

# Microbial biotransformation of cholic acid to the new potential steroid intermediate, 9 $\alpha$ - hydroxy – 3, 12-dioxo-23, 24-dinorchola-4,6-dienoic acid by *Micrococcus roseus*<sup>†</sup>

Shashi B. Mahato\* and Subhadra Garai

Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Calcutta-700032, India

Fermentation of cholic acid with a strain of *Micrococcus roseus* isolated from soil by enrichment culture technique using cholic acid as the sole source of carbon yielded the metabolite, 9 $\alpha$ -hydroxy-3, 12-dioxo-23, 24-dinorchola-4,6-dienoic acid as a single isolable product.

Microbial degradation of side chains of widely available phyosterols and bile acids is of much interest for commercial exploitation. The recent advances in microbial biotransformation of bile acids has helped the identification of a number of neutral and acidic steroidal compounds<sup>1,2</sup> which are useful as drugs and drug intermediates on a scale which would not have been possible by classical chemical transformations. A partial side chain cleavage of the bile acids to 20-carboxylic acids is important for practical use. Cholic acid is commonly available from the gall bladder bile of slaughtered cattle and sheep. In our studies directed at obtaining various physiologically important steroid derivatives by microbial transformations we isolated from soil a strain of *Micrococcus roseus* by an enrichment culture technique<sup>3</sup> using cholic acid (**1**) as the sole carbon source. This paper reports the synthesis of a potential new steroid intermediate, 9  $\alpha$  - hydroxy – 3, 12 – dioxo-23, 24-dinorchola-4,6-dienoic acid (**2**) as a single isolable product in good yield by fermentation of cholic acid (**1**) with this *M. roseus* strain.

Fermentation of cholic acid (**1**) with the *M. roseus* strain followed by usual workup and chromatographic purification led to the isolation of a single metabolite (**2**). The molecular formula, C<sub>22</sub>H<sub>28</sub>O<sub>5</sub> of the metabolite (**2**) was deduced from its elemental analysis and molecular ion peak in the mass spectrum. The <sup>1</sup>H NMR spectrum displayed two quaternary methyl signals at  $\delta$  1.14 and 1.27 and a secondary methyl signal at  $\delta$  1.60 assignable to the 18-CH<sub>3</sub>, 19-CH<sub>3</sub> and 21-CH<sub>3</sub> groups respectively. The downfield shift of the 21-CH<sub>3</sub> peak by 0.4 ppm in comparison to cholanic acid derivatives revealed the presence of a carboxyl group at C-20. The spectrum also showed a one-proton singlet at 6.0 and two one-proton doublets at 5.97 ( $J = 1.2, 9.9$  Hz) and 6.30 ( $J = 3.0, 9.9$  Hz)

assigned to the 4-H, 6-H and 7-H respectively. The position and splitting pattern of the signals are reminiscent of the presence of a 4,6-dien-3-one.<sup>4</sup> The presence of such a system in **2** was also supported by its UV spectrum which showed  $\lambda_{\max}$  at 283 nm. The <sup>13</sup>C NMR spectrum with DEPT (distortionless enhancement by polarisation transfer) studies revealed the presence of three carbonyls, three methyls, 5-methylenes, four sp<sup>3</sup> methines, three sp<sup>2</sup> methines, two sp<sup>3</sup> quaternary carbons, one sp<sup>2</sup> quaternary carbon and one quaternary carbon atom bearing an oxygen atom. These data were found to be in conformity with the structure **2**, which were assigned (shown on **2**, Fig.1) by the application of known chemical shift rules,<sup>5</sup> DEPT studies as well as by comparison with the <sup>13</sup>C data of model steroids.<sup>6,7</sup>

It is evident that the new metabolite **2** is produced from **1** by several enzymes generated by the microorganism. The product has potential for industrial application as compounds with this type of structural feature are suitable substrates for preparation of steroid drugs.<sup>8</sup> The generation of enzymes of such diverse character by the same organism is an interesting phenomenon which should be useful in further biochemical investigations.

## Experimental

The strain of *Micrococcus roseus* (IICB – 909) was isolated from soil by enrichment culture technique using cholic acid as the sole source of carbon. It was identified at the Institute of Microbial Technology, Chandigarh, India. It is being maintained on nutrient agar slants at the Culture Collection Unit of Steroid and Terpenoid Division of this Institute.

