by a strain of *Syncephalastrum* sp.

Some other unidentified dihydroxyprogesterone was also obtained in a small amount.

When Reichstein's compound S was used as asteroid substrate in similar techniques, most of the oxygenated product obtained was epi-hydrocortisone (V), m.p. 206–209°; $[\alpha]_D + 120^\circ$ (methanol), $\lambda_{\text{max}}^{\text{alc.}}$ 242 m μ ($\epsilon = 14,500$) and a minor amount of 14 α , 17 α , 21-trihydroxy-4-pregnene-3,20-dione (VI) was isolated, m.p. 213–218°; $[\alpha]_D + 155^\circ$ (methanol); $\lambda_{\text{max}}^{\text{alc.}}$ 241 m μ ($\epsilon = 11,000$). The 21-mono-acetate of (VI) was, m.p. 222–227° $[\alpha]_D + 177^\circ$ (chloroform). From these results, this fungus has considerable stereochemical specificity of steroid substrate in hydroxylation, that

is, it appears that 14 α -hydroxylation by this fungus is affected profoundly by the presence of 17 α -hydroxyl or the 21-hydroxyl function.

The authors wish to express their thanks to Professor K. Tsuda and Professor T. Asai, of the Institute of Applied Microbiology, University of Tokyo, and Mr. M. Matsui, Director of this Laboratory for their helpful advice and constant guidance throughout this work.

Makoto SHIRASAKA
Ryozo HAYASHI
Masako TSURUTA

Takamine Laboratory, Sankyo, Co., Ltd.

Received March 5, 1959

[Bull. Agr. Chem. Soc. Japan, Vol. 23, No. 3, p. 245~246, 1959]

Microbiological Hydroxylation of Progesterone and 17 α -Hydroxyprogesterone by *Sclerotinia libertiana*; 2 β -Hydroxylation.

Sir:

Among the numerous hydroxylation of steroids by microorganisms, 2 β -hydroxylation has only recently been discovered¹⁾.

In the previous paper²⁾ we have reported on a microbial hydroxylation of Reichstein's compound S (17 α -hydroxydesoxycorticosterone) to 2 β , 17 α , 21-trihydroxy-4-pregnene-3,20-dione utilizing *Sclerotinia libertiana*.

In the present communication we wish to report on the microbial hydroxylation of progesterone and 17 α -hydroxyprogesterone by this fungus in the 2 β -position.

When progesterone was used as the steroid substrate, the fermentation of this fungus yielded 2 β , 15 β -dihydroxyprogesterone and several unidentified dihydroxyprogesterones.

In case 17 α -hydroxyprogesterone was used, 2 β , 17 α -dihydroxy-, 15 β , 17 α -dihydroxy- and 11 α , 17 α -dihydroxyprogesterone were produced.

Among these isolated compounds, 2 β , 15 β -dihydroxy- and 2 β , 17 α -dihydroxyprogesterone have been recognized to be hitherto- undescribed new compounds.

In the experiment reported here, *Sclerotinia libertiana* was grown aerobically on a reciprocating shaker at 26° for 48 hours in 10 l of a medium of potato-decoct containing 3% of glucose.

Five grams of the requisite steroid was then added in methanol solution and the fermentation was allowed to proceed for a further 72 hours. The oxygenated products were extracted with ethyl acetate from the whole broth and the extract was first washed with 2% sodium bicarbonate solution and then with water. The ethyl acetate solution was then taken to dryness in vacuo and the residue was dissolved in chloroform. This chloroform solution was chromatographed on Florisil or alumina. Chloroform-acetate solvent mixtures were used as the developer.

When progesterone was incubated with *Sclero-*

1) H. L. Herzog, *J. Am. Chem. Soc.*, **79**, 3921 (1957).

G. Greenspan et al., *ibid.*, **79**, 3922 (1957).

2) M. Shirasaka et al., *Bull. Agr. Chem. Soc. Japan*, **22**, 273 (1958).

tinia libertiana, 2 β , 15 β -dihydroxyprogesterone (I) was isolated as the major product; m.p. 206–216°; $[\alpha]_D -66.7^\circ$ (chloroform); $\lambda_{\text{max}}^{\text{MeOH}}$ 243 m μ ($\epsilon=15,080$); $\lambda_{\text{max}}^{\text{KBr}}$ 1700 cm $^{-1}$, 1671 cm $^{-1}$ and 1621 cm $^{-1}$.

