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## 147. Katsumi Tanabe, Ryozo Hayashi, Rinji Takasaki, and Makoto Shirasaka : Steroid Series. II.[1] Microbial Oxidation of Progesterone by *Absidia regnieri*.*[2]

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Hydroxylation of 14$\alpha$-position in steroids by microörganisms has been effected largely by the genera of the order of Mucorales but some examples of the use of imperfect fungi such as *Curvularia lunata* and *Bacillus cereus* have also been reported.[2~5]

Microbial oxidation of progesterone was carried out in this Laboratory using *Absidia regnieri*, a member of Mucorales, and several kinds of hydroxylated product were isolated. As far as the substances whose structure had been determined are concerned, all were hydroxylated in 14$\alpha$-position.

Paper partition chromatography of the extract from microbial oxidation product, using propylene glycol-toluene as a solvent, indicated six spots and suggested that this oxidation is fairly complicated. The chloroform-insoluble portion of this extract was recrystallized from methanol to prismatic crystals (Ⅶ). The portion remaining after removal of (Ⅶ) was chromatographed over neutralized Florisil and six kinds of crystalline substances (I to Ⅵ) were isolated, as shown in Table I. The total of seven kinds of product thereby obtained all showed ultraviolet absorption maximum at around 240 m$\mu$, indicating that they all retain the 4-en-3-one system. Analytical values of (I) agreed with those for monohydroxyprogesterone, those of (Ⅵ) to trihydroxyprogesterone, and all the others to dihydroxyprogesterone. The structure of (Ⅳ) and (Ⅴ) was not determined due to lack of material but the structure of other oxidation products was clarified.

Table I.

| Subst. No. | m.p. (°C) | $[\alpha]_D$ (solvent) | $\lambda_{max}^{EtOH}$ m$\mu$ ($\varepsilon$) | Formula |
|---|---|---|---|---|
| ( I ) | 192.5~194 | +197.5 ($CHCl_3$) | 240.5 (17,000) | $C_{21}H_{30}O_3$ |
| (Ⅱ) | 249~253 | +149.2 ( 〃 ) | 240.0 (17,600) | $C_{21}H_{30}O_4$ |
| (Ⅲ) | 208~214 | +154.4 ( 〃 ) | 242.0 (16,900) | 〃 |
| (Ⅳ) | 212~213.5 | +153.8 (EtOH) | 241.5 (16,200) | 〃 |
| ( Ⅴ ) | 227~228.5 | + 88 (pyridine) | 240.0 (16,500) | 〃 |
| (Ⅵ) | 267~269 | + 75.1 ( 〃 ) | 239.5 (16,000) | $C_{21}H_{30}O_5$ |
| (Ⅶ) | 242~246 | +132 ( 〃 ) | 236.5 (13,500) | $C_{21}H_{30}C_4$ |

The substance (I) resists acetylation with acetic anhydride and pyridine, and oxidation with chromium trioxide in acetic acid, so that the hydroxyl function introduced microbiologically is thought to be tertiary. Of the known monohydroxyprogeterones with a tertiary hydroxyl, physical constants of 9$\alpha$-hydroxy compound, m.p. 190~192°, $[\alpha]_D$ +204°, and 14$\alpha$-hydroxy compound, m.p. 195~200°, $[\alpha]_D$ +188°,[3,4] are close to those of (I). Admixture with an authentic sample of 14$\alpha$-hydroxyprogesterone, kindly furnished by Dr. D.A. Shephard of the Upjohn Company, U.S.A., failed to show any depression

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*[2] A brief report of this work was published as a Communication to the Editor in Bull. Agr. Chem. Soc. Japan, **23**, 244(1959).

1) Part I : This Bulletin, **7**, 804(1959).

2) E. Vischer, A. Wettstein : "Advances in Enzymology," Vol., **20**, 247(1958). Interscience Publishers, Inc., U.S.A.

3) S.H. Eppstein, *et al.* : J. Am. Chem. Soc., **80**, 3382(1958).

