Table II. Analysis of Caffeine
(mg./10 shoots) $(^{15}NH_4)_2SO_4$······8.5 atom% ^{15}N excess

	(I) Young leaves	(II) Mature leaves	(III) Old leaves
(%)[a]	4.34	1.09	0.58
(mg.)	38.1	34.3	22.5
Caffeine-N (mg.)	11.0	9.9	6.5
$\frac{\text{Caffeine-N}}{\text{Total N}} \times 100$	17.7	9.4	5.2
^{15}N% excess	1.123	0.063	0.112
Newly formed caffeine (mg.)	5.04	0.25	0.30
$\frac{\text{Caffeine newly formed}}{\text{Amount of caffeine}} \times 100$	13.2	0.73	1.33
$\frac{\text{Caffeine-N newly formed}}{\text{Total N absorbed}} \times 100$	18.6	3.4	2.9

a) Content of caffeine in the leaves at the start of the experiment :
(I) 2.35%, (II) 0.56%, (III) 0.23%.

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Summary

The excised shoots of tea plants were cultivated for 1 week in $(^{15}NH_4)_2SO_4$ solution. ^{15}N was incorporated considerably more into protein and caffeine of young than into those of mature and old leaves. It was proved by these results that formation of caffeine can take place in the leaves of tea plant.

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Makoto Shirasaka and Masako Tsuruta : Microbiological Transformation of Steroid. VII.[1] Hydroxylation of Steroid by *Gibberella saubinetii*; 6β- and 15α-Hydroxylation.

(*Takamine Laboratory, Sankyo Co., Ltd.*[*1])

15α-Hydroxylation of steroids by microörganisms is a comparatively common reaction now and the substrate steroids used in such a case are usually progesterone and deoxycorticosterone. Only *Hormodendrum viride*[2] and *Helminthosporum sativum*[3] are known to effect 15α-hydroxylation of Reichstein's compound S. The microörganisms examined to date have only been tested with one kind of a substrate, except in the case of *Fusarium lini*[4] which was tested with four kinds of steroid, androstenedione, testosterone, progester-

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[*1] Nishi-shinagawa, Shinagawa-ku, Tokyo (白坂 亮, 鶴田雅子).
1) Part VI : This Bulletin, **9**, 203 (1961).
2) S. Bernstein, *et al.* : Chem. & Ind. (London), **1956**, 111.
3) K. Tsuda, *et al.* : This Bulletin, **7**, 534 (1959).
4) A. Gubler, *et al.* : Helv. Chim. Acta, **41**, 301 (1958).

one, and deoxycorticosterone, and was found to effect 15α-hydroxylation of all these steroids uniformly. However, it has not been examined with Reichstein's compound S and other steroids.

During the course of examination of fungi for oxidation of steroids, it was found that *Gibberella saubinetii* and *Fusarium lycopersiei* effected 15α-hydroxylation of progesterone. Application of these fungi to other steroids, e.g. 17α-hydroxyprogesterone, deoxycorticosterone, corticosterone, and Reichstein's compound S, showed the formation of a large amount of the 6β-hydroxylated compound rather than 15α-hydroxy compound from 17α-hydroxyprogesterone and Reichstein's compound S, while 6β-hydroxylated compounds were not obtained from other steroids, 15α-hydroxy compounds being the only product. These results show that these fungi have a marked substrate specificity and this experiment will be described herein.

Culture of *Gibberella saubinetii* by the method to be described later, with progesterone (I) as the substrate and paper chromatographic examination of its concentrated extract showed the presence of one spot with greater polarity than (I). Further purification of this product by alumina-column chromatography afforded some crystals (II) of m.p. 219~221°, whose elemental analytical values showed it to be monohydroxyprogesterone. A monoacetate was obtained by acetylation of (II) with acetic anhydride and pyridine.

Oxidation of (II) with chromium trioxide in acetic acid yielded a triketone compound (III), whose infrared spectrum showed absorption at $1733\,\text{cm}^{-1}$, indicating the presence of a five-membered ketone. Consequently, the newly introduced hydroxyl in (II) must be at 15- or 16-position, but 15β-hydroxyl in (I) is not acetylated, so that this hydroxyl must be at 15α-, 16α-, or 16β-position. Comparison of the constants of (II) and its acetate with those of hydroxyprogesterones showed that the constants of (II) are in good agreement with those of 15α-hydroxyprogesterone and their identity was established.

