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# SPECIALIA



## Transformation of progesterone by *Caldariella acidophila*, an extreme thermophilic bacterium

M. De Rosa, A. Gambacorta, G. Sodano and A. Trabucco

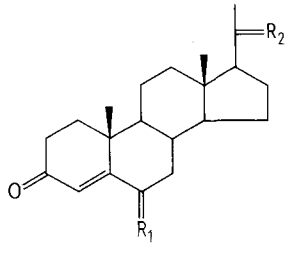
*Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R., Via Toiano 2, Arco Felice, Naples (Italy), 10 November 1980*

*Summary. Caldariella acidophila,* a thermophilic bacterium, converts progesterone (**1**) to pregn-4-en-3,6,20-trione (**2**), 5$\alpha$-pregnan-3,6,20-trione (**3**), pregn-4-en-20$\alpha$-ol-3-one (**4**), pregn-4-en-6$\beta$-ol-3,20-dione (**5**) and pregn-4-en-6$\alpha$-ol-3,20-dione (**6**). Different patterns of these metabolites were obtained by modifying the incubation conditions and using differently treated biocatalysts.

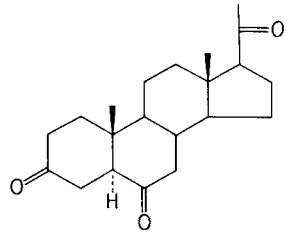
Despite the widespread use of microorganisms for the transformation of natural products[1,2], the potentiality of thermophilic microorganisms in this field has seldom been exploited[3]. We report here the microbiological transformation of progesterone by strain MT-4 of *Caldariella acidophila*, an extreme thermophilic bacterium growing optimally at 87 °C; it was previously called the MT organism of the *Caldariella* group[4]. *C. acidophila* was cultivated in standard media[4] and the cells were harvested at the end of the exponential growth phase. In a standard experiment (experiment 1, table) the resting cell suspension was prepared by suspending the wet cells (35 mg/ml) in a saline solution [$(NH_4)_2SO_4$, 2 g/l; $KH_2PO_4$, 3 g/l; $MgSO_4 \cdot 7\,H_2O$, 0.20 g/l; $MgCl_2 \cdot 2\,H_2O$, 0.25 g/l], adjusting the pH to 3.5 with $H_2SO_4$. A dimethylformamide solution of progesterone (30 mg/ml) was added to the cell suspension (2-l batches) to reach a final concentration of 0.13 mg/ml. The batches were maintained at 85 °C without agitation for 30 h; after this period the metabolites were recovered by extraction of the incubation mixture with diethyl ether. The ethereal extract was chromatographed on silica gel (benzene and increasing amounts of diethyl ether) and the single products further purified by preparative thin-layer chromatography. The transformation of progesterone (**1**) by the bacterium yielded 5 compounds (**2–6**), which were characterized by spectral means (NMR, MS, UV) and finally identified by comparison with authentic samples[5].

The yields were determined by using [4-$^{14}$C]-progesterone[6] in a 5-ml scale incubation and measuring the recovered radioactivity in the metabolites **1–6** after isolation by preparative TLC (table).

As the conditions adopted gave rise to 5 products, in different amounts (experiment 1, table), we selected condi-




**1**, $R_1 = H_2$, $R_2 = O$
**2**, $R_1 = R_2 = O$
**4**, $R_1 = H_2$, $R_2 = H$, $\alpha$-OH
**5**, $R_1 = H$, $\beta$-OH, $R_2 = O$
**6**, $R_1 = H$, $\alpha$-OH, $R_2 = O$




**3**

Progesterone biotransformation experiments with *C. acidophila* cells

| Experiment No. | Biotransformation conditions Biocatalyst | t (°C) | Agitation | Total conversion (%)* | Metabolites recovered (relative %)** 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Resting cells | 85 | No | 14 | 38 | 17 | 13 | 15 | 17 |
| 2 | Resting cells | 70 | No | 4 | – | – | 100 | – | – |
| 3 | Resting cells | 70 | Yes | 15 | – | – | – | 45 | 55 |
| 4 | Acetonized resting cells | 85 | No | 24 | 58 | 16 | – | 12 | 14 |
| 5 | Acrylamide trapped cells | 85 | No | 20 | 59 | 9 | – | 15 | 17 |

* 100 refers to the total labelled progesterone added; ** 100 refers to the sum of the radioactivity recovered in **2–6**.

tions for the preferential formation of the above metabolites, estimating the yields by [4-$^{14}$C]-progesterone incubations.

Bioconversion of progesterone at a lower temperature (70 °C; experiment 2, table) resulted in the recovery of pregn-4-en-20$\alpha$-ol-3-one (**4**) as the sole product, although the overall conversion was lower (4%) than at 85 °C (14%).

When at 70 °C the oxygen availability was increased by agitation of the incubation flask, only pregn-4-en-6$\alpha$-ol-3,20-dione (**5**) and pregn-4-en-6$\beta$-ol-3,20-dione (**6**) were recovered in about the same amount (experiment 3, table), the total yield being 15%.

