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# Use of 20,20-Dimethylacetal Protection in Microbial Hydroxylation and Dehydrogenation of Steroids

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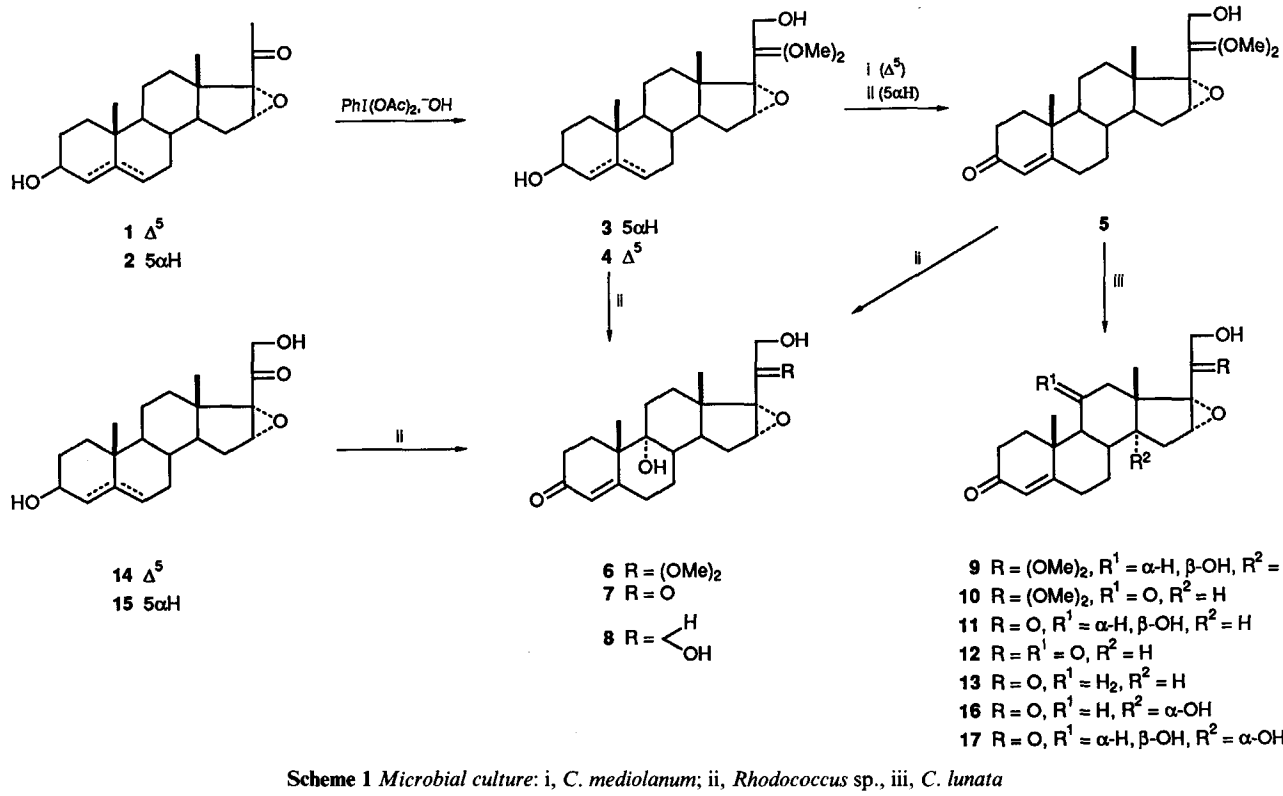
The 20,20-dimethylacetal group proposed for protection of the 20-oxo group in 21-hydroxylation of steroids by hypervalent iodine compounds has been successfully used to hamper side reactions in microbial 9α- and 11β-hydroxylation and 4-dehydrogenation of 21-hydroxy-16α,17α-epoxypregnanes by *Rhodococcus* sp., *Curvularia lunata* and *Corynebacterium mediolanum* cultures.

