Summary

9-Azahexahydrojulolidine (Ⅲ) was prepared by saponification and decarboxylation of 2-cyanoperhydropyrido[3,4,5-*i,j*]quinolizine (Ⅱ) with hydrochloric acid, obtained by reaction of 9-methyl-9-azahexahydrojulolidine (I) and cyanogen bromide. Series of these compounds was assumed to have the *cis*-quinolizidine ring and, in order to ascertain this assumption, (Ⅲ) was converted to 9-azajulolidine (Ⅳ).

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Steroid Series. I. Microbial Oxidation of Steroids by *Sclerotinia libertiana*.[*1]

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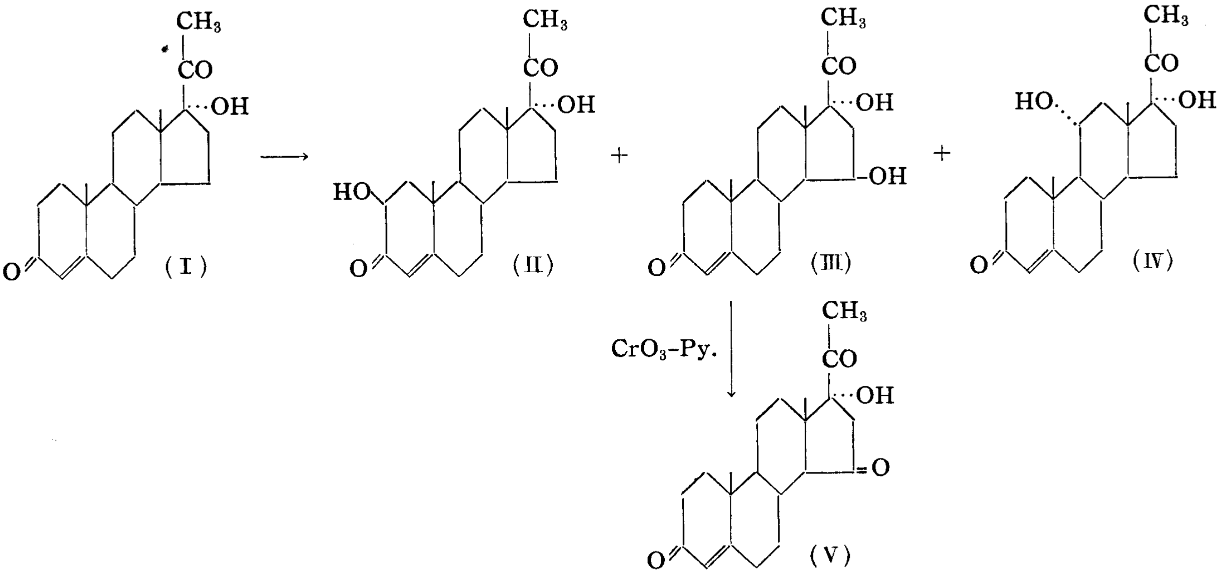
Since Peterson and others[1] succeeded in hydroxylation of 11α-position in progesterone by the use of the Phycomycetes of Rhizopus species and introduced a new process for synthesis of adrenocortical hormones, microbiological transformation of steroids has drawn the interest of many workers. Microbial oxidation of steroids of pregnane series with *Sclerotinia libertiana* was carried out by the present authors and it was found that this organism is capable of hydroxylating the 2β-position. 2β-Hydroxylation of steroids with microörganisms was reported only recently by two research groups, using Penicillium sp.,[2] *Rhizoctonia ferrugena*,[3] and Streptomyces sp.[4]



[*1] A brief report of this work was published as a Communication to the Editor in Bull. Agr. Chem. Soc. Japan, **22**, 273(1958); **23**, 245(1959).

[*2] Nishi-shinagawa, Shinagawa-ku, Tokyo (田辺克己, 高崎林治, 林 了三, 白坂 亮).

1) D.H. Peterson, *et al.* : J. Am. Chem. Soc., **74**, 5933(1952).

2) R.M. Dodson, A.H. Goldkamp, R.D. Muir : *Ibid.*, **79**, 3921(1957).

3) G. Greenspon, *et al.* : *Ibid.*, **79**, 3922(1957).

4) H.L. Herzog, *et al.* : *Ibid.*, **79**, 3921(1957).

In the present paper will be given results obtained from microbial oxidation using *Sclerotinia libertiana*, and 17α-hydroxyprogesterone, 17α-hydroxydesoxycorticosterone (Reichstein's Substance S), and progesterone as a substrate.

Oxidation of 17α-hydroxyprogesterone (I) afforded three kinds of crystalline products of m.p. 219~221°(II), m.p. 258~259°(III), and m.p. 218~221°(IV). The elementary analytical values of these substances all agreed with those calculated for dihydroxyprogesterone, showing the only one hydroxyl group had been introduced.