Table I. Comparison of Physical Constants of Hydroxyprogesterone and its Derivatives

Compound	m.p. (°C)	$[\alpha]_D$ ($CHCl_3$)
(II)	216~221	+190°
Acetate of (II)	169~170	+180°
Triketone of (II)	154~156	+202°
15α-Hydroxyprogesterone[a]	231~232	+218°
15α-Acetoxyprogesterone[a]	177~178	+176°
15-Oxoprogesterone[a]	157	+197°
16α-Hydroxyprogesterone[b]	225~226	+158°
16α-Acetoxyprogesterone[b]	134~135	+107°

a) B. Kluger, *et al.* : Naturwiss., **44**, 40 (1957).
b) D. Perlman, *et al.* : J. Am. Chem. Soc., **74**, 2126 (1952).

Similar fermentation of *Gibberella saubinetii*, with deoxycorticosterone as the substrate and paper chromatographic examination of its concentrated extract showed one spot with greater polarity than deoxycorticosterone. Purification of this product through Florisil-column chromatography gave crystals (IV) of m.p. 215~220°, whose analytical values indicated introduction of one hydroxyl into deoxycorticosterone. (IV) formed a diacetate on acetylation with acetic anhydride and pyridine, which indicated that the newly introduced hydroxyl is primary or secondary. Acetylation of (IV) followed by oxidation of its product, and saponification with methanolic potassium hydrogencarbonate gave a triketone compound (V), m.p. 211~216°. The infrared spectrum of (V) exhibited absorption at $1740\,\text{cm}^{-1}$, indicating the presence of a five-membered ketone. Consequently, the newly introduced hydroxyl in (IV) is at 15- or 16-position and, since (IV) does not show the Porter-Silber reaction,[5] this hydroxyl is known to be at 15-position. The comparison of the constants of (IV) with those

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[5] C. C. Porter, R. H. Silber : J. Biol. Chem., **210**, 923 (1954).

of 15α- and 15β-hydroxylated compounds of deoxycorticosterone obtained by Wettstein and others, as shown in Table II, showed (IV) to be 15α,21-dihydroxypregn-4-ene-3,20-dione.

Table II. Comparison of Physical Constants of Deoxycorticosterone Derivatives

Compound	Free steroid		Acetate	
	m.p. (°C)	$[\alpha]_D$	m.p. (°C)	$[\alpha]_D$ (EtOH)
(IV)	215∼220	+217° (EtOH)	160∼161	+160°
15α,21-Dihydroxypregn-4-ene-3,20-dione[a]	216∼222	+196° (EtOH)	160∼161	+159°
15β,21-Dihydroxypregn-4-ene-3,20-dione[a]	206∼216	+141.5° ($CHCl_3$)	184	—

[a] A. Wettstein: Experientia, **11**, 465 (1955).

The same fermentation of *Gibberella saubinetii* with 17α-hydroxyprogesterone (VI) as the substrate and paper chromatography of its extract showed the presence of (IV) and a spot with greater polarity than that. Separation of this product by column chromatography gave some of the unreacted (VI) and crystals (VII) of m.p. 230∼240°, whose analytical values indicated the presence of one newly introduced hydroxyl in (VI). This product gave a monoacetate by acetylation with acetic anhydride and pyridine, which meant that the newly introduced hydroxyl is primary or secondary. Treatment of (VII) with chromium trioxide in acetic acid gave a triketone compound (VIII). (VII) and its acetate were identified with $6\beta,17\alpha$-dihydroxyprogesterone and its 6-acetate, and (VIII) with 17α-hydroxypregn-4-ene-3,6,20-trione[6] through their physical constants and infrared spectra. It was thereby found that *Gibberella saubinetii* effected, not 15α-hydroxylation but 6β-hydroxylation in 17α-hydroxyprogesterone (VI).

The same fermentation of *Gibberella saubinetii* with Reichstein's compound S (IX) as the substrate and paper chromatographic examination of its extract showed the presence of two spots with greater polarity than (IX). Purification of this product through column chromatography afforded the crystals (X) of m.p. 210∼236° as the main oxidation product and a very minute amount of crystals (XI) of m.p. 215∼220°. Both were found to have newly introduced hydroxyl in (IX) and both gave a diacetate on acetylation with acetic anhydride and pyridine. (X) and its diacetate were identified with $6\beta,17\alpha$,21-trihydroxypregn-4-ene-3,20-dione and its 6,21-diacetate through physical constants[6] and infrared spectra.