Anal. Found. C, 72.82; H, 8.92. Calcd. for C₂₁H₃₀O₄: C, 72.80; H, 8.73%. The diacetate of (I), m.p. 127–128°; $[\alpha]_D -51.3^\circ$ (chloroform). The monoacetate of (I), m.p. 137–138°; $[\alpha]_D -22.7^\circ$ (chloroform).

The structure of (I) has been assigned on the basis of molecular rotation, the characteristic change of ultraviolet absorption in alkaline solution and the behavior by treatment with zinc and acetic acid affording the known 15 β -hydroxyprogesterone. The other three unidentified dihydroxyprogestones were also obtained in this experiment.

If 17 α -hydroxyprogesterone was used as the steroid substrate with this fungus *Sclerotinia libertiana*, 2 β , 17 α -dihydroxyprogesterone (II) was isolated. m.p. 219–221°; $[\alpha]_D -125^\circ$ (chloroform); $\lambda_{\text{max}}^{\text{MeOH}}$ 243 m μ ($\epsilon=16,000$). *Anal.* Found: C, 72.43; H, 8.28. Calcd. for C₂₁H₃₀O₄: C, 72.80; H, 8.73%.

The monoacetate of (II), m.p. 187.5–188.5°. The structure of this new compound (II) has

been decided on the basis described above.

Besides this, a small amount of 15 β , 17 α -dihydroxyprogesterone (III), m.p. 258–259° $[\alpha]_D +54.3^\circ$ (chloroform); $\lambda_{\text{max}}^{\text{MeOH}}$ 240.5 m μ ($\epsilon=15,000$) and 11 α , 17 α -dihydroxyprogesterone (IV), m.p. 216–218°; $[\alpha]_D +73^\circ$ (chloroform); $\lambda_{\text{max}}^{\text{MeOH}}$ 241.5 m μ ($\epsilon=17,000$) were also obtained.

From these results we have found that the fungus, *Sclerotinia libertiana* is capable of hydroxylating 15 β -, 11 α - and 11 β -positions of steroid nucleus besides 2 β -position.

In these experiments, we have prepared two kinds of new 2 β -hydroxyl steroid compounds, (I) and (II), with a strain of *Sclerotinia libertiana*.

We are greatly indebted to Dr. K. Tsuda, Dr. T. Asai, Professors of the University of Tokyo and Mr. M. Matsui, Director of this Laboratory for their helpful advice and constant guidance throughout this work.

Makoto SHIRASAKA
Rinzi TAKASAKI
Ryozo HAYASHI
Masako TSURUTA

Takamine Laboratory, Sankyo Co., Ltd.

Received February 19, 1959

[Bull. Agr. Chem. Soc. Japan, Vol. 23, No. 3, p. 246–248, 1959]

On the New Peptide-Type Ergot Alkaloids, Ergosecaline and Ergosecalinine

Sir:

Formerly, H. Rassbach et al.⁽¹⁾ reported that they isolated ergometrine from the saprophytic culture of *Claviceps purpurea* Tul. At about the same time, we⁽²⁾ also reported on the formation

of ergometrine and some other kinds of alkaloids in the saprophytic culture of a *Claviceps purpurea* strain obtained from the Spanish ergot of rye. We succeeded in isolating the following alkaloids in crystalline form from the mycelium filtrate (45 l.): peptide-type alkaloids (ca. 1.3 g. yield), ergometrine (45 mg.), ergometrinine (96 mg.), agroclavine (32 mg.) and secagroclavine

1) H. Rassbach, K. G. Büchel and H. Rochelmeyer, *Arzneimittel Forschung*, **6**, 690 (1956).

2) M. Abe, T. Yamano, S. Yamatodani, Y. Kōzu, M. Kusumoto, H. Komatsu and S. Yamada, *Ann. Rep. Develop. Res. (Agri.) Minist. Educ.*, **59** (1956).