TABLE I. Molecular Rotational Difference of 2β-Hydroxyl Groups

Compound	M_D	$M_D^{2\beta\text{-OH}}$	$\Delta M_D^{2\beta\text{-OH}}$
Androst-4-ene-3,13-dione[a]	+566	−100	−670
17α-Hydroxydesoxycorticosterone[b]	+381	−300	−681

a) See Footnote 2. *b*) See Footnote 4.

Herzog and others[4] stated that 2β-hydroxyl in 2β,17α-dihydroxydesoxycorticosterone makes larger levorotatory contribution than hydroxyls in any other position (Table I). (II) shows ultraviolet absorption maximum (in EtOH) of 243 mμ (ε 16,000) and infrared absorptions (in KBr) at 3520 (OH), 1706 (20-CO), 1678, and 1623 cm^{-1} (Δ^4-3-CO), with $[\alpha]_D^{29}$ −125°. The value of molecular rotational difference with (I) is −766°, which is in the same order of magnitude as the contribution of 2β-hydroxyl group.

(II) forms a monoacetate of m.p. 187.5~188.5° by treatment with acetic anhydride and pyridine, and reverts to (I) in a high yield when stirred at room temperature with zinc dust in acetic acid. It is known that the hydroxyl group in 2- and 6-position of Δ^4-3-oxosteroid are reductively liberated easily by treatment with zinc dust under foregoing conditions.[5] Considering these facts, (II) was found to be 2β,17α-dihydroxyprogesterone.

(III) showed ultraviolet absorption maximum at 240.5 mμ (ε 15,000) and infrared absorptions at 3448 (OH), 1700 (20-CO), 1656, and 1613 cm^{-1} (Δ^4-3-CO), with $[\alpha]_D$ +54.3° ($CHCl_3$). Oxidation of (III) with chromium trioxide at room temperature afforded crystals (V) of m.p. 247~248°, with infrared absorptions at 3390 (OH), 1736, (five-membered ring ketone), 1706 (20-CO), 1664, and 1618 cm^{-1} (Δ^4-3-CO). These spectral data indicate that the side chain at 17-position remains intact without oxidation and there is a five-membered ring with a carbonyl group, which shows the presence of a newly introduced hydroxyl group in the D-ring of (III) and the possible position of this hydroxyl would be 15α, 15β, 16α, or 16β. Rotational contribution of the hydroxyl substituted in these four

TABLE II. Molecular Rotational Difference for 15-Hydroxyl and 16-Hydroxyl Groups in Δ^4-3-Oxosteroids

Compound	M_D	$M_D^{15\beta\text{-OH}}$	$\Delta M_D^{15\beta\text{-OH}}$	$M_D^{15\alpha\text{-OH}}$	$\Delta M_D^{15\alpha\text{-OH}}$
17α,21-Dihydroxypregn-4-ene-3,20-dione[a]	+395	+348	− 47	+529	+130
Androst-4-ene-3,17-dione[a]	+546	+420	−126	+589	+ 43
Desoxycorticosterone[b]	+613	+490	−123	+679	+ 66
Progesterone[c]	+605	+499	−106	+724	+119

Compound	M_D	$M_D^{16\beta\text{-OH}}$	$\Delta M_D^{16\beta\text{-OH}}$	$M_D^{16\alpha\text{-OH}}$	$\Delta M_D^{16\alpha\text{-OH}}$
Progesterone[d]	+605	+634	+ 29	+522	− 83
3,17β-Estradiol[d]	+220	+253	+ 33	+176	− 44
17α-Hydroxyprogesterone[d]	+340			+329	− 11

a) S. Bernstein, *et al.*: Chem. & Ind. (London), **1956**, 111.
b) A. Wettstein: Experientia, **11**, 465(1955).
c) J. Fried, R. W. Thoma, D. Perlman, J. R. Gerke: U. S. Pat. 2,753,290.
d) D. K. Fukushima, T. F. Gallagher: J. Am. Chem. Soc., **73**, 196(1951).

