TABLE II

Paper Chromatography of Bile Acids[a]

Substance	R_f
Cholic acid	0.19
Trihydroxycoprostanic acid	0.43
Peak B	0.43
Deoxycholic acid	0.61
Peak A	0.73
Lithocholic acid	0.88

[a] Stationary phase, acetic acid–water (70:30); moving phase, isopropyl ether–heptane (60:40).

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The Microbial Reduction of Δ^4-3-Ketone Pregnene Compounds by a Fungus, *Alternaria bataticola*

Numerous studies on microbial reduction of steroids were carried out many years ago by Mamori and others (1). However, those papers dealt mostly with C_{19}-steroids, namely, androstenedione, testosterone, and others. Only a few reports on microbial reduction of pregnene compounds have been published.

Among these, microbial reductions of double bonds of pregnene compounds were demonstrated (2) in a small amount of by-products of microbial hydroxylation. Camerino and co-workers (3) reported reduction of the 3-ketone of pregnane compounds by yeast. According to their results, pregnane compounds without oxygen function at C-11 were not reduced to 3-hydroxyl, and the presence of a 17α-hydroxyl or 21-hydroxyl group prevented reduction of the 3-ketone.

During the course of an extensive investigation on microbial transformations of steroids, we have found that one of the fungi, *Alternaria bataticola*, is capable of reducing both double bond and the 3-ketone of pregnene compounds with or without an oxygen at C-11, namely, progesterone, deoxycorticosterone, corticosterone, Reichstein's Compound S, cortisone, hydrocortisone, etc. The double bonds of these steroids were reduced at first to the corresponding pregnane (normal series) compounds and then the 3-ketones were reduced to 3-hydroxyl by this microorganism. When progesterone, deoxycorticosterone, or Reichstein's Compound S were used as substrates, it was found that the corresponding 3-ketopregnane was produced at first, and after an additional period of incubation both isomers, 3α- and 3β-hydroxypregnane compounds, were obtained.

In contrast when corticosterone, cortisone, hydrocortisone, or 11α-hydroxyprogesterone were used as substrates these steroids were converted to the corresponding pregnane compounds as mentioned above, but only one isomer, the 3α-hydroxypregnane compound, was produced after an additional incubation.

In our experiment reported here, *Alternaria bataticola* was incubated in modified Czapek media containing 1.5% potato starch in place of sucrose. After incubation at 26° for a period of 48 hr. on a reciprocating shaker, 1.0 g. of steroid dissolved in methanol per 2 l. of medium was added and the incubation was allowed to proceed an additional 48 or 96 hr. The products were extracted with ethyl acetate and the extracts concentrated to dryness. The converted compounds were isolated from the extracts by alumina or Florisil partition chromatography. Identification of these compounds was made by physical constants, and by behavior on paper partition chromatography or infrared spectra. These results are given in Table I.

TABLE I

Microbial Reduction of C_{21} Steroids

Substrate	Products[a] (Corresponding derivatives)		
	3-keto-pregnane	3α-hydroxy-pregnane	3β-hydroxy-pregnane
Progesterone	+	+	+
Deoxycorticosterone	+	+	+
Compound S	+	+	+
17α-Hydroxyprogesterone	(+)	(+)	(+)
Corticosterone	+	+	—
Cortisone	+	+	—
Hydrocortisone	+	+	—
11α-Hydroxyprogesterone	(+)	(+)	—

[a] ( ): Not isolated, estimated by paper chromatography; +: produced; —: not produced.

The physical constants of the isolated compounds are given below.

Pregnane-3,20-dione (I) · m.p. 120–122°, $[\alpha]_D$ +124°, I.R. = identical.[1] 3α-Hydroxypregnane-20-one (II): m.p. 142°, $[\alpha]_D$ +96°, I.R. = identical. 3α-Acetate of (II): m.p. 98–101°, $[\alpha]_D$ +140°, I.R. = identical. 3β-Hydroxypregnane-20-one (III): m.p. 188–192°, $[\alpha]_D$ +90°. 3β-Acetate of (III): m.p. 140–144°, I.R. = identical. 21-Hydroxypregnane-3,20-dione-21-acetate:[2] m.p. 145–150°, $[\alpha]_D$ +100°, I.R. = identical. 3α,21-Dihydroxypregnane-20-one (IV): m.p. 139–145°, I.R. = identical. 3α,21-Diacetate of (IV): m.p. 85–87°, $[\alpha]_D$ +100°. 3β,21-Dihydroxypregnane-20-one-3,21-diacetate:[2] m.p. 99–107°, $[\alpha]_D$ +85°. 17α,21-Dihydroxypregnane-3,20-dione (V): m.p. 185–190°, $[\alpha]_D$ +40°. 21-Acetate of (V): m.p. 186–191°, $[\alpha]_D$ +60°. 3α,17α,21-Trihydroxypregnane-20-one (VI): m.p. 203–205°, $[\alpha]_D$ +62°. 3α,21-Diacetate of (VI): m.p. 198–201°, $[\alpha]_D$ +82.5°, I.R. = identical. 3β,17α,21-Trihydroxypregnane-20-one (VII): m.p. 215–225°, $[\alpha]_D$ +48°. 3β,21-Diacetate of (VII): m.p. 148–150°, $[\alpha]_D$ +44°, I.R. = identical. 11β,21-Dihydroxypregnane-3,20-dione-21-acetate:[2] m.p. 153–155°, $[\alpha]_D$ +128°, I.R. = identical. 3α,11β,21-Trihydroxypregnane-20-one (VIII): m.p. 196–197°, $[\alpha]_D$ +88°, I.R. = identical. 3α,21-Diacetate of (VIII): m.p. 90–96°, $[\alpha]_D$ +80°. 17α,21-Dihydroxypregnane-3,11,20-trione (IX): m.p. 214–217°, $[\alpha]_D$ +92°. 21-Acetate of (IX): m.p. 221°, $[\alpha]_D$ +72°, I.R. = identical. 3α,17α,21-Trihydroxypregnane-11,20-dione (X): m.p. 188–191°, $[\alpha]_D$ +60°. 3α,21-Diacetate of (X): m.p. 227–231°, $[\alpha]_D$ +95°, I.R. = identical. 11β,17α,21-Trihydroxypregnane-3,20-dione (XI): m.p. 192–198°, $[\alpha]_D$ +94°. 21-Acetate of (XI): m.p. 195–205°, $[\alpha]_D$ +80°, I.R. = identical. 3α,11β,17α,21-Tetrahydroxypregnane-20-one-3,21-diacetate:[2] m.p. 200–210°, $[\alpha]_D$ +82°, I.R. = identical.

