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SHORT COMMUNICATION

FORMATION OF PROGESTERONE AND 1-DEHYDROPROGESTERONE FROM CHOLESTEROL IN FERMENTATION CULTURES OF *MYCOBACTERIUM AURUM*

C. HÖRHOLD* and K.-H. BÖHME
Academy of Sciences of the G.D.R., Central Institute of Microbiology and Experimental Therapy, 6900 Jena, G.D.R.

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Summary—The formation of progesterone and 1-dehydroprogesterone from cholesterol in fermentation cultures of *Mycobacterium aurum* ATCC 25790 was studied with the aim of clarifying the microbial pathway.

The C_{22}-intermediate (20S)-20-carboxy-1,4-pregnadien-3-one was microbiologically converted via the undetectable corresponding aldehyde into the C_{22}-alcohol.

However in the fermentation broth without microorganisms, but containing 2,2′-bipyridyl and copper ions, synthetically prepared C_{22}-aldehyde was oxidized to the corresponding C_{21}-compound 1-dehydroprogesterone, suggesting that the enzymatically originated C_{22}-aldehydes may be immediately chemically oxidized to the corresponding C_{21}-ketones.

INTRODUCTION

Prome *et al.*[1] reported in 1983 for the first time the formation of progesterone and 1-dehydroprogesterone from cholesterol in fermentation cultures of *Mycobacterium aurum* in the presence of 2,2′-bipyridyl. Both products were detectable only in very small amounts by the use of combined gas chromatography–mass spectrometry of the corresponding *O*-trimethylsilylethers. The authors discussed two possible degradation pathways: direct splitting between carbon atoms 20 and 22 or the oxidative decarboxylation of a 20-carboxy-pregnane-intermediate, which is a known intermediate of the microbial side chain degradation of sterols.

However another possibility concerning the autoxidation of an aldehyde intermediate is possible.

It is known that 3-oxo-4-pregnene-20-carboxaldehyde is transformed into progesterone in the presence of oxygen and copper-II-acetate-2,2′-bipyridyl-complex as catalyst [2]. Analogous C_{22}-aldehyde compounds were elucidated as intermediates of the microbial sterol side chain degradation using mutants of Mycobacterium-species [3].

MATERIALS AND METHODS

Chemicals

Cholesterol was purchased from Union Chimique Belge, SA, and 2,2′-bipyridyl from Merck (Darmstadt, F.R.G.). (20S)-20-carboxy-4-pregnen-3-one (**1**), (20S)-20-carboxy-1,4-pregnadien-3-one (**2**), (20S)-20-hydroxymethyl-4-pregnen-3-one (**4**) and (20S)-20-hydroxymethyl-1,4-pregnadien-3-one (**6**) were produced by microbial side chain degradation of sterols [4, 5]. 4-Pregnen-3-one-20-carboxaldehyde (**3**) and 1,4-pregnadien-3-one-20-carboxaldehyde (**5**) were obtained by oxydation of the 20-hydroxymethyl compounds (**4** and **6**) with *t*-butylchromate [6].

Growth conditions and fermentation

Mycobacterium aurum ATCC 25 790 was inoculated (inoculation 1:10 with a 48 h preculture) into 50 ml culture medium, which consisted of glucose (10 g), casitone (5 g), KH_2PO_4 (1 g), $Na_2HPO_4 \cdot 2H_2O$ (3.1 g), Fe(III)-NH_4-citrate (5 mg), $MgSO_4 \cdot 7H_2O$ (10 mg), $CaCl_2$ (0.5 mg), $ZnSO_4 \cdot 7H_2O$ (0.1 mg), $CuSO_4 \cdot 5H_2O$ (0.1 mg) and distilled water to 1 l (pH 6.9). The culture was shaken for 48 h at 30°C, 25 mg steroid and 7.5 mg 2,2′-bipyridyl in 1 ml dimethylformamide were then added to each flask and the fermentation was continued for 96 h. The culture was acidified with HCl and extracted with chloroform. After washing with water the solvent was removed *in vacuo* at 30°C.