Fermentation of cholic acid was carried out as follows : Cholic acid (500 mg) was evenly distributed between 10 500 ml cotton-plugged Erlenmeyer flasks containing the medium (100 ml) [% w/v K<sub>2</sub> HPO<sub>4</sub>

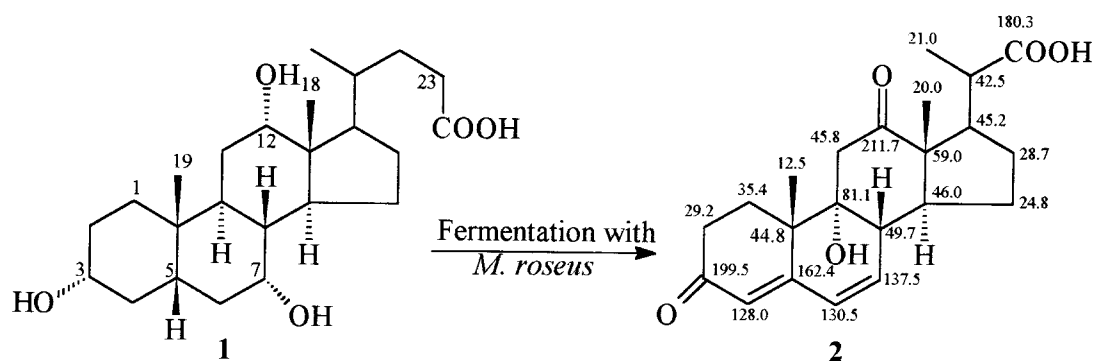


Fig. 1

\* To receive any correspondence.

<sup>†</sup> This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.

0.7,  $\text{KH}_2\text{PO}_4$  0.3,  $(\text{NH}_4)_2\text{SO}_4$  0.1,  $\text{MgSO}_4$  0.01, pH 7.0] and the contents were sterilised by autoclaving at 121°C for 15 minutes. Inoculation of the sterilised medium containing the substrate was made with a cell suspension obtained from a 20 h-old culture maintained on nutrient agar slants. The flasks thus inoculated were incubated on a rotary shaker at 110 rpm at 37°C in aerobic condition for 48 h. Fermentation was harvested after 48h of incubation. The broth was extracted with *n*-butanol after acidifying to pH 2.0 with concentrated HCl. The organic layer was washed with distilled water, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and the solvent evaporated under reduced pressure to yield a light brown semi solid residue (0.62 g). The residue was chromatographed on a column of silica gel (10 g). The column was eluted successively with petroleum ether, petroleum ether/ethyl acetate (3:1) and (1:1), ethyl acetate and ethyl acetate/methanol (19:1). The fractions eluted with petroleum ether/ethyl acetate (1:1) and ethyl acetate contained the metabolite (**2**) as revealed by TLC. These were collected and crystallized from methanol (yield 56%). No transformed product was obtained from the control. The metabolite **2** crystallised from methanol as prisms, m.p. 261–262°C,  $[\alpha]_D^{20} - 78.7^\circ$  (*c*, 0.75 in MeOH);  $\lambda_{\text{max}}$  283 nm ( $\epsilon = 20405$ );  $\nu_{\text{max}}$  (KBr pellet), 3420, 1705, 1638, 1610, 1275, 1195, 1025  $\text{cm}^{-1}$ ; MS  $m/z$  372 [ $\text{M}^+$ ]; (Found : C, 70.88; H, 7.55.  $\text{C}_{22}\text{H}_{28}\text{O}_5$  requires C, 70.94; H, 7.58).

Financial help was provided by the CSIR, New Delhi, in the form of a ES scheme.

Received 23 April 2000; accepted 22 May 2000  
Paper 00/269

## References

- 1 S. Hayakawa, *Adv. Lipid Research* 1973, **11**, 143.
- 2 S.B. Mahato, E. Mukherjee, S. Banerjee. *Biotech. Adv.* 1994, **12**, 357.
- 3 M.J. Pelczar, Jr., R.D. Reid, E.C.S. Chan, *Microbiology*, McGraw-Hill, New Delhi, 1977, pp. 138–139.
- 4 F. Abe, T. Yamauchi, *Phytochemistry*, 1976, **15**, 1745.
- 5 F.W. Wehrli, T. Wirthlin, *Interpretation of carbon-13 NMR spectra*; Heyden and Son, Ltd., London, 1978, pp. 64–128.
- 6 J.W. Blunt, J.B. Stothers, *Org. Magn. Reson.* 1977, **9**, 439.
- 7 E. Mukherjee, S. Banerjee, S.B. Mahato, *Steroids* 1993, **58**, 484.
- 8 K. Kieslich, *Arzneim.-Forsch.* 1986, **36**, 888.