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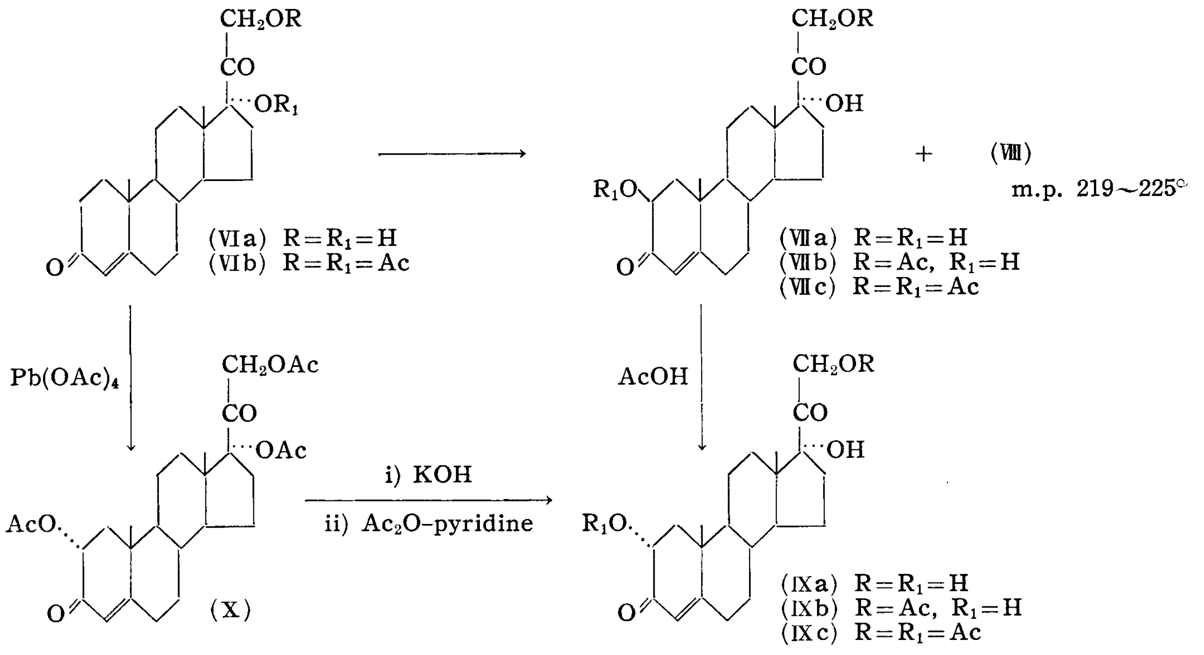
[5] F. Sondheimer, *et al.*: *Ibid.*, **75**, 4712(1953).

positions were calculated from known substances published in the literature and the values are listed in Table II.

The molecular rotation of (III) was +190° and difference with 17α-hydroxyprogesterone is −150°, which suggests that the hydroxyl in question should be at 15β- or 16α-position, as will be clear from Table II. However, 16α,17α-dihydroxyprogesterone is a known substance6) of m.p. 225°, $[\alpha]_D$ +95°, clearly different from (III), so that the structure of (III) should be 15β,17α-dihydroxyprogesterone.

The substance (IV), m.p. 218~221°, $[\alpha]_D$ +73° ($CHCl_3$), showed ultraviolet absorption maximum at 240.5 mμ (ε 17,000) and these data all agreed with those of 11α,17α-dihydroxyprogesterone. Comparison of the infrared spectrum of this substance with that of an authentic sample established their identity.

The oxidation of 17α-hydroxydesoxycorticosterone (VIa) produced two kinds of hydroxylated steroids, one of m.p. 215~222°(decomp.) (VIIa) and the other of m.p. 219~225°(decomp.) (VIII).



(VIIa), $[\alpha]_D$ −64°(dioxane), showed ultraviolet absorption maximum at 243 mμ (ε 16,000) and infrared absorptions at 3333 (OH), 1724 (20-CO), 1684, and 1623 cm^{-1} (Δ^4-3-CO). Its treatment with one mole of acetic anhydride in pyridine produced a monoacetate (VIIb) of m.p. 228~230°, while excess of acetic anhydride gave a diacetate (VIIc) of m.p. 215~218°. From these physical constants, (VIIa) was assumed to be 2β,17α-dihydroxydesoxycorticosterone[*3] obtained by Herzog and others4) by microbial oxidation of 17α-hydroxydesoxycorticosterone (VIa) with Streptomyces sp.

When the monoacetate (VIIb) was refluxed in acetic acid for one hour, a crystalline substance of m.p. 241~245° was produced which gave analyses identical with those of (VIIb), with ultraviolet absorption maximum at 240 mμ (ε 14,700) and infrared absorptions at 3425 (OH), 1748 (21-AcO), 1730 (20-CO), 1678, and 1618 cm^{-1} (Δ^4-3-CO). This substance was assumed to be a 2α-epimer (IXb) of (VIIb) and, therefore, 2α,17α-dihydroxydesoxycorticosterone (IXa) was prepared by the method of Rosenkranz and others.7) The mono-

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[*3] Constants for (VIIa) reported by Herzog, *et al.*5) : m.p. 225.5~228°(decomp.); $[\alpha]_D$ −58°(dioxane). UV λ_{max} 243 mμ (ε 14,500).