Treatment of (XI) with sodium bismuthate in acetic acid gave monohydroxyandrostenedione (XII) and its constants agreed with those of 15α-hydroxyandrost-4-ene-3,20-dione.[7] (XI) and its diacetate were identified with $15\alpha,17\alpha$,21-trihydroxypregn-4-ene-3,20-dione[2,3] and its 15,21-diacetate through their constants and infrared spectra.

These experimental results indicate that *Gibberella saubinetii*, same as in the case of 17α-hydroxyprogesterone, carry out 6β-hydroxylation of the compound S in the main and a very slight reaction of 15α-hydroxylation occurs in this case.

Finally, application of this fungus to corticosterone (XIII) and direct crystallization of the extract residue from acetone afforded crystals (XIV) of m.p. 180∼182°, whose analytical values indicated introduction of two hydroxyls into (XIII). (XIV) gave a diacetate on acetylation with acetic anhydride and pyridine. Acetylation of (XIV) with equivalent of acetic anhydride, followed by oxidation of its product with chromium trioxide, and hydrolysis of its product with potassium hydrogencarbonate yielded a tetraketone compound (XV), m.p. 195∼205°, whose infrared absorption at 1750 cm^{-1} indicated the presence of a five-membered cyclic ketone. Consequently, one of the two newly introduced hydroxyls in (XIV) is at 15- or 16-position, and since (XIV) does not show the Porter-Silber reaction, this hydroxyl must

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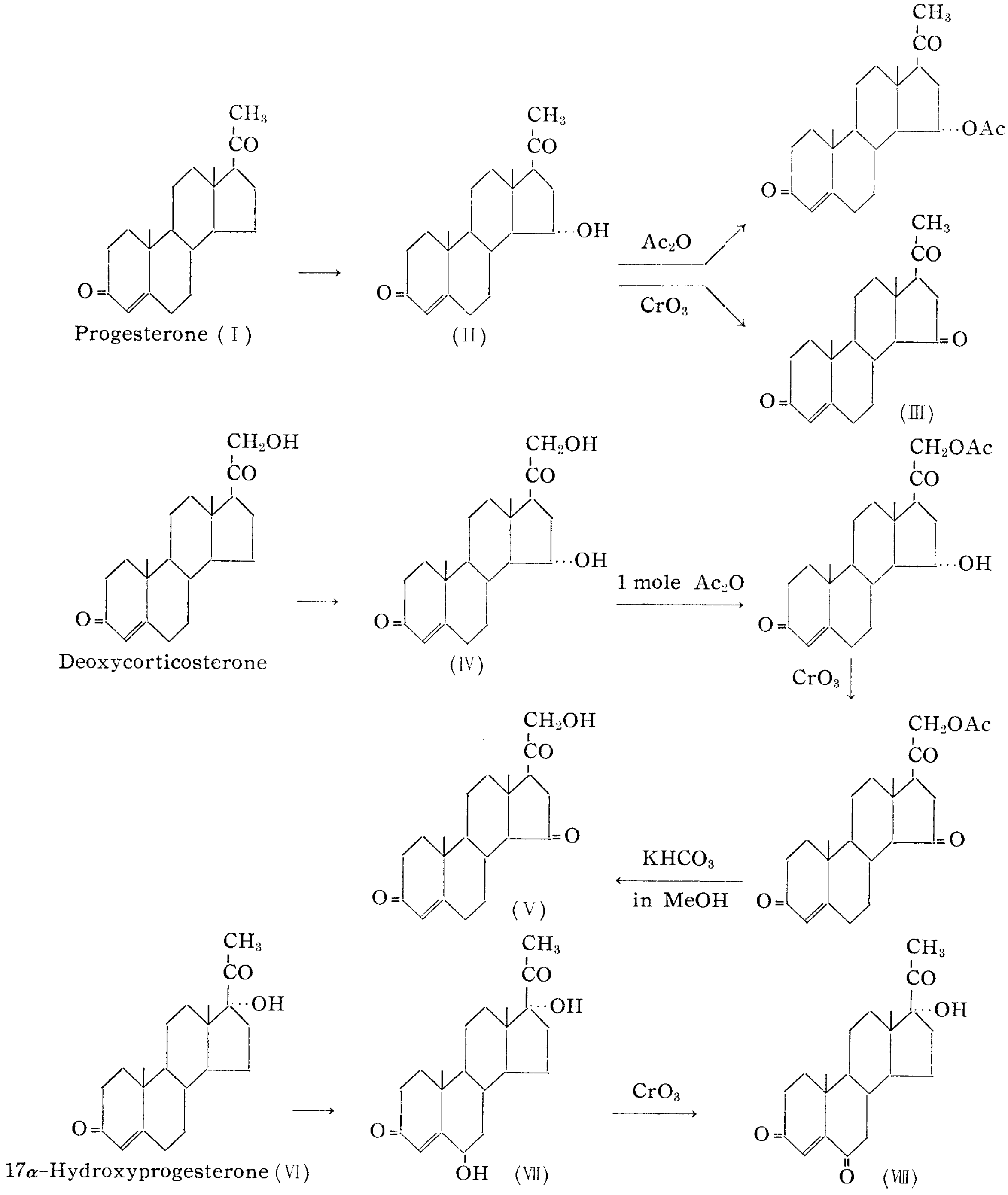
6) Part III: This Bulletin, **9**, 152 (1961).
7) R. B. Burton, *et al.*: J. Biol. Chem., **188**, 763 (1951).

