tively slow rate of reaction of **1b** with glyoxylate was predicted from our earlier results on substituent effects upon the rate of the reaction[2]; and it was on this basis that we thought it might be possible to detect certain discrete intermediates during the reaction of **1b** with glyoxylate. However, as was the case for other nitrosobenzenes the only significant product observed at any time in the present reaction was the hydroxamic acid **2b**. This single product of the reaction was readily observed in HPLC chromatograms as a peak with identical retention times and peak height ratios to those observed for authentic **2b** (table). The figure illustrates the time-dependent conversion of **1b** to **2b** for a 6-h period, during which time period a close material balance between the starting material (**1b**) and the hydroxamic acid product (**2b**) was observed. A very slight deviation from linearity in the time-dependent formation of **2b** and disappearance of **1b** was accounted for by the tendency of **1b** to volatilize from aqueous solutions, as is typical of many aromatic nitroso compounds[2]. We conclude that *p*-nitronitrosobenzene (**1b**) behaves like other nitroso aromatics towards glyoxylate, and that the nitroso-glyoxylate reaction is a general chemical reaction for aromatic C-nitroso compounds.

1 This study was supported by grant No.CA 32395 from the National Cancer Institute, and by Research Career Development Award ES 00120 from the National Institute of Environmental Health Sciences, DHHS.
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Microbiological reduction of steroidal ketones using the thermophilic bacterium *Caldariella acidophila*

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Summary. Twelve steroidal ketones have been subjected to reduction with *C. acidophila* resting cells, regio- and stereospecific reduction of the 3-keto groups being observed, as well as reduction of the Δ^4-double bond. The presence of oxo groups at C-11 or C-12 and the presence of hydrophobic side chains on the steroidal molecules inhibit the reduction.

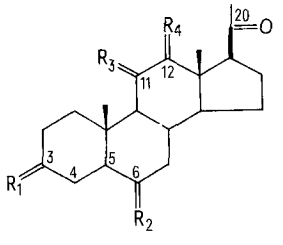
In a previous communication[1] we have reported that the main microbiological transformation induced by MT-4 strain of *Caldariella acidophila* on progesterone (**1**) consists in hydroxylation-oxidation at the allylic carbon (C-6), some reduction products also being isolated.

From these preliminary results it could be predicted that the reduction processes would predominate when a steroidal molecule lacking an available allylic position is used as a substrate. In this report we describe the observed modifications of steroid substrates which have been chosen with the purpose of verifying the practicability of reductions of steroidal ketones with *C. acidophila*.

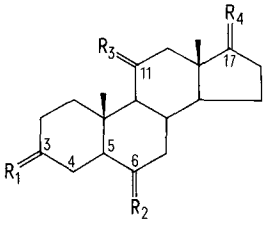
The substrates were incubated with resting cells of *C. acidophila* at 85 °C without agitation for 30 h, as previously described[1], and the metabolites were recovered by extraction of the incubation mixture with chloroform. The extracts were chromatographed on silica gel columns (benzene and increasing amounts of diethyl ether or chloroform and increasing amounts of methaol) and the single products further purified by preparative TLC. The identity of the metabolites was established mainly by the chemical shifts of the angular methyl groups and the chemical shifts and coupling constants of the C*H*OH protons in the ^{1}H NMR-spectra[2,3], by mass spectroscopy[4] and by comparison with authentic samples, when possible. The results are reported in the table and can be summarized as follows.

When carbonyl reduction occurs, mainly 3β-alcohols are formed (substrates **2**, **4**, **7**), indicating the presence in the bacterium of a constitutive oxo-steroid reductase activity which allows a regio- and stereo-specific reduction of steroidal polyketones.

Compounds containing carbonyl groups at C-11 or C-12



1, $R_1=0$; $R_2=R_3=R_4=H_2$; Δ^4;
2, $R_1=R_2=0$; $R_3=R_4=H_2$;
3, $R_1=H$, βOH; $R_2=0$; $R_3=R_4=H_2$;
7, $R_1=R_3=0$; $R_2=R_4=H_2$;
8, $R_1=H$, βOH; $R_2=R_4=H_2$; $R_3=0$;
9, $R_1=R_4=0$; $R_2=R_3=H_2$; 5βH;
12, $R_1=0$; $R_2=H$, βOH; $R_3=R_4=H_2$; Δ^4;
13, $R_1=R_2=0$; $R_3=R_4=H_2$; Δ^4;
16, $R_1=H$, βOH; $R_2=0$; $R_3=R_4=H_2$; Δ^4;



4, $R_1=R_4=0$; $R_2=R_3=H_2$;
5, $R_1=H$, βOH; $R_2=R_3=H_2$; $R_4=0$;
6, $R_1=R_4=H$, βOH; $R_2=R_3=H_2$;
10, $R_1=R_3=R_4=0$; $R_2=H_2$; Δ^4;
11, $R_1=R_3=R_4=0$; $R_2=H_2$; Δ^1; Δ^4;
14, $R_1=R_4=0$; $R_2=H$, βOH; $R_3=H_2$; Δ^4;
15, $R_1=R_2=R_4=0$; $R_3=H_2$;
17, $R_1=R_2=R_4=0$; $R_3=H_2$; Δ^4;