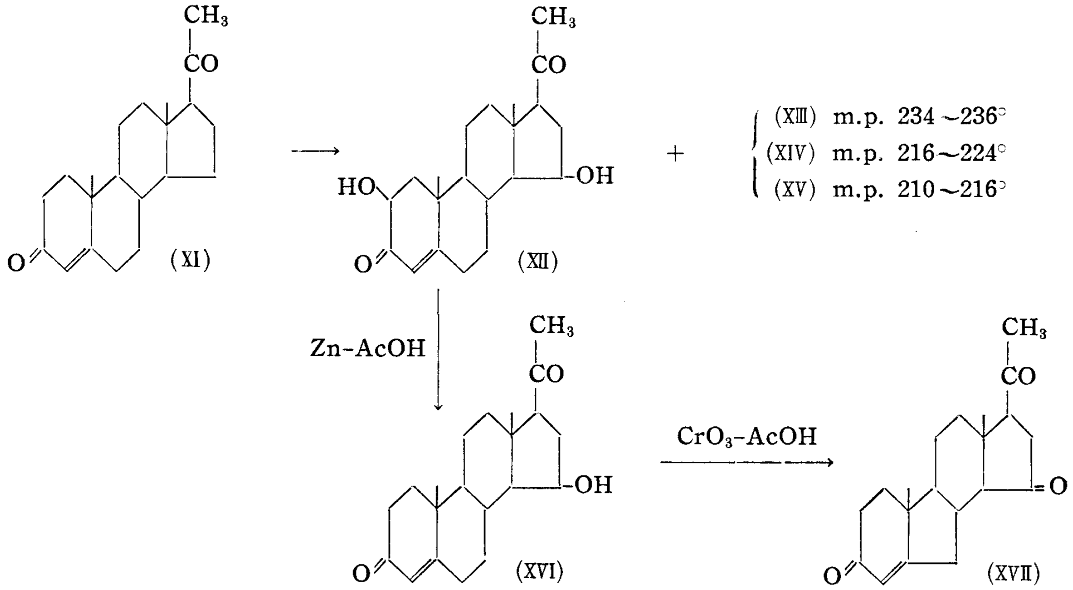
6) G. Cooley, B. Ellis, F. Hartley, V. Petrow : J. Chem. Soc., **1955**, 4373.

7) G. Rosenkranz, O. Mancera, F. Sondheimer : J. Am. Chem. Soc., **77**, 145(1955).

acetate obtained by its acetylation was identified with (IXb) by mixed fusion and infrared spectra of the two substances also agreed completely. It follows, therefore, that (VIIIa) is 2β, 17α-dihydroxydesoxycorticosterone.

Analytical values of (VIII) agreed with those for dihydroxydesoxycorticosterone but no further examinations could be made due to small amount of the sample available.

The product from the microbial oxidation of progesterone (XI) was chromatographed over Florisil and separated into four kinds of crystalline substances of m.p. 206~216° (XII), m.p. 234~236° (XIII), m.p. 216~224° (XIV), and m.p. 210~216° (XV). Structural determination was not carried out except on (XII) due to lack of material.



Analytical values of (XII) agreed with those for dihydroxyprogesterone. It showed ultraviolet absorption maximum at 243 mμ (ε 15,000) and [α]$_D$ −66.7°(CHCl$_3$). Since it forms a diacetate by treatment with acetic anhydride and pyridine at room temperature, the two hydroxyls must be primary or secondary. Presence of α-ketol was indicated by the positive reaction of (XII) to the triphenyltetrazolium chloride test[8] and the strong levorotation of its M$_D$ value,[4] together with the ability of *Sclerotinia libertiana* to hydroxylate 2β-position, suggests the presence of one of the hydroxyls in 2β-position.

Treatment of (XII) with zinc dust in acetic acid at room temperature effected reduction of the hydroxyl in α-ketol, as anticipated,[5] and a crystalline substance (XVI) of m.p. 202~203.5°, [α]$_D^{28}$ +149°(CHCl$_3$), was obtained. This monohydroxyprogesterone (XVI) no longer gave positive reaction to the triphenyltetrazolium chloride test and formed a triketosteroid by oxidation with chromium trioxide. This ketosteroid was assumed to be 15-oxoprogesterone (XVII) from its physical constants and the fact was confirmed by the presence of an absorption for a five-membered ring ketone at 1733 cm^{-1} in its infrared spectrum and the absence of an absorption for 1,3-diketone in its ultraviolet spectrum taken in alkaline ethanol solution, showing that it is not 16-oxosteroid.

It therefore follows that (XVI) is either 15α-[*4,9] or 15β-hydroxyprogesterone.[9] The physical constants agree well with those of the latter and it is concluded that the structure of (XII) is 2β,15β-dihydroxyprogesterone.