be at 15-position. The other hydroxyl in (XIV) is tertiary since it is not oxidized by chromium trioxide but its position has not been determined as yet. The configuration of the hydroxyl at 15-position is also undetermined but, considering the experimental results obtained with other substrate steroids as described above, it is presumably 15α. Although the structure of (XIV) remains undetermined, it is certain that at least one hydroxyl had been introduced into the 15-position.

Application of *Fusarium lycopersici* to the various steroids described above and examination of the products by paper chromatography showed that the results were entirely the same as in the case of *Gibberella saubinetii*. It was thereby concluded that these two kinds of fungus have entirely the same behavior to steroids and that they both have a



Progesterone (I)

(II)

(III)

Deoxycorticosterone

(IV)

(V)

17α-Hydroxyprogesterone (VI)

(VII)

(VIII)

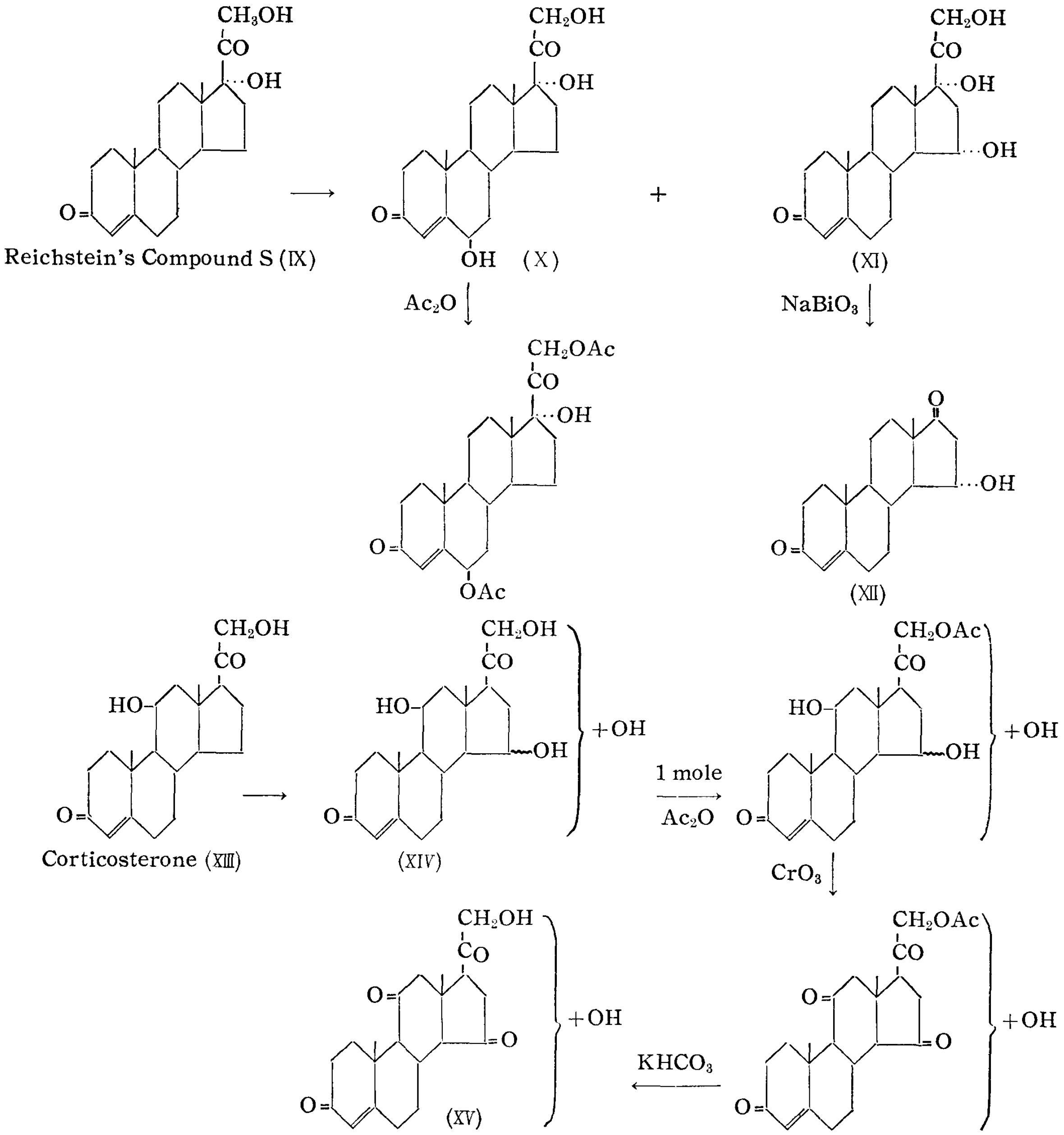


Chart 1.

marked substrate specificity. These fungi carry out almost 15α-hydroxylation alone in progesterone, deoxycorticosterone, and corticosterone, while 6β-hydroxylation alone in 17α-hydroxyprogesterone and Reichstein's compound S, with a very slight evidence of 15α-hydroxylation in these steroids. These results indicate these fungi are almost incapable of effecting 15α-hydroxylation in steroids possessing a 17α-hydroxyl group and this is probably due to the fact that the comparative proximity of 17α-hydroxyl group sterically interferes with introduction of a hydroxyl into 15α-position.

Gibberella and *Fusarium* spp. are taxonomically, i.e. morphologically, clearly different but they are Ascomycetes and imperfect fungi of the same system. It is quite interesting, from the taxonomy of microörganisms, especially of fungi, that such different species of fungi show extremely similar behavior in the oxidation of steroids.