The reduction of the 20-oxo group or the degradation of the corticoid side chain are usually secondary processes in corticosteroid production using methods based on microbial hydroxylation or dehydrogenation of steroids. To reduce these undesirable processes partially, substituents have been introduced at C-17, or protection based on C-17–C-21-orthoester or C-20 ethylene ketal formation has been used.[1] We have for the first time shown that 20,20-dimethyl acetal protection, employed in the 21-hydroxylation of 20-oxopregnanes,[2,3] may be used to stop the above mentioned side reactions in the microbiological transformations. As starting materials we used 21-hydroxy-20,20-dimethoxy-16α,17α-epoxypregnanes **3–5**, obtained in one step from the 21-methyl-16α,17α-epoxypregnan-20-ones **1** and **2**.[4–7] The presence of the epoxide ring in **3–5** should allow them to be used as intermediates in the synthesis of drugs such as corticosterone, dexamethasone, triamcinolone *etc.* Three strains of microorganisms: *Rhodococcus* sp., *Curvularia lunata* and *Corynebacterium mediolanum* which are capable of inducing 9α,11β-hydroxylation and $\Delta^5 \rightarrow \Delta^4$ isomerisation of steroid substrates[1,8,9] have been tested for their ability to transform **3–5**, and the results are summarized in Table 1 which also shows a comparison of our results with those reported[9–11] for the corresponding transformations of 20-oxo analogues.

The oxidation of the 3β-hydroxy-$\Delta^5$ steroid **4**[4,5] into its $\Delta^4$-3-oxo derivative **5**[6] was performed using *C. mediolanum* B-964[9] in 70% yield at a substrate concentration of 2 g dm$^{-3}$ a pH of *ca.* 8.0. It is worth noting that the known[6] synthetic route to **5** is no so efficient; also our experiments with Oppenaur oxidation of **3** into **5** were unsatisfactory. Ethyl acetate is required for extraction of the 20,20-dimethyl acetals from the culture broth.

A wild strain of *Rhodococcus* sp. (10C-77) was used for conversion of the 3β-hydroxy-5αH steroid **3** into **5**. The capability of this strain to dehydrogenate $\Delta^5$-steroids into $\Delta^4$-3-oxo derivatives with subsequent 9α-hydroxylation has been demonstrated previously.[8] Our investigations have shown that: (*i*) the strain of *Rhodococcus* sp. appeared to be a highly suitable microorganism also for transformation of the 5α-H steroid **3** into its $\Delta^4$-3-oxo analogue; (*ii*) the processes of $\Delta^4$-dehydrogenation of 5αH steroids and the subsequent 9α-hydroxylation can be separately done in the presence of a 9α-hydroxylase inhibitor in the culture broth ($CoCl_3$). The ability of *Rhodococcus* sp. to convert 5αH steroids into $\Delta^4$-3-oxo derivatives is of practical interest because little is known about microorganisms with such function.[12,13] Moreover, one-step activation of rings *A* and *C* in 5αH steroids is unknown so far. The enzymatic activity of *Rhodococcus* sp. depends on whether the steroid substrate is used in the form of the 5αH acetal **3** or its $\Delta^5$-analogue **4**; the latter is transformed with a higher rate. Thus, upon exposure of the acetal **4** to the culture of *Rhodococcum* sp. at a steroid concentreation of 1.2–2.0 g dm$^{-3}$ and pH 8.0, the 9α-hydroxy-$\Delta^4$-3-ketone **6** was isolated as the sole product after 12–16 h incubation. A longer incubation period




**Scheme 1** *Microbial culture*: i, *C. mediolanum*; ii, *Rhodococcus* sp., iii, *C. lunata*

**Table 1** Microbial transformation of 20,20-dimethylepoxides **3–5** and their 20-oxo analogues **13–15**

| Substrates | Conc./g $dm^{-3}$ | Microorganism | Incubation time/h | Products | M.p., /°C | Yield (%) |
|---|---|---|---|---|---|---|
| **4** | 2.0 | *C. mediolanum* | 22 | **5** | 155–157[6] | 70 |
| **3** | 0.5 | *Rhodococcus* sp. ( + $CoCl_3$) | 12 | **5** | | 35 |
| **3** | 0.5–1.0 | *Rhodococcus* sp. | 20–26 | **6** | 205–210 | 75 |
| **4** | 1.2–2.0 | *Rhodococcus* sp. | 12–16 | **6** | | 90 |
| **5** | 1.0 | *Rhodococcus* sp. | 40 | **6** | | 62 |
| **5** | 2.0 | *Rhodococcus* sp. | 48 | **6** | | 19 |
| **5** | 0.4 | *C. lunata* | 28 | **9** | 185–187 | 75 |
| | | | | **10** | 160–168 | 23 |
| **14** | 1.0 | *Rhodococcus* sp. | 17 | **7** | 222–223 | 16 |
| | | | | **8** | 188–193 | 18 |
| **15** | 3.0 | *Rhodococcus* sp. | 12 | **7** | | 27 |
| **13** | 0.5 | *C. lunata* | 168 | **11–17**[a] | — | — |