Reduction of steroidal ketones by *C. acidophila*

Substrates[a]	Products (yields %)[b]
5α-Pregnan-3,6,20-trione (**2**)	3β-Hydroxy-5α-pregnan-6,20-dione (**3**; 45%)
5α-Androstan-3,17-dione (**4**)	3β-Hydroxy-5α-androstan-17-one (**5**; 10%) 3β,17β-Dihydroxy-5α-androstane (**6**; 2%)
5α-Pregnan-3,11,20-trione (**7**)	3β-Hydroxy-5α-pregnan-11,20-dione (**8**; 3%)
5β-Pregnan-3,12,20-trione (**9**)	None
Androst-4-en-3,11,17-trione (**10**)	None
Androst-1,4-dien-3,11,17-trione (**11**)	None
6β-Hydroxy-pregn-4-en-3,20-dione (**12**)	Pregn-4-en-3,6,20-trione (**13**; 4%) 5α-Pregnan-3,6,20-trione (**2**; 25%)
6β-Hydroxy-androst-4-en-3,17-dione (**14**)	5α-Androstan-3,6,17-trione (**15**; 35%)
Pregn-4-en-3,60,20-trione (**13**)	5α-Pregnan-3,6,20-trione (**2**; 4%) 3β-Hydroxy-5α-pregnan-6,20-dione (**3**; 25%) 3β-Hydroxy-pregn-4-en-6,20-dione (**16**; 13%)
Androst-4-en-3,6,17-trione (**17**)	5α-Androstan-3,6,17-trione (**15**; 10%)
5α-Cholestan-3,6-dione (**18**)	None
5β-Cholanic acid-3,7-dione-methyl ester (**19**)	None

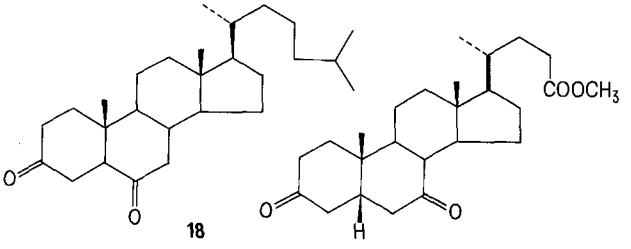
[a] Purchased from Steraloids, Wilton, USA. [b] Isolated yields.

(substrates **7** and **9**) are poorly or not reduced; the presence of carbonyl groups at C-11 also inhibits the hydroxylation-oxidation at C-6 (substrates **10** and **11**) expected because of similarity with the progesterone framework[1]. Δ^4, 6β-hydroxy compounds (substrates **12** and **14**) are oxidized to the corresponding 6-oxo derivatives and, subsequently, reduction of the Δ^4-double bond takes place.

The reduction of the Δ^4-double bond also occurs in Δ^4, 6-oxo derivatives (substrates **13** and **17**); in 1 case (substrate **13**), the product of reduction at C-3 occurring before Δ^4 reduction was also isolated.

Compounds containing a voluminous hydrophobic side chain (substrates **18** and **19**) are not reduced; this result agrees with a previous report[5] of inhibition of the bacterial reductase activity by steroids carrying an aliphatic side chain.

No attempts were made to optimize the single processes; however, acetonized[1] resting cells failed to transform the given substrates, indicating that, unlike other enzymatic activities[6], the reductase activity is lost when *C. acidophila* cells are treated with organic solvents.



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Helminthosporoside, a host-specific toxin from *Helminthosporium sacchari:* a structure revision and a new partial structure[1]

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Summary. Reevaluation of the previously proposed structure of helminthosporoside, a host-specific toxin from *Helminthosporium sacchari*, reveals a sesquiterpenoid bis-digalactoside. The carbohydrate portion of the toxin was characterized by ^{13}C-NMR spectroscopy, methylation analyses, FD and FAB mass spectroscopy. The ring size and anomeric configuration of the galactose moieties were determined by utilizing a ^{13}C-NMR structural analysis method. A new partial structure is proposed.

A host-specific toxin of sugarcane produced by *Helminthosporium sacchari* was isolated by Steiner and Byther[3]. An isolated fraction was characterized by NMR, IR, and mass spectroscopy. A linkage assignment was made after enzymatic analyses, and a structure was proposed (**1**). The trivial name of helminthosporoside was suggested for this toxin[4].

In an attempt to verify the validity of the proposed structure for helminthosporoside, we had to repeat the early work. Isolation techniques and bioassays were similar to the original methods of Steiner and Strobel[4]. The yellow-colored toxin preparation obtained by their procedure was decolorized by treatment with activated charcoal in boiling