[*4] Constans reported for 15α-hydroxyprogesterone: m.p. 231~232°, [α]$_D^{22}$ +219°(CHCl$_3$), λ_{max}^{EtOH} 240 mμ (ε 16,500).

8) W. J. Mader, R. R. Buck: Anal. Chem., **24**, 666(1952).

9) J. Fried, R. W. Thoma, D. Perlman, J. R. Gerke: U. S. Pat. 2,753,290.

Meyer[10] recorded periodical change of ultraviolet absorption spectra of several hydroxylated Δ^4-3-oxosteroids in alkaline ethanol solution and described the characteristic change of the absorption curve of a 2-hydroxylated compound. Measurement of ultraviolet absorption curves of (II), (VIIa), and (XII) according to the direction of Meyer showed that absorption coefficient increased with the shift of absorption maximum near 240 mμ to around 230 mμ, and at the same time typical shoulder at around 255 mμ and a second minimum near 360 mμ appeared. These offer further evidences for 2-hydroxylated steroids.

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Experimental

Oxidation of 17α-Hydroxyprogesterone (I) with *Sclerotinia libertiana*—Ten liters of potato juice containing 300 g. of glucose was placed in shake flasks of 500-cc. capacity, 100 cc. to each flask, and sterilized. About 5 cc. of 48-hr. culture of *Sclerotinia libertiana* was seeded in each flask and this was shake-cultured at 27° for 3 days. To each flask, 2 cc. of 2.5% MeOH solution of 17α-hydroxyprogesterone was added and again shake-cultured for 3 days. After completion of culture, cells were separated by filtration, and the filtrate and cells were each extracted twice with AcOEt. The combined extract was washed consecutively with 2% $NaHCO_3$ and water, dried over anhyd. Na_2SO_4, and the solvent was evaporated, leaving 4.25 g. of an oily residue. The residue was dissolved in 70 cc. of MeOH and the crystals that separated out were 0.52 g. of the starting material.

Concentration of the mother liquor left 3.72 g. of a solid which was dissolved in 70 cc. of $CHCl_3$, the solution was passed through a column of 120 g. of silicic acid, and the column was eluted with $CHCl_3$ and a mixture (100:1) of $CHCl_3$ and MeOH. The effluents afforded 550 mg. of the recovered starting material, m.p. 213~215°, and crystalline products of 330 mg. of (II), m.p. 216~220°, 100 mg. of (III), m.p. 254~258°(decomp.), and 710 mg. of (IV), m.p. 215~219°.

(II) was recrystallized from acetone-hexane mixture to plates, m.p. 219~221°; $[\alpha]^{29}_D$ −125°(c=0.85, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$ (2β,17α-Dihydroxyprogesterone): C, 72.80; H, 8.73. Found: C, 72.43; H, 8.23. UV λ^{EtOH}_{max} 243 mμ(ε 16,000). IR ν^{KBr}_{max} cm^{-1}: 3520 (OH), 1706 (20-CO), 1678, 1623 (Δ^4-3-CO).

Treatment of (II) with Ac_2O in pyridine by the usual method afforded the monoacetate of m.p. 187.5~188.5°. *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.11; H, 8.36. Found: C, 71.00; H, 8.36.

Recrystallization of (III) from acetone-hexane mixture gave prismatic crystals of m.p. 258~259° (decomp.); $[\alpha]^{28}_D$ +54.3°(c=0.59, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$ (15β,17α-Dihydroxyprogesterone): C, 72.80; H, 8.73. Found: C, 72.84; H, 8.87. UV: λ^{EtOH}_{max} 240.5 mμ (ε 15,000). IR ν^{KBr}_{max} cm^{-1}: 3448 (OH), 1700 (20-CO), 1656, 1613 (Δ^4-3-CO).

Recrystallization of (IV) from Et_2O-hexane mixture gave rhomboprismatic crystals, m.p. 218~221°, $[\alpha]^{29}_D$ +73°(c=1.1, $CHCl_3$), whose infrared absorption spectrum agreed with that of the known 11α,17α-dihydroxyprogesterone. UV: λ^{EtOH}_{max} 241.5 mμ (ε 17,000). IR ν^{KBr}_{max} cm^{-1}: 3448 (OH), 1700 (20-CO), 1667, 1616 (Δ^4-3-CO).

Formation of 17α-Hydroxyprogesterone (I) from 2β,17α-Dihydroxyprogesterone (II)—To a solution of 103 mg. of (II) dissolved in 3 cc. of AcOH, 200 mg. of Zn dust and 0.6 cc. of water were added and the mixture was stirred at room temperature for 10 min. After filtration, the filtrate was concentrated, a small amount of water was added to the residue, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with dil. $NaHCO_3$ and water, dried over anhyd. Na_2SO_4, and $CHCl_3$ was evaporated. The residue was dissolved in a small amount of $CHCl_3$ and passed through a column of Florisil. From its effluent, crystals melting at 116~117.5° were obtained which showed no depression of m.p. on admixture with 17α-hydroxyprogesterone, and the ultraviolet and infrared absorption spectra of the two were identical.