Experimental

Fermentation and Extraction—A potato decoction containing 3% of glucose was placed in twenty 500-cc. shake flasks, 100 cc. to each flask, the flasks were sterilized, inoculated with the fungus, and shake-cultured for ca. 48 hr. at 26°. To each of the flasks, 2 cc. of 2.5% MeOH solution of the substrate steroid was added and the flasks were again shaken at the same temperature for 48~72 hr. After completion of the fermentation, the culture liquid was filtered to separante the fungal cells which were extracted with AcOEt. The combined extract and filtrate was extracted with AcOEt, the extract was washed with 2% $NaHCO_3$ and H_2O, and dried over anhyd. Na_2SO_4. After evaporation of AcOEt in a reduced pressure, the residue was crystallized directly from a solvent or purified by adsorption column chromatography.

Paper Chromatography—Paper chromatography was carried out as reported previously,[8] using propyleneglycol-toluene system, by the descending method.

Hydroxylation of Progesterone—Fermentation of *Gibberella saubinetii* was carried out as above, with 1 g. of progesterone (I) as the substrate and 2.06 g. of a concentrated extract was obtained. The extract was submitted to chromatography over 50 g. of alumina and the column was eluted with various mixtures of benzene, Et_2O, and $CHCl_3$. The fraction eluted with benzene-Et_2O (90:10 and 50:50) was recrystallized from Me_2CO and 322 mg. of the unreacted progesterone was recovered.

The next fraction eluted with $CHCl_3$-Me_2CO (95:5) afforded 120 mg. of 15α-hydroxyprogesterone (II), m.p. 219~221°; $[\alpha]_D$ +190° ($CHCl_3$). *Anal.* Calcd. for $C_{21}H_{20}O_3$: C, 76.32; H, 9.15. Found : C, 76.11; H, 8.80. UV : λ_{max}^{MeOH} 240 mμ (ε 17,900). IR ν_{max}^{KBr} cm^{-1} : 3400 (OH), 1703 (20-CO), 1665, 1617 (Δ^4-3-CO).