4) A. Schubert, D. Onken, R. Siebert, K. Heller : Ber., **91**, 2549(1958).

5) M. Nishikawa, H. Hagiwara : Yakugaku Zasshi, **78**, 1256(1958).

of the melting point and infrared absorption spectra in chloroform solution of the two were in complete agreement, confirming their identity.

The substance (II) is not oxidized by chromium trioxide in acetic acid or acetylated by the usual method, suggesting a progesterone with two tertiary hydroxyls. Treatment of this substance with periodic acid also results in recovery of the starting material. Recently, Schubert and others[4] reported microbial oxidation of progesterone by *Circinella* strain and the product isolated from it, 9α,14α-dihydroxyprogesterone, m.p. 272~273°, $[\alpha]_D^{25}$ +179°($CHCl_3$), gave infrared absorption spectrum which agreed with that of (II) over the whole range, although the constants were somewhat different. It is therefore assumed that (II) is 9α,14α-dihydroxyprogesterone contaminated with a small amount of some impurities.

Dihydroxyprogesterone (III) formed a monoacetate, m.p. 191.5~192.5°, by the usual method. Its oxidation with chromium trioxide in acetic acid failed to afford the anticipated ketosteroid, only forming a small quantity of an acid substance. When heated with potassium hydroxide in methanol, (III) lost one mole of water to form the substance (X) of m.p. 180.5~181.5°. Its ultraviolet absorption maximum at 283.5 mμ (ε 27,000) and infrared absorptions at 1650, 1623, and 1585 $cm^{-1}$ indicated the presence of a partial structure of 4,6-dien-3-one.*3 This shows that the liberated hydroxyl was in 7-position. Reduction of (X) over palladium-carbon in methanol results in absorption of two moles of hydrogen to form a tetrahydro compound which is identical with 14α-hydroxypregnan-20-one (IX), obtained by catalytic reduction of (I) under the same condition. This shows that the other hydroxyl group in (III) is in 14α-position.

Treatment of (III) with benzaldehyde in the presence of anhyd. zinc chloride affords a benzylidene compound (XI), m.p. 181.0~184.5°, whose infrared spectrum already lacks the absorption of alcohol and indicates out-of-plane vibration of a monosubstituted benzene at 705 and 763 $cm^{-1}$. These data suggest that (XI) is 7α,14α-benzylidene derivative and the hydroxyl in 7-position is in a position *cis* to the 14-hydroxyl, taking α-configuration. This is also supported by the fact that the molecular rotational difference ($\Delta M_D$ −117°) between (III) ($M_D$ +535°) and (II) ($M_D$ +652°) is a levorotatory contribution.[6]

Incubation of progesterone with *Syncephalastrum* sp. for a long period of time results in formation of 7α,14α,15β-trihydroxyprogesterone, m.p. 269~275°; $[\alpha]_D^{28}$ +103°(c=0.5, MeOH); UV : $\lambda_{max}$ 240.4 mμ (ε 12,500).[7] Similarity of physical constants of (VI) with these data suggested their identity and this was confirmed through mixed fusion. The infrared spectra of the two substances also agreed completely.

The substance (VII) colored pale red with triphenyltetrazolium chloride. It formed a monoacetate, m.p. 176.5~177.5°, by treatment with acetic anhydride and pyridine, and its oxidation with chromium trioxide in acetic acid produced a monohydroxylated dioxosteroid (VIII), m.p. 198~203°. It was therefore assumed that one of the two newly introduced hydroxyl groups is secondary and the other, tertiary. (VIII) exhibited ultraviolet absorption maximum at 251 mμ (ε 10,300) which corresponds to the absorption of $\Delta^4$-3,6-dioxosteroid*4 and the secondary hydroxyl is assumed to be in 6-position.

