINS OF MYCOBACTERIUM SP.

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SUMMARY

A new metabolite, 5-alpha-androstane-3,6,17-trione, was isolated as a product of microbial conversion of upgraded neutral fraction of the Polish tall oil by *Mycobacterium* NRRL B-3683, NRRL B-3805, MB 3683, and MB 3805.

INTRODUCTION

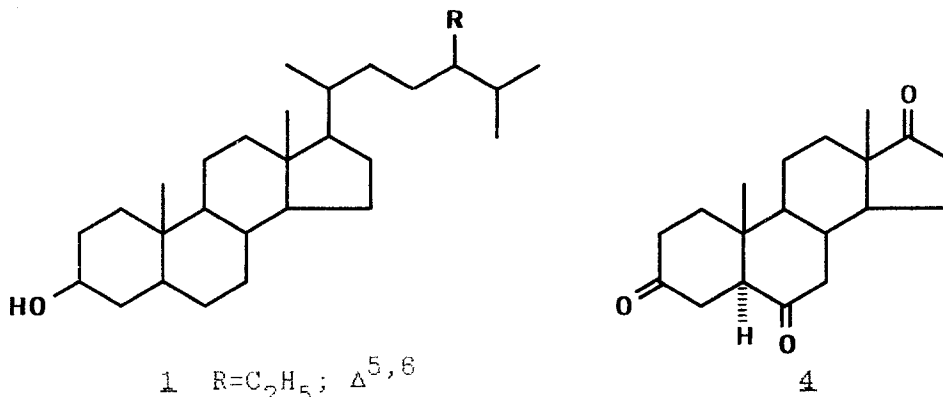
Owing to the importance of steroid hormones in therapy, all processes converting steroids of plant or animal origin into steroid drugs are of considerable significance. *Mycobacterium* sp. are widely used for microbiological transformations of steroids (Schubert et al., 1961; Ambrus et al., 1969). Classical mutagenic techniques have been successfully applied for strain improvement of the steroid transforming *Mycobacterium*, resulting in industrially important strains which degrade naturally occurring sterols to produce metabolites for synthesis of steroid drugs (Marsheck et al., 1972; Wovcha et al., 1978). This paper reports another type of metabolite isolated during microbial conversion of upgraded neutral fraction of the Polish tall oil.

## MATERIALS AND METHODS

The upgraded neutral fraction (UNF) containing ca.83.7% of degradable sterols ( $\beta$ -sitosterol (1) - 70.3%, sitostanol (2) - 8.9%, and campesterol (3) - 4.5%) was subjected to microbial conversion by means of *Mycobacterium* NRRL B-3683, NRRL B-3805, MB 3683 or MB 3805, respectively. The UNF was obtained by recrystallization of neutral fraction isolated from the Polish tall oil in ethanol (Szykuła et al., 1989). *Mycobacterium* was grown in a slightly modified medium described by Conner et al. (1976) on a rotary shaker at 30°C with shaking at 200 rpm. After 72 hr, the second stage culture was inoculated along with addition of UNF at the concentration of 1g/l and was shaken for 120 hrs. Metabolites were extracted from the broth with ethyl acetate and the crude extract was subjected to flash chromatography on silica gel with a discrete gradient of ethyl acetate in hexane.

## RESULTS AND DISCUSSION

Along with the earlier reported products of microbial conversion of  $\beta$ -sitosterol (1) (Kieslich, 1985), a new metabolite, 5- $\alpha$ -androstane-3,6,17-trione (4) was detected. Structure of (4) was confirmed by spectroscopic (MS,  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, IR, UV) and chromatographic (GLC, HPLC) data which were identical with those reported by Oberman et al. (1970). As presented in Table 1, 5- $\alpha$ -androstane-3,6,17-trione (4) was isolated in the 4.9 to 14.8% yield (accounted for the total transformation products) respectively to the strain used.



- 1 R=C<sub>2</sub>H<sub>5</sub>; Δ<sup>5,6</sup>  
 2 R=C<sub>2</sub>H<sub>5</sub>  
 3 R=CH<sub>3</sub>; Δ<sup>5,6</sup>

4

Strain	<i>MB 3683</i>	<i>MB 3805</i>	<i>NRRL B-3683</i>	<i>NRRL B-3805</i>
Yield %	4.9	5.3	11.2	14.8

Table 1. Yield of 5- $\alpha$ -androstane-3,6,17-trione respectively to the strain used.

Presented 5- $\alpha$ -androstane-3,6,17-trione is a new metabolite from the conversion of UNF of the tall oil sterols. Attempts to transform pure form of tall oil sterols under identical conditions failed to achieve (4). However, this metabolite has been reported to be a product of cholesterol transformation by *Mycobacterium parafortuitum* MC1-0801 (Imata et al., 1979).

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