*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.49; H, 9.34.

Fried[1] gives IV, m.p. 204–205°, $[\alpha]_D$ + 151°.

*Cortisone from 1-dehydrocortisone.* Incubation of 2.0 g. of 1-dehydrocortisone with *B. megaterium* for 48 hr. followed by isolation of the steroidal products in the usual way afforded 1.6 g. of crude solids. Paper chromatography[10] indicated that a substance with the same mobility as cortisone was present. Chromatography on 15 g. of Florisil and elution with 50% ether-in-hexane afforded small amounts of crystalline solids, which were pooled and recrystallized from acetone-hexane. There resulted 15 mg. of cortisone, m.p. 215–220° dec., whose infrared spectrum was identical with that of an authentic sample. No additional crystalline products other than some starting material were obtained on completing the chromatogram.

*Cortisol from 1-dehydrocortisol.* From 2 g. of 1-dehydrocortisol by incubation with *B. megaterium*, a crude mixture of 1.5 g. of oily steroids was obtained. Initial chromatography on 15 g. of Florisil and elution with 5% methanol in methylene chloride afforded a series of crystalline fractions of m.p. > 200°, which were pooled (420 mg.) and rechromatographed on 15 g. of Florisil. Elution with 1% methanol in methylene chloride afforded a series of fractions which had the same mobility as cortisol in a paper chromatogram. Recrystallization from acetone-hexane gave 45 mg. of cortisol, m.p. 210–215°, whose infrared spectrum was identical with an authentic sample.

SCHERING CORP.
BLOOMFIELD, N. J.

## Microbiological Transformation of Steroids. VI. Stereospecific Reductions of the 20-Carbonyl Group

FERNANDO CARVAJAL, ONOFRIO FRED VITALE, MARGARET JEVNIK GENTLES, HERSHEL L. HERZOG, AND E. B. HERSHBERG

*Received October 8, 1958*

Reduction of the 20-carbonyl to 20β-hydroxyl by microbiological means was first noted by Fried, Thoma, and Klingsberg[1] from the action of *Streptomyces lavendulae* on progesterone. Szpilfogel, Van Hemert, and DeWinter[2] have described the simultaneous reduction at 20- and 1,2-dehydrogenation of cortisone with *Fusarium* and *Calonectria* strains to give 1-dehydro Reichstein's U. None of these organisms is suitable for the generalized reduction of 4-pregnene-3,20-diketosteroids to the corresponding 4-pregnene-3-keto-20β-hydroxysteroids because of the other chemical transformations promoted simultaneously by these organisms.

We have found that various species of *Streptomyces*, in particular *Streptomyces griseus* (Schering FC No. 103), *Streptomyces sp.* (FC No. B222), and *Streptomyces sp.* (QM No. 1086), and an unidentified bacterium (FC No. C78) are capable of reducing the 20-carbonyl to 20β-hydroxyl in a variety of corticosteroids in good yield without producing other chemical alterations in the molecule. In this way we have transformed 4-pregnene-17α,21-diol-3,20-dione (I) (Reichstein's Compound S) into 4-pregnene-17α,20β,21-triol-3-one (II),[3a,3b] cortisone (III) into 4-pregnene-17α,20β,-21-triol-3,11-dione (IV),[4] cortisol (V) into 4-pregnene-11β,17α,20β,21-tetrol-3-one (VI),[5] 1-dehydrocortisone (VII)[6] into 1,4-pregnadiene-17α,-20β,21-triol-3,11-dione (VIII),[7] and 1-dehydrocortisol (IX)[6] into 1,4-pregnadiene-11β,17α,20β,21-tetrol-3-one (X).