It is known that the hydroxyl in 6-position of $\Delta^4$-3-oxosteroid, same as that in 2-position, is reductively liberated easily by stirring the steroid with zinc in acetic acid at room temperature.[8] Treatment of (VII) according to this process resulted in its reduc-

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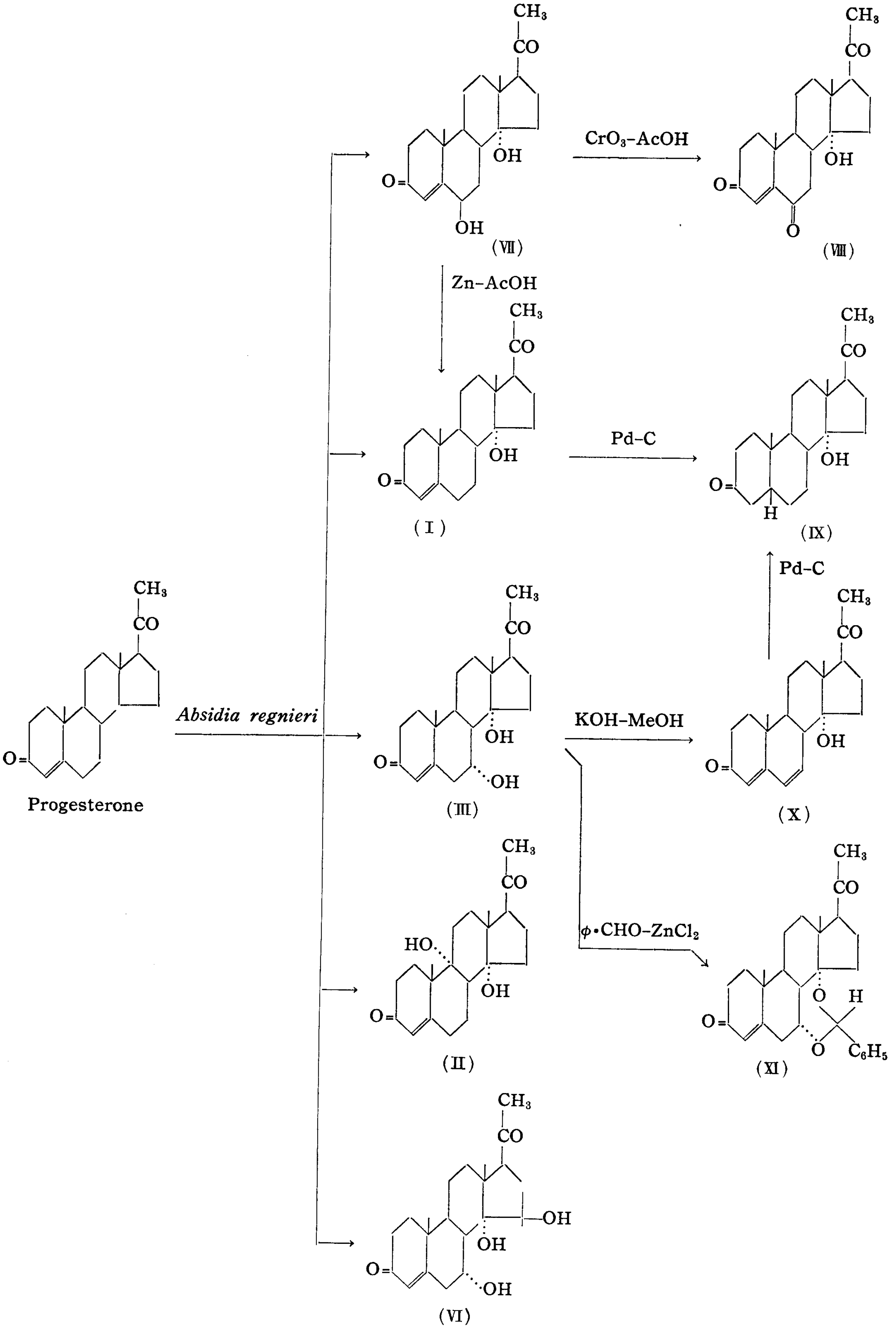
*3 e.g. 4,6,22-Ergostatrien-3-one, UV : $\lambda_{max}^{EtOH}$ 280 mμ; 6-dehydrocorticosterone, UV : $\lambda_{max}^{EtOH}$ 283 mμ.