[a] Literature results.[10,11]

(20–26 h) and lower substrate concentration (0.5–1.0 g $dm^{-3}$) are necessary for analogous conversion of the 5αH acetal **3** into **6**. The conversion rate of **3** into **6** depends on how the substrate is administered (steroid dissolved in dimethylformamide or sonicated). Use of a fine suspension of **3**, obtained by the action of ultrasound led to a greater yield of **6** (75%) than that (50%) obtained for the solution. TLC analysis during transformations of **3** and **4** indicates that their conversion into **6** proceeds *via* formation of the $\Delta^4$-3-ketone **5**, whose accumulation in the culture broth seems to be undesirable, because it slows the 9α-hydroxylation process (at a concentration above 0.5 g $dm^{-3}$). When the $\Delta^4$-3-ketone **5** was used as a substrate at a concentration of 1.0 and 2.0 g $dm^{-3}$, the reaction mixture contained only 62 and 91% of **6** respectively. Moreover, even after 48 h the starting substrate **5** was only partially transformed into **6** at a steroid concentration of 2.0 g $dm^{-3}$. Prolonging of the transformation period for more than 17 h increases the risk of loss of the required products owing to their destruction.

The oxidation of the acetal **5** by the action of *C. lunata* VCPM-70 at pH 8.0 proceeds quantitatively and gives rise to a steroid mixture in which the 11β-hydroxyepoxide **9** predominated (75%), with the 11-ketone **10**, as a byproduct; the formation of **10** is typical for species of *Curvularia*.[1]

The acetal group in **5**, **6**, **9** and **10** may be removed easily by treatment with toluene-*p*-sulphonic acid or pyridinium toluene-*p*-sulphonate in acetone or benzene[5–7] to give rise to the free 20-oxo epoxides **13**,[6] **7**,[8] **11**[10,11] and **12**[14] in 70–90% yield.† Practically the same results were obtained using chloroform for extraction of the culture broth and magnesium sulphate as drying agent. During such treatment the 20,20-dimethyl acetals are spontaneously hydrolysed into the corresponding 20-ketones.

A comparison of the results for microbial conversions of the 20,20-dimethyl acetals **3–5** by the cultures tested with those for the 20-oxo analogues **14**, **15** and **13** (Table 1) shows convincingly the advantage of 20-acetal protection. Thus, when the 20-oxo epoxide **14** is incubated with cells of *Rhodococcus* sp., reduction of the carbonyl group with concomitant decomposition of the steroid, which allows **7** to be isolated in a yield not exceeding 16% and the 20-epimeric alcohol **8** in about 18% yield. In an attempt to increase the yield of **7** and to stop the decomposition, the microbial transformation of **15** was carried out under various conditions (using microdispersed **15**, variations in incubation time and substrate concentration), but without visible success (the yield of **7** was not more than 35%).

† A solution of the 20,20-dimethyl acetal (5 mmol) in acetone (70 ml) containing toluene-*p*-sulphonic acid (1 mmol) was kept at 0 °C. Acetone is then removed *in vacuo*, and water added. The residue was filtered off and crystallized from MeOH.

According to the literature[10,11] the oxidation of the 20-oxo epoxide **13** by the fungus *C. lunata* gives a mixture of the 11β-hydroxyepoxide **11**, the 14α-hydroxyepoxide **16** and 11β,14α-dihydroxyepoxide **17**, yields and physicochemical data for which were not reported. The foregoing results demonstrated unequivocally that 20,20-dimethyl acetal protection, applied to hydroxylation of **5** by *C. lunata*, hinders the by-process of 14-hydroxylation which is typical for species of *Curvularia*.[1]

The structures of compounds **6–12** were confirmed by mass, IR and $^1H$ NMR spectroscopy. They all show molecular ions in their mass spectra (*m/z*) 406, 360, 362, 406, 404, 360 and 358, respectively. In their $^1H$ NMR spectra singlet signals from the epoxide protons are observed at δ 3.44–3.82 and the 9α-hydroxy protons at δ 2.42–2.48. The equatorial 11α-protons in compounds **9** and **11** give multiplets at δ 4.38–4.42, with $W_{1/2}$ = 9–10 Hz.

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