Formation of the 20β-carbinols was carried out by aerobic incubation of the appropriate steroid with the *Streptomyces* strain in a yeast extract–dextrose–corn steep liquor medium. Progress of the reaction was measured by the disappearance of the substrate as estimated by paper chromatography according to Shull,[8] and by appearance of a more polar spot which did not stain with "red tetrazolium".[9] When the starting material had been consumed (usually 1–3 days), the reaction mixture was extracted with chloroform, and the product was isolated by concentration of the extract and crystallization from a suitable solvent (usually acetone-hexane). Yields of the reduced product varied between 20% and 75%. The poorest results occurred in the 11 β-hydroxyl series.

The structure of II was assigned on the basis of the absence of the 20-carbonyl band in the infrared spectrum (and the presence of the other appropriate bands), the correspondence of physical constants with those reported by Julian,[3b] and the preparation of the known diacetate.[3b] The structure of IV was confirmed by comparison of its melting point with that given by Reichstein and von Euw,[4] and by preparation of the diacetate.[4] The structure of VI was assigned by similar techniques. Compound VIII was characterized by preparation of the previously described diacetate[6] and by the changes in the molecular rotation accompanying this reaction[10] (see Table I). Compound

(1) J. Fried, R. W. Thoma, and A. Klingsberg, *J. Am. Chem. Soc.*, **75**, 5764 (1953).

(2) S. A. Szpilfogel, P. A Van Hemert, and M S. DeWinter, *Rec. trav. chim.*, **75**, 1227 (1956).

(3a) L. Ruzicka and P. Muller, *Helv. Chim. Acta*, **22**, 755 (1939).

(3b) P. L. Julian, E. W. Mayer, W. J. Karpel, and W. Cole, *J. Am. Chem. Soc.*, **75**, 1982 (1951).

(4) T. Reichstein and J. von Euw, *Helv. Chim. Acta*, **24**, 247E (1941).

(5) T. Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936); **20**, 953 (1937).

(6) H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto, and E. B. Hershberg, *J. Am. Chem. Soc.*, **77**, 4781 (1955).

(7) H. L. Herzog, Gordon Conference on Steroids and Natural Products, August, 1955.

(8) G. M. Shull, Abstracts of Papers, 126th Meeting of the American Chemical Society, September 1954, New York, p. 9A.

(9) W. J. Mader and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952).

(10) *Cf.* L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrane*, Rheinhold Publishing Corp., New York, N. Y., 1949, 3rd ed., p. 434.

X, isolated only as its 20,21-diacetate, has not been prepared previously and the structure has been assigned by analogy with the other transformations in this series.

Although reduction of the 20-carbonyl group to 20α-hydroxyl has been demonstrated with mammalian enzyme systems,[11] so far as we are aware no microbiological reductions of this kind have been reported.[11a] The chemical reduction has been solved in certain special cases,[3b,12] but the methods employed are not suitable for the direct, one-step reduction of the 20-carbonyl in the cortical steroid series. We now find that a strain of the yeast *Rhodotorula longissima* (Schering OFV No. 2[12a]) can transform I into 4-pregnene-17α,20α,21-triol-3-one (XI), III into 4-pregnene-17α,20α,21-triol-3,11-dione (XII), and VII into 1,4-pregnadiene-17α,20α,21-triol-3,11-dione (XIII). The reactions were carried out in an Edamin-dextrose–corn steep liquor medium in essentially the way described earlier in this article. As a rule, the reaction was slower than in the reductions leading into the 20β-series (usually 1–7 days) and the yields were somewhat lower (ca. 10–25%).

The structure of XI was determined by comparison of physical constants with literature values,[3b] by preparation of the known diacetate,[3b] and by degradation to 4-androstene-3,17-dione with sodium bismuthate.[13] A side reaction occurring during this microbiological transformation involves reduction of the 3-carbonyl group. A small amount of an unidentified steroid, possessing no important carbonyl absorption bands in the infrared, was isolated from the reaction mother liquors. The structure of XII was derived by analogy with that of XI, and confirmed by the anticipated changes in rotation on acetylation[10] (see Table I). The structure of XIII was assigned in the same way.