*4 e.g. Cholest-4-ene-3,6-dione, UV : $\lambda_{max}^{CHCl_3}$ 252 mμ (log ε 4.0)

6) Ch. Meystre, E. Vischer, A. Wettstein : Helv. Chim. Acta, **38**, 381(1955).

7) K. Tsuda, E. Ohki, A. Tanaka, T. Matsuhisa : Paper presented at Kanto Local Meeting of the Pharmaceutical Society of Japan, December 6, 1958. This Bulletin, 7, 369(1959).

8) F. Sondheimer, *et al.* : J. Am. Chem. Soc., **75**, 4712(1953).


Progesterone
*Absidia regnieri*
(Ⅶ)
$CrO_3$-AcOH
(Ⅷ)
Zn-AcOH
(Ⅰ)
Pd-C
(Ⅸ)
Pd-C
(Ⅲ)
KOH-MeOH
(Ⅹ)
$\phi$•CHO-$ZnCl_2$
(Ⅺ)
(Ⅱ)
(Ⅵ)

tion to form 14$\alpha$-hydroxyprogeterone (I) in a high yield and this is another evidence that the secondary hydroxyl is in 6-position, as stated above, and also proves the presence of the tertiary hydroxyl in 14$\alpha$-position.

The ultraviolet absorption maximum of (VII) shows a hypsochromic shift of ca. 4~5 mμ from that of normal $\Delta^4$-3-oxosteroid and molecular absorption coefficient also indicates a lower value by ca. 2000. Such abnormality of ultraviolet absorption spectrum indicates that the hydroxyl in 6-position is $\beta$-oriented[9] and this fact is endorsed from the difference in molecular rotation as shown in Table II.

Table II. Molecular Rotation Increments of 6$\beta$- and 6$\alpha$-Hydroxyl Groups

| Progesterone substituent | $M_D$ | $\Delta M_D$ 6$\beta$- and 6$\alpha$-OH |
|---|---|---|
| Progesterone | +605[a] | |
| 6$\beta$-Hydroxy | +353[b] | −252 |
| 6$\beta$-Acetoxy | +376[b] | −229 |
| 6$\alpha$-Hydroxy | +495[b] | −110 |
| 6$\alpha$-Acetoxy | +517[b] | − 98 |
| 14$\alpha$-Hydroxy (I) | +653 | |
| 6$\beta$,14$\alpha$-Dihydroxy (VII) | +395 | −258 |
| 6$\beta$-Acetoxy-14$\alpha$-hydroxy | +452 | −201 |

*a*) D.K. Fukushima, T.F. Gallagher; J. Am. Chem. Soc., **73**, 196(1951).
*b*) C.P. Balant, E. Ehrenstein : J. Org. Chem., **17**, 1587(1952).

Camerino[10] had already isolated 6$\beta$,14$\alpha$-dihydroxyprogesterone by incubation of progesterone with *Mucor corymbifer*. Its constants of m.p. 255~265°, $[\alpha]_D$ +142°±4°($CHCl_3$), are somewhat higher in value than those of (VII) but the values of its monoacetate, m.p. 175~178°, $[\alpha]_D$ +125°±4°($CHCl_3$), are almost the same as those of the monoacetate obtained in the present series of experiments.

Meyer[11] examined periodical change of ultraviolet absorption spectrum of hydroxylated $\Delta^4$-3-oxosteroids in alcoholic alkali and stated that there is a marked difference in shift in the position of absorption maximum and in other spectral changes by the difference in the configuration of 6-hydroxyl group. Periodical change in ultraviolet absorption spectrum was examined by Meyer's method with 0.066*N* ethanolic potassium hydroxide solution of (VII) at 23°. The result of this measurement is shown in Table III.