Oxidation of 15β,17α-Dihydroxyprogesterone (III) with Chromium Trioxide—A solution of 30 mg. of CrO_3 dissolved in 1 cc. of pyridine was added to a solution of 300 mg. of (III) dissolved in 2 cc. of pyridine and the mixture was allowed to stand over night. The reaction mixture was treated as usual and 15-oxo-17α-hydroxyprogesterone (V), m.p. 247~249°, was obtained. *Anal.* Calcd. for C_{21}-

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[10] A.S. Meyer: J. Org. Chem., **20**, 1240(1955).

$H_{28}O_4$: C, 73.22; H, 8.19. Found : C, 72.90; H, 7.63. UV λ_{max}^{EtOH} 239.5 mμ (ε 17,000). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1736 (five-membered ring ketone), 1706 (20-CO), 1664, 1618 (Δ^4-3-CO).

Oxidation of 17α-Hydroxydesoxycorticosterone (VIa) with *Sclerotinia libertiana*—Under exactly the same conditions as those used for microbial oxidation of 17α-hydroxyprogesterone, (VIa) was cultured with the microörganism, treated in the same way, and 5.36 g. of an oxidation product was obtained. This was dissolved in 100 cc. of $CHCl_3$, the solution was passed through a column of 180 g. of silica gel, and the column was eluted with $CHCl_3$ and a mixture (100:1) of $CHCl_3$ and MeOH. The eluates afforded 0.76 g. of recovered starting material, m.p. 212~216°, and (VIIa) of m.p. 210~220° (decomp.). Recrystallization of the latter from $CHCl_3$-Et_2O mixture afforded needle crystals, m.p. 215~222° (decomp.); $[\alpha]_D^{26.5}$ −64°(c=0.56, dioxane). *Anal.* Calcd. for $C_{21}H_{30}O_5$ (2β,17α-Dihydroxydesoxycorticosterone) : C, 69.58; H, 8.34. Found : C, 69.34; H, 8.39. UV : λ_{max}^{EtOH} 243 mμ (ε 16,000). IR ν_{max}^{KBr} cm^{-1} : 3333 (OH), 1724 (20-CO), 1684, 1623 (Δ^4-3-CO).

Further elution with $CHCl_3$:MeOH mixture (100:2) afforded crystals (VIII) of m.p. 219~225°(decomp.). *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found : C, 69.63; H, 8.56.

Acetylation of 2β,17α-Dihydroxydesoxycorticosterone (VIIa)—To a solution of 500 mg. of (VIIa) dissolved in 6 cc. of pyridine, 155 mg. (1.10 moles) of Ac_2O was added and the mixture was allowed to stand over night. The mixture was poured into ice-water, extracted with Et_2O-$CHCl_3$ (4:1) mixture, and the extract solution was washed consecutively with dil. HCl, water, dil. $NaHCO_3$, and water. After drying over anhyd. Na_2SO_4, the solvent was evaporated from the extract and 570 mg. of a residue was obtained. The residue was dissolved in $CHCl_3$, passed through a column of Florisil, and the column was eluted with $CHCl_3$. The crystals obtained from the initial fraction were recrystallized from acetone-hexane mixture to the diacetate (VIIc) as rhomboprismatic crystals, m.p. 215~218°; $[\alpha]_D^{27}$ +7°(c=1.0, dioxane). *Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found : C, 66.88; H, 7.63. UV : λ_{max}^{MeOH} 244 mμ (ε 15,000). IR ν_{max}^{KBr} cm^{-1} : 3521, 2278 (OH), 1748 (AcO), 1724 (20-CO), 1672, 1618 (Δ^4-3-CO).

The crystals obtained from later fractions were recrystallized from Et_2O-hexane mixture to 21-monoacetate (VIIb) as needles, m.p. 228~230°; $[\alpha]_D^{29}$ −22°(c=0.9, dioxane). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7,97. Found : C, 68.21; 7.94. H, UV : λ_{max}^{MeOH} 243 mμ (ε 14,200). IR ν_{max}^{KBr} cm^{-1} : 3472 (OH), 1745 (CO in AcO), 1727 (20-CO), 1678, 1623 (Δ^4-3-CO).