As previous work on this microorganism showed that several organic solvents have permeabilizing effects on the cell membrane without causing any significant lysis[7], we performed incubation experiments (experiment 4, table) at 85 °C with acetonized[7] resting cells. In these conditions a strong increase of the total conversion (24%) of progesterone was observed, while the metabolite pattern resembled that of experiment 1, the only difference being the lack of **4** and the relative increase of pregn-4-en-3,6-20-trione (**2**) and 5$\alpha$-pregnan-3,6,20-trione (**3**).

To evaluate the usefulness of acrylamide trapped cells[7] in this process, progesterone (**1**) was incubated in the conditions of experiment 1, using an amount of acrylamide beads (300 g) containing about 70 g of wet cells. The results of this experiment (experiment 5, table) were about the same as in experiment 4, in which acetonized resting cells were used, and are in accordance with the conjecture[7] that polymerization conditions cause a permeabilization of the cellular membrane similar to that obtained by solvent treatment.

In conclusion the results of the above experiments suggest that at the lower temperature (70 °C) the hydroxylative and oxidative systems are inactive; however when the cells are shaken at 70 °C the increased oxygen availability gives rise only to the hydroxylated metabolites. The permeabilization of cell membranes results in a generalized increase of progesterone conversion.

Further work is in progress to optimize the bioconversion yield and to select the conditions better for obtaining particular metabolites.

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5 Purchased from Steraloids, Wilton, NH, USA.
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## The structure of ceroplastodiol, a new tricyclic sesterterpene isolated from insect wax[1]

L. Quijano, J.S. Calderón and T. Ríos

*Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México 20, D.F. (Mexico), 21 April 1980*

*Summary.* The isolation and structure elucidation of cereoplastodiol, a new tricyclic sesterterpene isolated from wax of the insect *Ceroplastes albolineatus* are reported.

In continuation of our search for new sesterterpenic compounds in the wax of the scale insect *Ceroplastes albolineatus* Cockerell (Coccidae)[2], which has been previously shown to be a source of different types of compounds formed from 5 isoprene units (sesterterpenes), we have undertaken the study of a new collection of the insect's wax and succeeded in isolating a new tricyclic sesterterpene, which we have named ceroplastodiol (**1a**), and its corresponding diacetate (**1b**) and a mixture of the monoacetates (**1c**) (**1d**).

Ceroplastodiol (**1a**), m.p. 120–2°, $[\alpha]_D + 143.7(CHCl_3)$ has the composition $C_{25}H_{40}O_2$ and its MS displayed a molecular ion peak at m/Z 372 and a base peak at m/Z 354 ($M^+ - H_2O$) along with peaks at m/Z 336 ($M^+ - 2H_2O$), 227 ($M^+ - H_2O - C_8H_{15}O$). The IR-spectrum showed absorptions at 3450, 1650, 855, 840 $cm^{-1}$ and UV-absorption at 213 nm (log $\varepsilon$, 3.038) indicating the presence of OH groups and double bonds.

The PMR of ceroplastodiol (**1a**), revealed signals due to 2 vinyl methyl groups at $\delta$ 1.62 and 1.70, a secondary methyl group doublet at 0.80 ($J = 6.5$ Hz) and a tertiary methyl group singlet at 0.74. The last 2 methyl groups of the tricyclic skeleton, ceroplastane (**2**) have to be methylenes bearing hydroxy groups as indicated by the appeareance of 2 broad singlets at 4.02 and 4.22 which shifted downfield (4.38 and 4.57) upon acetylation. The PMR-spectrum also showed signals due to vinyl protons as triplets at $\delta$ 5.42 and 5.55 ($J = 7.0$ Hz), a broad triplet at 3.98 ($J = 8.0$ Hz) was assigned to H–6 doubly allylic and a doublet at 2.48 ($J = 14.0$ Hz) to one of H–1 protons.

Acetylation of **1a** afforded the diacetate **1b**. Catalytic hydrogenation in $EtOH/HClO_4$ using $PtO_2$ as catalyst gave the tricyclic hydrocarbon **2**, $M^+$ 346 ($C_{25}H_{46}$), which was identical with that obtained from ceroplastol acetate (**3**) under the same conditions.

Evidence for the position of the OH groups was provided by Sarett's oxidation of ceroplastodiol (**1a**), which led to the dialdehyde (**1e**) and the monoaldehyde (**1f**).

The IR-spectrum of **1e** contained bands at 2720, 1680, 1660 $cm^{-1}$ and UV-absorption at 235 (log $\varepsilon$, 4.244), 260 nm (log $\varepsilon$, 4.184) indicating the presence of 2 different $\alpha$, $\beta$-unsaturated systems. The PMR-spectrum showed 2 different aldehydic proton absorptions at 9.27 and 9.90. The downfield chemical shift at 6.36 of one of the vinyl proton signals as a triplet of quartets ($J = 7.0$ Hz, $J = 1.5$ Hz) indicated that one of the OH groups was at C–25 as in all the other sesterterpenes isolated from the wax, therefore the other one should be at C–20 since the vinyl proton broad triplet remained at 5.66. Furthermore the AB doublet due to one of H–1 protons was shifted downfield from 2.48 to 3.21 due to the anisotropic effect of the carbonyl group at C–20.

The most polar compound from the oxidation of ceroplastodiol (**1a**) was the monoaldehyde (**1f**). IR, $\nu$max 2780,