Table III. Spectral Change of (VII) in Alakline Medium over varying Periods of Time

| Time | First $\lambda_{max}$, mμ ($\varepsilon$) | $\lambda_{min}$, mμ ($\varepsilon$) | Second $\lambda_{max}$, mμ ($\varepsilon$) |
|---|---|---|---|
| 1 min. | 236.5 (12,500) | — | — |
| 5 min. | 236.5 (11,800) | — | 384 (942) |
| 1 hr. | 238.5 (10,700) | 306 | 379 (2290) |
| 4 hr. | 244.5 (8,800) | 304 | 380 (3540) |
| 8 hr. | 256 (7,660) | 304 | 377 (6280) |

According to Meyer, such spectral changes are characteristic to 6$\alpha$-hydroxyl group and there is only a slight (3~4 mμ) bathochromic shift of absorption maximum in the 6$\beta$-hydroxylated compound. As described above, (VII) is most probably a 6$\beta$-hydroxylated compound and such discrepancy is hard to understand.

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[9] C. Amendolla, G. Rosenkranz, F. Sondheimer : J. Chem. Soc., **1954**, 1226.
[10] B. Camerino, C.G. Alberti, A. Vercellone : Gazz. chim. ital., **83**, 684(1953); A. Wettstein : Experientia, **11**, 465(1955).
[11] A.S. Meyer : J. Org. Chem., **20**, 1240(1955).

Microbiology for the donation of 7α,14α,15β-trihydroxyprogesterone. They are indebted to Misses C. Furukawa and H. Ohtsuka, and Messrs. O. Amakasu, H. Higuchi, and N. Higosaki, all of this Laboratory, for elemental analyses and for infrared and ultraviolet spectral measurements.

## Experimental

**Oxidation of Progesterone with *Absidia regnieri***—A medium consisting of 5% of glucose, 2% of peptone, and 0.5% of corn steep liquor was poured into a 500-cc. shake flasks, 100 cc. to each flask, and sterilized. Each flask was seeded with ca. 5 cc. of 24-hr. culture of *Absidia regnieri* and incubated at 27° for 2 days. Two cc. each of 2.5% MeOH solution of progesterone was added to each flask and the flasks were incubated for further 3 days. At the end of this period, cellular material was filtered off, and the filtrate and cells were each extracted twice with AcOEt. The combined extract was washed with 2% $NaHCO_3$ solution and water, dried over anhyd. $Na_2SO_4$, and the solvent was evaporated, leaving 11.6 g. of an oily substance. This residue was diluted with 250 cc. of $CHCl_3$ and the solution was warmed for a while, from which 1.10 g. of insoluble matter, m.p. 220～240°, was separated. Recrystallization from MeOH afforded 6β,14α-dihydroxyprogesterone (VII) as prismatic crystals, m.p. 242～246°(decomp.); $[\alpha]_D^{28}$ +132°(c=0.725, pyridine), +114°(c=0.53, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$ : C, 72.80; H, 8.73. Found : C, 72.61; H, 8.72. UV : $\lambda_{max}^{EtOH}$ 236.5 mμ ($\varepsilon$ 13,500). IR $\nu_{max}^{KBr}$ $cm^{-1}$ : 2497, 3367 (OH), 1701 (20-CO), 1669 (3-CO), 1619 ($\Delta^4$).

6β-Monoacetate of (VII) : Prepared by the usual method with $Ac_2O$ in pyridine, m.p. 176.5～177.5° (from MeOH); $[\alpha]_D^{29}$ +116.7°(c=0.79, $CHCl_3$), +101.8°(c=1.35, pyridine). *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found : C, 71.26; H, 8.27. UV : $\lambda_{max}^{EtOH}$ 235.0 mμ ($\varepsilon$ 13,900).

The $CHCl_3$ solution of the oxidation products, after removal of (VII), was concentrated to 55 cc. and chromatographed over 300 g. of neutralized Florisil.[*5] The $CHCl_3$ eluate afforded a small amount of fluorescent oil.