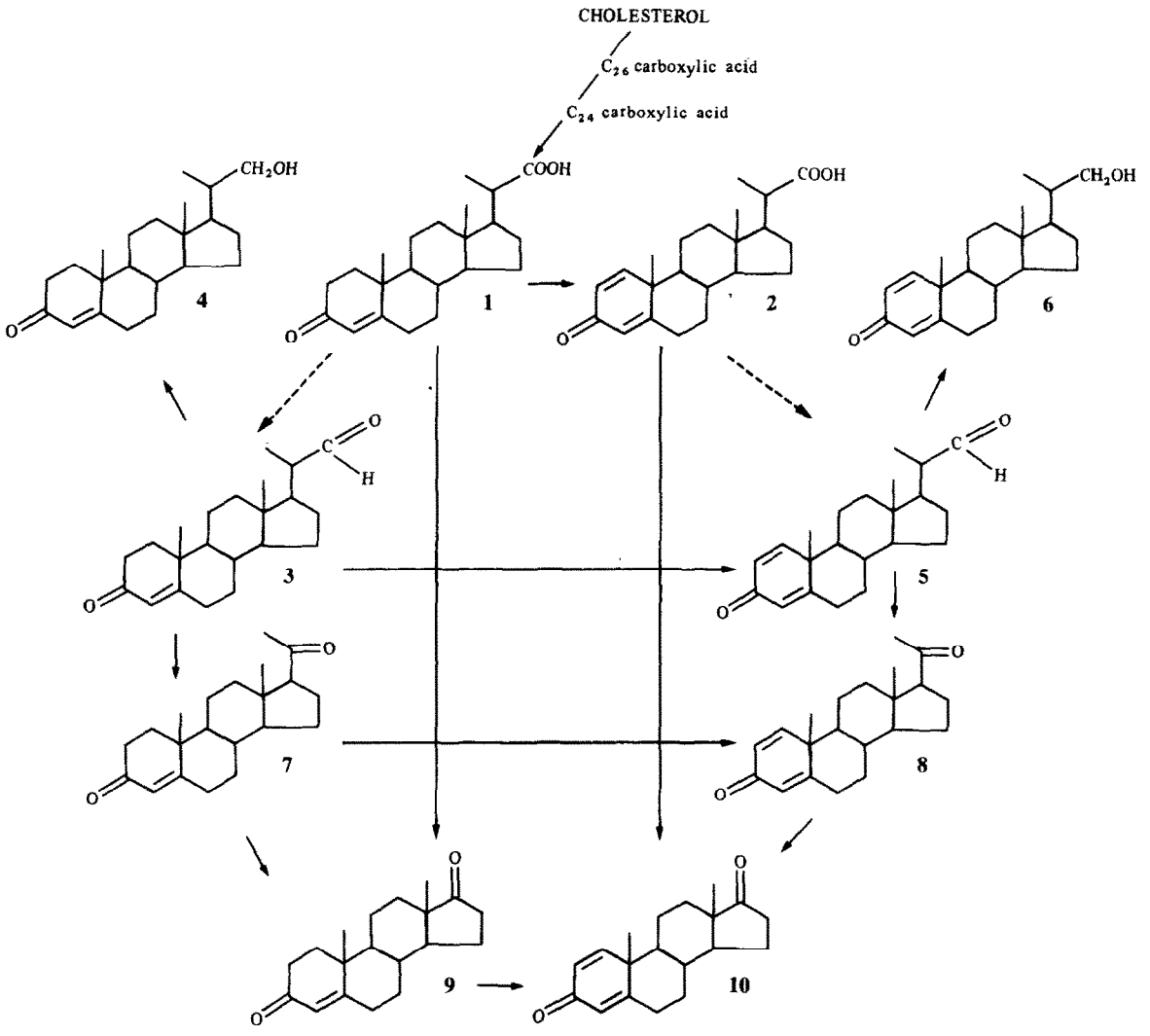
Analytical methods

Analysis by gas chromatography (GLC) was performed at 270°C using 3.5% OV-17 gaschrom Q on 80/100 mesh in a 1.8 m × 3 mm column with a flow rate of 60 ml/m^{-1} nitrogen in a gas chromatograph GCMF 18.3 with FID from VEB Chromatron, Berlin, G.D.R. Analysis by thin-layer chromatography (TLC) was performed on 0.25 mm layers of Kieselgel GF 254 (Merck) in chloroform–ethylacetate (85:15, v/v). Products were detected under u.v. light and by spraying with 10% H_2SO_4/ethanol and heating at approx. 120°C for 10 min.