Isomerization of 2β,17α-Dihydroxydesoxycorticosterone 21-Acetate (VIIb) to 2α,17α-Dihydroxydesoxycorticosterone 21-Acetate (IXb)—A solution of 50 mg. of (VIIb) dissolved in 3 cc. of glacial AcOH was refluxed for 1 hr. in an oil bath and AcOH was distilled off under a reduced pressure. Water was added to the residue and extracted with Et_2O. The extract solution was washed with dil. $NaHCO_3$ and water, dried over anhyd. Na_2SO_4, and Et_2O was evaporated. The residue was dissolved in $CHCl_3$ and the solution was chromatographed over 15 g. of silicic acid. The crystals obtained from $CHCl_3$ effluent were recrystallized from acetone-hexane mixture to needle crystals (IXb), m.p. 241~245°(decomp.). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found : C, 68.27; H, 7.75. UV : λ_{max}^{EtOH} 240 mμ (ε 14,700). IR ν_{max}^{KBr} cm^{-1} : 3472 (OH), 1745 (CO in AcO), 1727 (20-CO), 1678, 1615 (Δ^4-3-CO).

A solution of 166 mg. of 2α,17α-dihydroxydesoxycorticosterone (IXa), prepared by the method of Rosenkranz, dissolved in 2 cc. of pyridine and added with 50 mg. of Ac_2O was allowed to stand over night, the mixture was poured into ice water, and extracted with $CHCl_3$. This was treated by the usual method and 180 mg. of the residue hereby obtained was dissolved in $CHCl_3$. This solution was chromatographed over 6 g. of Florisil and the initial fraction eluted with $CHCl_3$ afforded the diacetate (IXc) of m.p. 202~204°. *Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found : C, 66.52; H, 7.59.

Crystals obtained from later fractions were recrystallized from acetone-hexane mixture to 21-monoacetate (IXb) as needles, m.p. 242~245°. UV : λ_{max}^{EtOH} 240 mμ (ε 14,700). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found : C, 68.12; H, 7.91.

This substance (VIIb) showed no depression of m.p. on admixture with the 21-monoacetate obtained by isomerization, as described above, and infrared absorption spectra of the two were completely identical.

Oxidation of Progesterone with *Sclerotinia libertiana*—Progesterone (XI) was cultured under identical conditions as in microbial oxidation of 17α-hydroxyprogesterone, treated similarly, and 5.08 g. of oxidation product was obtained. This was dissolved in 30 cc. of AcOEt and allowed to stand, from which 1.56 g. of crude crystals melting at 204~211° were obtained. Further crop of 0.47 g. of crystals, m.p. 191~206°, was obtained from the filtrate. The combined crystals were recrystallized from MeOH to 2β,15β-dihydroxyprogesterone (XII) as prismatic crystals of m.p. 206~216°; $[\alpha]_D^{27.5}$ −66.7° (c=1.05, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H,8.73. Found : C, 72.82; H, 8.92. UV : λ_{max}^{EtOH} 243 mμ (ε 15,080). IR ν_{max}^{KBr} cm^{-1} : 3500 (OH), 1700 (20-CO), 1671, 1621 (Δ^4-3-CO).

Triphenyltetrazolium chloride test of this substance gave pink coloration.

AcOEt was distilled off from the filtrate obtained after removal of (XII), the residue was dissolved in 28 cc. of a mixture (100:2) of $CHCl_3$-acetone, and the solution was chromatogaphed over 130 g. of Florisil. $CHCl_3$ effluent afforded 0.22 g. of an oily substance. A fraction eluted with $CHCl_3$-acetone (100:4) afforded 0.3 g. of crude crystals melting at 122~124° and its recrystallization from petr. benzine gave the starting progesterone (XI) as prisms, m.p. 122~124°. The fraction eluted with $CHCl_3$-acetone (100:8) mixture gave 0.2 g. of crude crystals (XIII), m.p. 228~238°(decomp.); $[\alpha]_D^{27}$ +190° (c=1.20, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found : C, 72.55; H, 8.58. UV : λ_{max}^{EtOH} 238.5 mμ (ε 14,500). IR ν_{max}^{KBr} cm^{-1} : 3550 (OH), 1745, 1707 (20-CO), 1677, 1628 (Δ^4-3-CO).

The fraction eluted with $CHCl_3$-acetone (100:20) mixture afforded 1.45 g. of crude crystals, m.p. 206~214°, which were recrystallized from MeOH to (XIV), m.p. 216~224°; $[\alpha]_D^{27}$ −17.8°(c=0.80, pyridine). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found : C, 72.71; H, 8.50. UV : λ_{max}^{EtOH} 242 mμ (ε 14,900). IR ν_{max}^{KBr} cm^{-1} : 3490 (OH), 1746 (20-CO), 1693, 1619 (Δ^4-3-CO).