The fraction eluted with $CHCl_3$-acetone (100:2) gave 1.14 g. of crude crystals melting at 175～185° which recrystallized from acetone-hexane mixture to 14α-hydroxyprogesterone (I) as prismatic crystals, m.p. 192.5～194°; $[\alpha]_D^{27}$ +197.5°(c=1.54, $CHCl_3$), +186°(c=0.94, EtOH). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found : C, 76.19; H, 9.03. UV : $\lambda_{max}^{EtOH}$ 240.5 mμ ($\varepsilon$ 17,000). IR $\nu_{max}^{KBr}$ $cm^{-1}$ : 3584 (OH), 1690(20-CO), 1674(3-CO), 1625($\Delta^4$); $\nu_{max}^{CHCl_3}$ $cm^{-1}$ : 3400～3500(OH), 1669(20-CO), 1667(3-CO), 1618($\Delta^4$).

The fraction eluted with $CHCl_3$-$Me_2CO$ (100:4) afforded 0.75 g. of crude crystals melting at 205～230° which recrystallized from MeOH to 9α,14α-dihydroxyprogesterone (II) as prismatic crystals, m.p. 249～253° (decomp.); $[\alpha]_D^{29}$ +149.2° (c=1.03, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$ : C, 72.80; H, 8.73. Found : C, 72.58; H, 8.57. UV : $\lambda_{max}^{EtOH}$ 240 mμ ($\varepsilon$ 17,600). IR[*6] $\nu_{max}^{KBr}$ $cm^{-1}$ : 3290 (m), 1698 (s), 1667 (vs), 1623 (w), 1274 (w), 1238 (m), 1208 (m), 1193 (m), 1116 (w), 1080 (w), 1058 (w), 959 (m), 955 (m), 926 (m), 916 (w), 899 (w), 882 (w), 865 (m).

The fraction eluted with $CHCl_3$-$Me_2CO$ (100:8) afforded 2.03 g. of crude crystals melting at 201～210° which recrystallized from $Me_2CO$ to 7α,14α-dihydroxyprogesterone (III) as needles, m.p. 208～214°; $[\alpha]_D^{27}$ +154.4°(c=0.94, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{20}O_4$ : C, 72.80; H, 8.73. Found : C, 72.91; H, 8.57. UV : $\lambda_{max}^{EtOH}$ 242 mμ ($\varepsilon$ 16,900). IR $\nu_{max}^{KBr}$ $cm^{-1}$ : 3425 (OH), 1700 (20-CO), 1677 (3-CO), 1631 ($\Delta^4$).

7α-Monoacetate of (III) : Obtained by the usual method with $Ac_2O$ and pyridine and recrystallized from $Me_2CO$-hexane to needles, m.p. 191.5～192.5°; $[\alpha]_D^{27}$ +161°(c=0.99, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{32}O_5$ : C, 71.10; H, 8.30. Found : C, 71.19; H, 8.30.

From the first eluate with $CHCl_3$-$Me_2CO$ (100:20), 0.63 g. of crude crystals of m.p. 185～203° was obtained and recrystallized from $Me_2CO$-hexane mixture to (IV) as prismatic crystals, m.p. 212～213.5°; $[\alpha]_D^{28}$ +153.8°(c=0.89, in EtOH). *Anal.* Calcd. for $C_{21}H_{30}O_4$ : C, 72.80; H, 8.73. Found : C, 72.60; H, 8.53. UV : $\lambda_{max}^{EtOH}$ 241.5 mμ ($\varepsilon$ 16,200). IR $\nu_{max}^{KBr}$ $cm^{-1}$ : 3378 (OH), 1699 (20-CO), 1672 (3-CO), 1618 ($\Delta^4$).