In the eosinophil test[14] IV displayed an activity equal to cortisone while XIII was about one third as active. Biological and clinical studies with these and related compounds will be described by Drs. S. Tolksdorf and P. L. Perlman of these laboratories, to whom we are indebted for these data, and by Dr. Maurice Pechet of Harvard University.

TABLE I

CHANGES IN MOLECULAR ROTATION OF 20-HYDROXYSTEROIDS UPON ACETYLATION

| Compound | $M_D$[a] | $M_D$ of 20,21-diacetate[a] | $\Delta^{Ac}$ |
|---|---|---|---|
| 4-Pregnene-17α,20β,21-triol-3-one | 226 | 554 | +328 |
| 4-Pregnene-17α,20α,21-triol-3-one | +192 | 82 | −110 |
| 4-Pregnene-17α,20α,21-triol-3,11-dione | 571 | 478 | −93 |
| 1,4-Pregnadiene-17α,20β,21-triol-3,11-dione | 425 | 710 | +285 |
| 1,4-Pregnadiene-17α,20α,21-triol-3,11-dione | 421 | 333 | −88 |
| Average of 17α,20β,21-triols[10] | | | +240 to +410° |

[a] All rotations in dioxane.

## EXPERIMENTAL[15]

*Representative procedure for reduction of 20-carbonyl to 20β-hydroxyl.* The appropriate microorganism (for ex. *S. griseus*, Schering FC No. 103) was propagated on nutrient agar medium at 28° for 7 days. A medium, prepared from 3 g. of yeast extract (Difco), 10 g. of dextrose, and 1 g. of corn steep liquor made up to 1 l. with tap water, was adjusted to *p*H 7 and distributed among ten 300-ml. Erlenmeyer flasks (100 ml. of medium per flask). The flasks and contents were sterilized and then inoculated with spores (from the agar slants) which were suspended in distilled sterile water. Incubation on a rotary shaker (28°, 220 r.p.m.) was continued for 1–2 days, and then there was added to each flask a solution of 25–100 mg. of steroid in 1–6 ml. of 95% ethanol (methanol or acetone may also be used). After 1–3 days of incubation with shaking, paper chromatography[8] of the chloroform extract of an aliquot revealed complete consumption of the starting material and the appearance of a more polar ultraviolet absorbing spot which did not stain with "red tetrazolium." The reaction mixture was then extracted thoroughly with chloroform and the extracts were washed with water, dried, and concentrated to a residue. Recrystallization of the residue from the appropriate solvent (usually acetone) afforded crystalline steroid in yields averaging between 20% and 75%.

In general, transformation was less rapid and yields were poorer with 11β-hydroxylated steroids than with 11-unsubstituted or 11-ketosteroids.

*Procedure for reduction of 20-carbonyl to 20α-hydroxyl. Rhodotorula longissima* (Schering OFV No. 2) was propagated on nutrient agar at 28° for 7 days. A medium, prepared from 20 g. of Edamin, 3 g. of corn steep liquor, and 50 g. of dextrose made up to 1 l. with tap water, was adjusted to *p*H 5.3. The growth of the culture and the transformation of the steroids were carried out as described in the first example. Transformation time tended to be longer in this reduction than in that of the first example, occasionally extending to as much as 7 days. The methods of control and isolation were

(11) R. I. Dorfman and F. Ungar, *Metabolism of Steroid Hormones*, Burgess Publishing Co., Minneapolis, Minn., 1953, p. 45.

(11a) NOTE ADDED IN PROOF: Our attention has since been drawn to the reductions of some 16,17-oxido-20-keto-steroids to 20α-hydroxy-steroids by an unidentified yeast, which were accompanied by Wagner-Meerwein rearrangement in all instances; B. Camerino *et al.*, *Gazz. Chim. ital.*, **86**, 260 and 1219 (1956).