15-Acetate of (II) : Usual acetylation of (II) with Ac_2O-pyridine gave the monoacetate of m.p. 169~170°; $[\alpha]_D$ +188° ($CHCl_3$). *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found : C, 74.01; H, 8.71. IR ν_{max}^{KBr} cm^{-1} : 1740 (acetyl CO), 1700 (20-CO), 1670, 1620 (Δ^4-3-CO).

Oxidation of (II) with CrO_3—A solution of (II) in AcOH was oxidized with CrO_3 in the usual manner and 15-oxoprogesterone, m.p. 154~156°; $[\alpha]_D$ +202° ($CHCl_3$), was obtained. *Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found : C, 76.61; H, 8.30. UV : λ_{max}^{MeOH} 239 mμ (ε 20.500). IR ν_{max}^{KBr} cm^{-1}: 1733 (5-membered ring CO), 1704 (20-CO), 1668, 1630 (Δ^4-3-CO).

Hydroxylation of Deoxycorticosterone—The same fermentation of *Gibberella saubinetii* with 1 g. of deoxycorticosterone as the substrate gave 1.65 g. of extract residue. This residue was dissolved in 100 cc. of $C_2H_4Cl_2$, passed through a column of 80 g. of Florisil, and the column was serially eluted with various mixtures of $C_2H_4Cl_2$ and Me_2CO. The fraction eluted with 5:1 and 2:1 mixture of $C_2H_4Cl_2$ and Me_2CO was recrystallized from Me_2CO and afforded 260 mg. of 15α,21-dihydroxypregn-4-ene-3,20-dione (IV). Further recrystallization from Me_2CO gave crystals of m.p. 215~220°; $[\alpha]_D$ +217° ($CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.74. Found : C, 72.12; H, 8.51. UV : λ_{max}^{MeOH} 242 mμ (ε 17,100). IR ν_{max}^{KBr} cm^{-1} : 3570, 3470 (OH), 1713 (20-CO), 1645, 1612 (Δ^4-3-CO).

15,21-Diacetate of (IV) : Usual acetylation of (IV) with Ac_2O and pyridine gave the diacetate of m.p. 160~161°; $[\alpha]_D$ +160° ($CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found : C, 69.38; H, 7.73.

Oxidation of (IV) with CrO_3—Acetylation of 150 mg. of (IV) with 1 equivalent of Ac_2O and pyridine was carried out by the usual method and the concentrated extract therefrom was dissolved in 8 cc. of AcOH. To this solution, 3 cc. of AcOH containing 25 mg. of CrO_3 was added and the mixture was allowed to stand for 5 hr. at room temperature. The reaction solution was evaporated, diluted with 20 cc. of H_2O, and extracted with CH_2Cl_2. The extract was washed with 7% $NaHCO_3$ and H_2O, dried over anhyd. Na_2SO_4, and evaporated in a reduced pressure to leave 130 mg. of a residue. The residue was dissolved in hydr. MeOH containing 150 mg. of $KHCO_3$, the mixture was allowed to stand over night, and MeOH was evaporated. The residue was diluted with H_2O, extracted twice with H_2O, extracted twice with CH_2Cl_2, and the extract was evaporated after drying over anhyd. Na_2SO_4. Recrystallization of the residue gave 67 mg. of crude crystals which was dissolved in 1 cc. of MeOH with application of heat and the solution was applied as a band on a filter paper for chromatography, treated with propyleneglycol-MeOH (1:2). The paper was developed for ca. 4 hr. by the usual method, dried in air, and the portions showing absorption of ultraviolet ray were cut out. The strips of filter paper were digested with warm MeOH, the solution was evaporated, and the residue was crystallized from Me_2CO to 13 mg. of the triketone compound (V), m.p. 211~216°; *Anal.* Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found : C, 73.41; H, 8.28. IR ν_{max}^{KBr} cm^{-1} : 3400 (OH), 1740 (5-membered ring CO), 1715 (20-CO), 1660, 1615 (Δ^4-3-CO).