The latter eluate with the same solvent afforded 0.49 g. of crude crystals, m.p. 210～218°, which recrystallized from MeOH to (V) as plate crystals, m.p. 227～228.5°; $[\alpha]_D^{28}$ +88°(c=1.12, pyridine). *Anal.* Calcd. for $C_{21}H_{30}O_4$ : C, 72.80; H, 8.73. Found : C, 72.64; H, 8.42. UV $\lambda_{max}^{EtOH}$ 240 mμ ($\varepsilon$ 16,500). IR $\nu_{max}^{KBr}$ $cm^{-1}$ : 3521 (OH), 3350 (OH), 1701 (20-CO), 1672 (3-CO), 1629 ($\Delta^4$).

Fractions eluted with $CHCl_3$-$Me_2CO$ (2:1) and $Me_2CO$ afforded 1.06 g. of crude crystals, m.p. 225～240°. This recrystallized from EtOH to 7α,14α,15β-trihydroxyprogesterone (VI) as prismatic crystals, m.p. 267～269°(decomp.); $[\alpha]_D^{28}$ +75.1°(c=1.02, pyridine). *Anal.* Calcd. for $C_{21}H_{30}O_5$ : C, 69.58; H, 8.34.

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[*5] Washed with 5% AcOH solution and water, and dried at 140° for 20 hr.

[*6] Absorption spectrum reported by Schubert, *et al.*5) IR $\nu_{max}^{Nujol}$ $cm^{-1}$ : 3290 (m), 1700(s), 1675 (vs), 1623 (w), 1278 (w), 1243 (m), 1212 (m), 1198 (m), 1118 (w), 1082 (w), 1060 (w), 960 (m), 950 (m), 932 (m), 920 (w), 904 (w), 887 (w), 862 (m).

Found: C, 69.45; H, 8.02. UV: $\lambda_{\max}^{\mathrm{EtOH}}$ 239.5 mμ (ε 16,000). IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3521 (OH), 3367 (OH), 1689 (20-CO), 1678 (3-CO), 1629 ($\Delta^4$).

**14α-Hydroxy-4,6-pregnadiene-3,20-dione (X)**—A solution of 110 mg. of 7-monoacetate of (III), 15 cc. of MeOH, and 100 mg. of KOH was refluxed for 1 hr., MeOH was evaporated under a reduced pressure, 10 cc. of water was added to the residue, and extracted with $CHCl_3$. The extract was washed with water, dried, and the solvent was evaporated, leaving a crystalline substance of m.p. 173.5~178°. This solid was chromatographed over 10 g. of alumina and a fraction eluted with benzene-$CHCl_3$ (3:1) afforded 57 mg. of needle crystals (from MeOH), m.p. 180.5~181.5° (reported$^{10)}$ m.p. 195~197°). *Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.69; H, 8.33. UV: $\lambda_{\max}^{\mathrm{EtOH}}$ 283.5 mμ (ε 27,000). IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3425 (OH), 1709 (20-CO), 1650 (3-CO), 1623, 1585 ($\Delta^{4,6}$).

**14α-Hydroxypregnane-3,20-dione (IX)**—i) A solution of 37 mg. of (X) dissolved in 8 cc. of MeOH and added with 50 mg. of 5% Pd-C was submitted to reduction and 6 cc. of $H_2$ (27°, 758 mm. Hg), equivalent to 2.1 double bonds per molecule, was absorbed. After removal of the catalyst by filtration, MeOH was evaporated from the filtrate and the residue was chromatographed over 8 g. of alumina. Elution with benzene-$CHCl_3$ (2:1) afforded crude crystals melting at 154~157° and its recrystallization from $Me_2CO$-petr. ether gave 14α-hydroxypregnane-3,20-dione (IX), m.p. 162.5~164°. $[\alpha]_D^{26}$ +146.5°(c=0.77, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.96; H, 9.56. IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3472 (OH), 1701 (3- and 20-CO).

ii) A solution of 95 mg. of (I) dissolved in 6 cc. of MeOH and 50 mg. of 5% Pd-C was submitted to reduction and 9.0 cc. of $H_2$ was absorbed (27°, 759 mm. Hg), equivalent to 1.25 double bonds per molecule. The product was recrystallized from $Me_2CO$-petr. ether mixture to needle crystals, m.p. 166.5~168°; $[\alpha]_D^{27}$ +141°(c=1.20, $CHCl_3$). No depression of m.p. was observed on admixture with the hydroxypregnanedione (IX) obtained by the foregoing method (i) and infrared spectra of the two were in complete agreement.