RESULTS AND DISCUSSION

In fermentation cultures of *Mycobacterium aurum* ATCC 25 790 without using the inhibitor 2,2′-bipyridyl cholesterol was completely degraded without accumulation of intermediates. In the presence of the inhibitor 1,4-andostadiene-

*To whom correspondence should be addressed (present address): Professor Cläre Hörhold, Akademie der Wissenschaften der D.D.R., Zentralinstitut für Mikrobiologie und experimentelle Therapie, Jena Beutenbergstr. 11, Schließfach 73, 6900 Jena, D.D.R.



Scheme 1. Proposed pathway of progesterone (**7**) and 1-dehydroprogesterone (**8**) formation from cholesterol and further degradation by *Mycobacterium aurum* ATCC 25 790. Suggested reactions ↓ and demonstrated reactions ↓.

3,17-dione (ADD) (**10**), 4-androstene-3,17-dione (AD) (**9**), (20S)-20-hydroxymethyl-4-pregnen-3-one (**4**), (20S)-hydroxymethyl-1,4-pregnadien-3-one (**6**), (20S)-20-carboxy-4-pregnadien-3-one (**2**) and additionally 1-dehydroprogesterone (**8**) and progesterone (**7**) were detected by thin-layer chromatography and gas chromatography in yields of 0.01–0.025%. No C_{22}-aldehyde was detectable. Starting from (20S)-20-carboxy-4-pregnen-3-one (**1**) the fermentation yielded an identical product spectrum (Table 1). Using the same conditions and starting from the chemically

Table 1. Transformation pattern of cholesterol and intermediates of the side chain degradation pathway by *M. aurum*

Substrate		1,2′-Bi-pyridyl	Pattern of metabolites
Cholesterol		−	Total degradation
Cholesterol		+	**1,2,4,6,7,8,9,10**
(20S)-20-Carboxy-4-pregnen-3-one	(**1**)	+	**4,6,7,8,9,10**
(20S)-20-Carboxy-1,4-pregnadien-3-one	(**2**)	+	**4,6,7,8,10**
(20S)-20-Hydroxymethyl-4-pregnen-3-one	(**4**)	+	**6**
(20S)-20-Hydroxymethyl-1,4-pregnadien-3-one	(**6**)	+	Unchanged
(20S)-20-Hydroxymethyl-1,4-pregnadien-3-one	(**6**)	−	Unchanged
(20S)-20-Carboxaldehyde-4-pregnen-3-one	(**3**)	+	**4,5,6,8**
(20S)-20-Carboxaldehyde-1,4-pregnadien-3-one	(**5**)	+	**6,8**
Progesterone	(**7**)	+	**8,10**
1-Dehydroprogesterone	(**8**)	+	**10**
Androst-4-ene-3,17-dione	(**9**)	+	**10**
Androsta-1,4-diene-3,17-dione	(**10**)	+	Unchanged
Control experiment without M. aurum			
(20S)-20-Carboxaldehyde-4-pregnen-3-one	(**3**)	+	**7**
(20S)-20-Carboxaldehyde-1,4-pregnadien-3-one	(**5**)	+	**8**

prepared 4-pregnen-3-one-20-carboxyaldehyde (**3**) and the corresponding 1-ene-compound (**5**), progesterone and 1-dehydroprogesterone respectively were formed. However the same products were obtained without microorganisms by an autocatalytical mechanism. This means enzymatically originated C_{22}-aldehydes may be immediately oxidized by the catalytic action of 2,2'-bipyridyl and copper ions present in the medium. Therefore we conclude that the traces of C_{21}-compounds isolated from fermentation cultures of Mycobacteria are connected with the byway of the sterol side chain degradation which proceeds starting from C_{22}-carboxylic acids (**1**, **2**) via the corresponding aldehyde (**3**, **5**) to the C_{22}-alcohols (**4**, **6**). Progesterone and 1-dehydroprogesterone were formed autocatalytically only from the small, undetectable amount of intermediately produced C_{22}-aldehyde. The small amounts are further diminished by the degradation to AD and ADD respectively.

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