Hydroxylation of 17α-Hydroxyprogesterone (VI)—Usual fermentation of *Gibberella saubinetii* with 1 g. of (VI) as the substrate gave 2.1 g. of a concentrated extract. This extract was passed

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[8] Part I : This Bulletin, **9**, 54 (1961).

through a column of 80 g. of Florisil and the column was eluted with various mixtures of $C_2H_4Cl_2$ and Me_2CO. The initial eluate afforded ca. 260 mg. of unreacted (VI). The next eluted fraction was recrystallized from Me_2CO and gave 312 mg. of 6β,17α-dihydroxyprogesterone (VII), m.p. 230~242°; $[\alpha]_D$ +10°(Me_2CO). *Anal.* Calcd. for C, 72.80; H, 8.74. Found : C, 72.10; H, 8.62. UV : λ_{max}^{MeOH} 236 mμ (ε 13,500). IR ν_{max}^{KBr} cm^{-1} : 3370 (OH), 1700 (20-CO), 1664, 1625 (Δ^4-3-CO).

Oxidation of (VII) with CrO_3—To a solution of 70 mg. of (VII) dissolved in 4 cc. of AcOH, 2 cc. of AcOH containing 17 mg. of CrO_3 was added and the mixture was allowed to stand for 3 hr. A small amount of MeOH was added to this mixture, which was concentrated, the residue was diluted with 20 cc. of H_2O, and extracted with Me_2Cl_2. The extract was washed with 7% $NaHCO_3$ and H_2O, dried over anhyd. Na_2SO_4, and evaporated in reduced pressure. The residue was recrystallized from Me_2CO to 13 mg. of 17α-hydroxypregn-4-nen-3,6,20-trione (VIII), m.p. 238~242°. *Anal.* Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found : C, 73.10; H, 8.31.

Hydroxylation of Reichstein's Compound S (IX)—The same fermentation of *Gibberella saubinetii* with 1 g. of (IX) as the substrate gave 2.6 g. of concentrated extract. This was purified through 80 g. of Florisil and the last-eluted fraction gave 412 mg. of (X), m.p. 230~236°; $[\alpha]_D$ +72°(MeOH). *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.24. Found : C, 69.10; H, 8.40. UV : λ_{max}^{MeOH} 238 mμ (ε 13,200). IR ν_{max}^{KBr} cm^{-1} : 3430 (OH), 1710 (20-CO), 1660, 1615 (Δ^4-3-CO).

6,21-Diacetate of (X) : Usual acetylation of (X) with Ac_2O and pyridine gave the diacetate of m.p. 190~193°; $[\alpha]_D$ +76°($CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found : C, 66.97; H, 7.55.

The second-eluted fraction gave 36 mg. of (XI) which was recrystallized repeatedly from Me_2CO to give crystals of m.p. 215~220; $[\alpha]_D$ +150°($CHCl_3$). *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found : C, 69.51; H, 8.10. UV : λ_{max}^{MeOH} 241 mμ (ε 14,000). IR ν_{max}^{KBr} cm^{-1} : 3430 (OH), 1715 (20-CO), 1660, 1625 (Δ^4-3-CO).

15,21-Diacetate of (XI) : Usual acetylation of (XI) with Ac_2O and pyridine gave the diacetate of m.p. 198~200°; $[\alpha]_D$ +140°(MeOH). *Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found : C, 67.61; H, 7.21.

Oxidation of 15α,17α-21-Trihydroxypregn-4-ene-3,20-dione (XI) with $NaBiO_3$—A solution of 52 mg. of (XI) dissolved in 8 cc. of AcOH was diluted with 8 cc. of H_2O, 1 g. of $NaBiO_3$ was added, and the mixture was reacted for 16 hr. The reaction mixture was filtered, the filtrate was extracted with CH_2Cl_2, and the extract was washed with 10% $NaHCO_3$ and H_2O. The residue obtained on evaporation of CH_2Cl_2 was recrystallized several times from Me_2CO and yielded the crystals of 15α-hydroxyandrost-4-ene-3,17-dione (XII), m.p. 194~198°; $[\alpha]_D$ +211°. *Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found : C, 75.51; H, 8.43.

Hydroxylation of Corticosterone (XIII)—The same fermentation of *Gibberella saubinetii* with corticosterone (XIII) as the substrate gave 2.4 g. of a concentrated extract. This was dissolved in Me_2CO and the insoluble matter was collected by filtration. Repeated recrystallization of this product from MeOH afforded 322 mg. of (XIV), m.p. 180~182°; $[\alpha]_D$ +216°(MeOH). *Anal.* Calcd. for $C_{21}H_{30}O_6$: C, 66.64; H, 7.99. Found : C, 66.31; H, 7.89. UV : λ_{max}^{MeOH} 241.5 mμ (ε 17,000). IR ν_{max}^{KBr} cm^{-1} : 3400 (OH), 1730 (20-CO), 1655, 1625 (Δ^4-3-CO).