(12) E. L. Shapiro, D. Gould, and E. B. Hershberg, *J. Am. Chem. Soc.*, **77**, 2912 (1955); D. K. Fukushima and E. D. Meyer, *J. Org. Chem.*, **23**, 174 (1958).

(12a) This culture was deposited at the Culture Collection of the Northern Regional Research Laboratories (USDA) at Peoria, Ill., where it was entered as *Rhodotorula longissima* NRRL No-Y 2343.

(13) C. J. W. Books and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(14) R. S. Speirs and R. K. Meyer, *Endocrinology*, **48**, 316 (1951); E. Rosemberg, *et al.*, *Endocrinology*, **54**, 363 (1954).

(15) All melting points are corrected. Analyses and optical data were obtained by the Physical Chemistry Department of Schering Corporation and by the Galbraith Laboratories, Knoxville, Tenn., Dr. Jo-Yun Chen and Mr. Edward Townley interpreted the infrared spectra.

the same, and the products could usually be purified by crystallization, although those experiments with I as substrate did not proceed to completion and required chromatographic purification.

*4-Pregnene-17α,20β,21-triol-3-one* (II) *from Reichstein's Compound S.* From the action of *Streptomyces sp.* FC No. B222 on I (0.9 g.) (final concentration of steroid in medium 0.5 g./l.; added in 40 ml. ethanol; complete transformation in 48 hr.) there was isolated a nicely crystalline crude product which, after recrystallization from acetone-hexane, afforded 0.43 g. of II, m.p. 175°, resolidification 178°, remelt 190°, $\lambda_{max}^{EtOH}$ 242 mμ (ε = 16,300), $[\alpha]_D^{25}$ +65° (dioxane), $\lambda_{max}^{Nujol}$ 2.92 mμ (OH), 6.05 and 6.17 μ (Δ⁴-3-one). Julian[3b] gives m.p. 188–190°, $[\alpha]_D^{25}$ +65° (dioxane).

*Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.21; H, 9.35.

*4-Pregnene-17α,20β,21-triol-3-one 20,21-diacetate.* A solution of 240 mg. of II in 5 ml. of pyridine and 5 ml. of acetic anhydride was allowed to stand overnight at room temperature. Dilution of the reaction mixture with water caused the precipitation of needles which were removed by filtration and recrystallized from acetone-hexane. There resulted 240 mg. of 20,21-diacetate, m.p. 191–193°, $[\alpha]_D^{25}$ + 128° (dioxane); $\lambda_{max}^{EtOH}$ 241 mμ (ε = 16,600), $\lambda_{max}^{Nujol}$ 2.93 μ (OH), 5.71 and 5.75 μ (split acetate carbonyl bands), 6.02 and 6.18 μ (Δ⁴-3-one) and 8.12 μ (C—O—C of acetate). Julian[3b] reports m.p. 189–191°, $[\alpha]_D^{25}$ + 150° (chloroform).

*Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; N, 8.39. Found: C, 69.28; H, 8.50.

*4-Pregnene-17α,20β,21-triol-3,11-dione* (IV) *from cortisone.* From the action of *Streptomyces griseus* (FC No. 103) on 925 mg. of III (final concentration of steroid in medium 0.5 g./l.; added in 40 ml. of ethanol; transformation in 24 hr.) there was isolated an oily crude product which afforded 0.46 g. of IV, m.p. 203–205° dec. on crystallization from acetone-hexane. Further recrystallization raised the m.p. to 206–207° dec. Fieser[16] cites Reichstein and von Euw[4] as reporting m.p. 208°.

*4-Pregnene-17α,20β,21-triol-3,11-dione 20,21-diacetate.* Acetylation of IV by the aforedescribed procedure gave a diacetate which, after recrystallization from acetone-hexane, melted at 255.5–257°, $[\alpha]_D^{25}$ + 186° (dioxane). Reichstein and von Euw[4] give m.p. 252–253°, $[\alpha]_D^{21}$ + 178.5° (acetone).