The fraction eluted with $CHCl_3$-acetone (100:50) mixture gave 0.15 g. of crude crystals, m.p. 200~215°, which recrystallized from MeOH to prismatic crystals (XV), m.p. 210~216°; $[\alpha]_D^{27}$ −65.0°(c=0.85, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found : C, 72.83; H, 8.50. UV : λ_{max}^{EtOH} 239 mμ (ε 19,350). IR ν_{max}^{KBr} cm^{-1} : 3530 (OH), 1707(20-CO), 1695,1622 (Δ^4-3-CO).

Acetylation of 2β,15β-Dihydroxyprogesterone (XII)—A solution of 100 mg. of (XII) dissolved in 3 cc. of pyridine, added with 2.36 g. of Ac_2O, was allowed to stand at room temperature for 22 hr., 20 cc. of 2*N* HCl was added under ice cooling, and the mixture was extracted with $CHCl_3$. The extract was washed with water, dried over anhyd. Na_2SO_4, and $CHCl_3$ was evaporated. The residue was dissolved in benzene, the solution was chromatographed over alumina, and eluted with benzene, from which the diacetate (XII) was obtained as prisms, m.p. 127~128°; $[\alpha]_D^{28.5}$ −51.3°(c=0.93, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_6 \cdot \frac{1}{2}H_2O$: C, 68.31; H, 8.03. Found : C, 68.02; H, 7.90. UV : λ_{max}^{EtOH} 242.5 mμ (ε 15,400). IR ν_{max}^{KBr} cm^{-1} : 1760 (AcO), 1731 (20-CO), 1704, 1623 (Δ^4-3-CO).

A solution of 69.3 mg. of (XII) dissolved in 2 cc. of pyridine and added with 23.5 mg. (1.15 moles) of Ac_2O was allowed to stand for 22 hr. at room temperature, 12 cc. of 2*N* HCl was added, and this was treated as described above. The crude crystals thereby obtained were recrystallized from acetone-hexane mixture to 2β-monoacetate of (XII) as needle crystals of m.p. 137~138°. There was no coloration to triphenyltetrazolium chloride test. $[\alpha]_D^{27}$ −22.7°(c=0.97, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found : C, 69.97; H, 8.07. UV : λ_{max}^{EtOH} 243.5 mμ (ε 15,700). IR ν_{max}^{KBr} cm^{-1}: 3560 (OH), 1753 (AcO), 1700 (20-CO), 1678, 1621 (Δ^4-3-CO).

Formation of 15β-Hydroxyprogesterone (XVI) **from 2β,15β-Dihydroxyprogesterone** (XII)—To a solution of 250 mg. of (XII) dissolved in a mixture of 10 cc. of glacial AcOH and 3 cc. of water, 1.0 g. of Zn dust was added and the mixture was stirred for 10 min. at room temperature. After removal of Zn dust by filtration, AcOH was evaporated under a reduced pressure, water was added to the residue, and extracted with $CHCl_3$. The extract solution was washed with water, dried over anhyd. Na_2SO_4, and $CHCl_3$ was evaporated, leaving crude crystals of m.p. 196~198°. This was dissolved in $CHCl_3$-benzene (1:2) mixture and the solution was chromatographed over alumina, from which 0.15 g. of crystals melting at 199~200.5° was obtained. Recrystallization from MeOH gave 15β-hydroxyprogesterone (XVI) as needle crystals, m.p. 202~203.5°; $[\alpha]_D^{28}$ +149°(c=0.82, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found : C, 76.51; H, 9.32. UV : λ_{max}^{EtOH} 240.5 mμ (ε 17,300). IR ν_{max}^{Nujol} cm^{-1}: 3440 (OH), 1700 (20-CO), 1660, 1619 (Δ^4-3-CO).

Oxidation of 15β-Hydroxyprogesterone (XVI)—A solution of 40 mg. of CrO_3 dissolved in 4 cc. of 90% AcOH was added to a solution of 110 mg. of (XVI) dissolved in 3 cc. of 90% AcOH and the mixture was allowed to stand for 24 hr. at room temperature. Treatment of this mixture in a usual manner afforded 100 mg. of crude crystals melting at 143~149°. This was chromatographed over alumina and elution with benzene afforded crystals of m.p. 152~154°, which was recrystallized from acetone-hexane to 15-oxoprogesterone (XVII) as prismatic crystals, m.p. 156~157°; $[\alpha]_D^{28}$ +208°(c=0.91, $CHCl_3$) (reported[9]) m.p. 155~157°; $[\alpha]_D$ +200°).

Summary

Sclerotinia libertiana was found to hydroxylate 2β-position of some Δ^4-3-oxosteroids. This microörganism was incubated with 17α-hydroxyprogesterone, 17α-hydroxydesoxycorticosterone (Reichstein's Substance S), and progesterone as a substrate, and the corresponding 2β-hydroxylated products were obtained. Some additional hydroxylated steroids were obtained and their structure was determined.

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