15,21-Diacetate of (XIV) : Usual acetylation of (XIV) with Ac_2O and pyridine gave the 15,21-diacetate of m.p. 166~170°; $[\alpha]_D$ +190°(MeOH). *Anal.* Calcd. for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found : C, 65.00; H, 7.81.

Oxidation of (XIV) with CrO_3—Acetylation of 85 mg. of (XIV) with equivalent (0.25 cc.) of Ac_2O and pyridine was carried out in the usual manner. The reaction mixture was extracted, the evaporated extract was dissolved in 5 cc. of AcOH, and 3 cc. of AcOH containing 15 mg. of CrO_3 was added to effect oxidation. The oxidation product was extracted with Me_2Cl_2, the extract was washed with $NaHCO_3$ solution and H_2O, and dried over anhyd. Na_2SO_4. The residue obtained by evaporation of CH_2Cl_2 hydrolyzed with hydr. MeOH containing 70 mg. of $KHCO_3$ by standing over night and an oily residue was obtained from its extract. This oily residue was dissolved in a small amount of MeOH and the solution was applied as a band on the filter paper treated with propyleneglycol-MeOH. This was developed and eluted as described above, and the product obtained therefrom was recrystallized from Me_2CO to 11 mg. of a tetraketone compound (XV), m.p. 195~205°. IR ν_{max}^{KBr} cm^{-1} : 3370 (OH), 1750 (5-membered ring CO), 1730 (11-CO), 1715 (20-CO), 1647, 1625 (Δ^4-3-CO).

The authors express their gratitude to Prof. K. Tsuda and Prof. T. Asai, both of The Institute of Applied Microbiology, University of Tokyo, for their unfailing guidance throughout the course of the present work, and to Mr. M. Matsui, the Director of this Laboratory, and Mr. H. Okazaki, Chief of this Section, both of this company, for their kind encouragement.

Summary

Application of *Gibberella soubinetii* to various steroid showed the formation of 15α-hydroxylated compound from progesterone and deoxycorticosterone, and 6β-hydroxylated compound from Reichstein's compound S and 17α-hydroxyprogesterone. Corticosterone afforded, by this reaction, 15α-hydroxycorticosterone derivative with one tertiary hydroxyl, whose structure has not been determined. *Fusarium lycopersici* also showed the same reaction as *Gibberella soubinetii*.

(Received March 16, 1960)

UDC 547.495.9.07

Genzo Ito: Über die Synthese von N^1-substituierten N^3-Acylguanidinen (Vorläufige Mitt.). Benzoylverbindungen.

(*Pharmazeutische Hochschule Meiji*[*1])

Unter den vielen, bisher in der Literatur erschienenen N^1,N^3-disubstituierten Guanidinen sind diejenigen, die eine Acylgruppe als einen Substituenten enthalten, nicht so zahlreich,[1~5] mit Ausnahme von N^1-(*p*-Aminobenzolsulfo)-N^3-acylguanidinen.[6]

Vom Gesichtspunkt der Arzneimittelforschung aus hat der Verfasser eine allgemeine Methode entwickelt, aliphatische und aromatische Amine durch einstufige Reaktion in die entsprechenden Monoacylguanidin-derivate mit schwacher Basizität und luftbeständigen Eigenschaften zu verwandeln.

Die vorliegende Abhandlung beschreibt solche Synthesen von einigen Benzoylverbindungen.

A. Reaktion der aliphatischen Amine

Traube und Gorniak[1] stellten N^1-Methyl-N^3-benzoylguanidin durch Einwirkung von Benzoesäure-äthylester auf Methylguanidin dar. Dieselbe Substanz ist auch aus Methylguanidin-hydrochlorid und Benzoylchlorid durch Erhitzen im Rohr erhalten worden.[2] Der Verfasser versuchte Monobenzoylierung des Benzylguanidins :

Durch Umsetzung von Benzylguanidin mit äquimolaren Mengen von Benzoylchlorid nach Schotten-Baumann wurde das N^2,N^3-Dibenzoyl-derivat erhalten, das sich mit dem Reaktionsprodukt von Benzylamin und 2-Methyl-1,3-dibenzoylisothioharnstoff[7,8] identifizierte.

Eine Synthese des N^1-Benzyl-N^3-benzoylguanidins (II) gelang dem Verfasser durch

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