**7α,14α-Benzylideneprogesterone (XI)**—A mixture of 0.19 g. of freshly fused $ZnCl_2$ added to a solution of 66 mg. of (III) dissolved in 4.0 cc. of freshly distilled benzaldehyde was stirred for 2 hr. at room temperature by which $ZnCl_2$ disappeared and the reaction mixture became reddish orange solution. This mixture was allowed to stand for 12 hr. at room temperature and excess of benzaldehyde was distilled off. The residue, added with 10 cc. of 5% $NaHCO_3$ solution, was extracted with $CHCl_3$, the extract was washed with water, dried, and the solvent was evaporated. The residue so obtained was chromatographed over 15 g. of alumina, the column was eluted with benzene, and 20 mg. of crude crystals, m.p. 175~184.5°, was obtained. Recrystallization from MeOH afforded 7α,14α-benzylideneprogesterone (XI) as needle crystals, m.p. 181~184.5°. *Anal.* Calcd. for $C_{28}H_{34}O_4$: C, 77.39; H, 7.89. Found: C, 77.20; H, 7.82. IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 1706 (20-CO), 1675 (3-CO), 1623 ($\Delta^4$), 763, 765 (CH out-of-plane vibration in the monosubstituted benzene).

**6-Oxo-14α-hydroxyprogesterone (VIII)**—A solution of 14 mg. of $CrO_3$ dissolved in 0.8 cc. of 90% AcOH was added to the solution of 34 mg. of (VII) dissolved in 2.0 cc. of 90% AcOH and the mixture was allowed to stand for 15 hr. at room temperature. Excess of the reagent was decomposed by addition of 0.5 cc. of EtOH, AcOH was distilled off under a reduced pressure, and 5 cc. of water was added to the residue. This was extracted with $CHCl_3$, the extract was washed with water, dried, and the solvent was evaporated to leave 6-oxo-14α-hydroxyprogesterone (VIII) which was recrystallized from MeOH to needles, m.p. 198~203°; $[\alpha]_D^{27}$ +85.7°(c=0.20, $CHCl_3$). UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε): 251 (10,300), 312 (2,150). IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3576 (OH), 1695, 1681 (3,6-dioxo-4-ene, 20-CO), 1621 ($\Delta^4$).

**Reduction of (VII) with Zinc and Acetic Acid**—A solution of 64 mg. of (VII) dissolved in a mixture of 3.0 cc. of glacial AcOH and 1.0 cc. of water, added with 0.3 g. of zinc dust was stirred for 10 min. at room temperature. Zn was filtered off and AcOH was evaporated from the filtrate under a reduced pressure. Ten cc. of water was added to the residue, this was extracted with $CHCl_3$, and the solvent was evaporated from the extract after washing with water and drying. Crude crystals, m.p. 180~188°, so obtained were chromatographed over 5 g. of alumina and the fraction eluted with $CHCl_3$ afforded crystals melting at 188.5~192°. This was recrystallized from $Me_2CO$-hexane mixture to needles, m.p. 192~194°; $[\alpha]_D^{27}$ +204.9°(c=0.74, $CHCl_3$). This showed no depression of m.p. on admixture with 14α-hydroxyprogesterone (I) and infrared spectra of the two compounds agreed completely.

## Summary

Microbial oxidation of progesterone with *Absidia regnieri* was carried out and seven kinds of hydroxylated product were isolated. Five of these products possessed at least one hydroxyl in 14α-position and were found to be 14α-hydroxy-, 9α,14α-dihydroxy-, 7α,14α-dihydroxy-, 6β,14α-dihydroxy-, and 7α,14α,15β-trihydroxyprogesterone.

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