The order of increasing polarity of these free compounds on paper partition chromatography was: 3β-Hydroxypregnane > 3α-hydroxypregnane > 3-keto-Δ^4-pregnene > 3-ketopregnane. Microanalytical data showed that these compounds incurred no change in the oxygen content when compared to the original Δ^4-3-ketosteroids.

In these microbial reductions we have found that both the 4,5-double bond and the carbonyl of the conjugated ketone of pregnenes, with and without 21-, 17α-hydroxy or 11-oxygen, could be reduced to the corresponding 3-hydroxypregnane analogous.

And it is notable to state that this fungus, *Alternaria bataticola*, reduced pregnene compounds which have no oxygen function at C-11 to both the 3α- and 3β-hydroxypregnane derivatives, but those which have an oxygen at C-11 were reduced to the 3α-hydroxypregnane only. From these results, it appears that an oxygen function at C-11 of steroids influences the stereochemistry of the hydrogenation of pregnane compounds to 3-hydroxyl by this microorganism.

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[1] Infrared is identical with that of authentic compound.

[2] These compounds were crystallized as acetate derivatives.

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Sequence of Components in the Succinic Chain of the Mitochondrial Electron Transport System

In the electron transport chain of beef heart mitochondria, electrons can enter the chain through two entry points: (1) succinate → flavoprotein of the succinic dehydrogenase (f_S); and (2) DPNH → flavoprotein of the DPNH dehydrogenase (f_D). f_S and f_D are not linked directly one to the other but are independently linked to the electron transfer chain (1).

In a companion communication (2) it has been shown that the cytochromes are not obligatory components in the reduction of coenzyme Q by the succinic flavoprotein. In the present communication evidence will be presented bearing on the nature of the components involved in the reoxidation of reduced coenzyme Q (QH_2), and on the over-all sequence of components in the segment of the chain between succinate and oxygen.

The observation of Hatefi (3) which was independently confirmed by Pumphrey and Redfearn (4) that the cytochrome *c*-dependent oxidation of QH_2 by oxygen was completely inhibited by antimycin A, suggested that coenzyme Q preceded cytochrome c_1 in the electron transfer chain. In the SDC preparation of Rabinowitz and de Bernard (5), known to be free of cytochromes *c* and *a*, QH_2 reduces only cytochrome c_1, and this reduction is antimycin-sensitive (cf. Fig. 1). Cytochrome *b* does not appear to be required for the reaction between QH_2 and c_1.

A particle ($f_Sf_Dc_1c$), essentially free of cytochrome *b*, catalyzing the reduction of cytochrome *c* by QH_2 or succinate can be prepared by repeated extraction of the succinic dehydrogenase complex (SDC) (1) with 10% isobutanol at 15°. After this treatment the particle contained 3.2 mμmoles cytochrome c_1, 1.0 mμmoles cytochrome *c*, 0.15 mμmoles cytochrome *b*, and 1.21 mμmoles flavin per mg. protein. Thirty five per cent of the flavin was released only after tryptic digestion. With cytochrome *c* as the electron acceptor the $f_Sf_Dc_1c$ particle catalyzed the antimycin-sensitive oxidation of 0.8 μmoles succinate or 0.6 μmoles QH_2 per min. per mg. protein. While there was a ten-fold decrease in the cytochrome *b* in going from the SDC to the $f_Sf_Dc_1c$, the succinate-cytochrome *c* activity dropped by less than half, and the QH_2 to cytochrome *c* activity increased (6). The QH_2 → cytochrome *c* reaction appears to be a function of the cytochrome c_1, not the cytochrome *b*, content of the particle. It has been shown (2) that cytochrome *b* is not required for the reduction of coenzyme Q by succinate, and the data given above suggests that cytochrome *b* does not participate in the reoxidation of QH_2. Since it has been shown that coenzyme Q is an essential component in the antimycin-sensitive oxidation of succinate by oxygen (7), these observations lead to the following formulation: $f_S \rightarrow Q \rightarrow c_1$.