*4-Pregnene-11β,17α,20β,21-tetrol-3-one* (VI) *from cortisol.* From the action of *Streptomyces griseus* (FC No. 103) on 915 mg. of V (final concentration of steroid in medium 0.5 g./l. added in 40 ml. of ethanol; transformation in 4 days) there was isolated 150 mg. of VI, m.p. 105–110° after crystallization from acetone-hexane. Recrystallization from ethanol water raised the m.p. to 133–135°, $[\alpha]_D^{25}$ + 85° (dioxane), $\lambda_{max}^{Nujol}$ 2.94 and 3.07 μ (OH), 6.07 and 6.21 μ (Δ⁴-3-one). Reichstein and von Euw[4] report m.p. 124–129°, $[\alpha]_D^{25}$ + 87° (ethanol).

*1,4-Pregnadiene-17α,20β,21-triol-3,11-dione* (VIII) *from 1-dehydrocortisone.* The product from the action of *Streptomyces griseus* (FC No. 103) on VII (2 g.) (final concentration of steroid in medium 0.5 g./l.; added in 40 ml. of ethanol; transformation in 48 hr.) was crystallized from methylene chloride–hexane to give 1.5 g. of crystalline VIII, m.p. 115–125°, resolidifying and remelting at 182–183°. Recrystallization from acetone-hexane raised the m.p. to 184–185° without intermediate phase change, $[\alpha]_D^{25}$ + 118° (dioxane), $\lambda_{max}^{methanol}$ 238 mμ (ε = 15,300), $\lambda_{max}^{Nujol}$ 2.94 and 3.06 μ (OH), 5.87 μ (11-carbonyl and solvent carbonyl), 6.01, 6.19, and 6.24 μ (Δ¹,⁴-3-one).

*Anal.* Calcd. for $C_{21}H_{28}O_5.C_3H_6O$: C, 68.87; H, 8.19. Found: C, 69.12; H, 7.91.

*1,4-Pregnadiene-17α,20β,21-triol-3,11-dione 20,21-diacetate.* Acetylation of VIII in the usual way yielded a 20,21-diacetate which, after recrystallization from acetone-hexane, melted at 239–242°, $[\alpha]_D^{25}$ + 160° (dioxane), $\lambda_{max}^{Nujol}$ 2.91 μ (OH), 5.74 and 5.77 μ (split acetate carbonyls), 5.90 μ (11-carbonyl), 6.01, 6.14, and 6.22 μ (Δ¹,⁴-diene-3-one) and 8.20 μ (C—O—C of acetate). This sample was identical with that obtained from the action of *Corynebacterium simplex*[7] on cortisone, followed by acetylation.

*1,4-Pregnadiene-11β,17α,20β,21-tetrol-3-one 20,21-diacetate from 1-dehydrocortisol.* From the action of *Streptomyces griseus* (FC No. 103) on 925 mg. of IX (final concentration of steroid in medium 0.5 g./l.; added in 40 ml. of ethanol; transformation in 4 days) a glassy product was obtained. Acetylation of this product in the usual way afforded 175 mg. of 20,21-diacetate of X, m.p. 228–230°. Several recrystallizations from acetone-hexane raised the m.p. to 243–244°.

*Anal.* Calcd. for $C_{25}H_{34}O_7.C_3H_6O$: C, 66.64; H, 7.99. Found: C, 66.53; H, 7.57.

*4-Pregnene-17α,20α,21-triol-3-one* (XI) *from Reichstein's Compound S.* From the action of *Rhodotorula longissima* (Schering OFV No. 2) on 2.0 g. of I (final concentration of steroid in medium 0.5 g./l.; added in 40 ml. of ethanol; transformation in 5 days) the chloroform concentrate was chromatographed on Florisil (40 g.). From methylene chloride elution 920 mg. of I was recovered and elution with 1–5% methanol in methylene chloride gave a total of 840 mg. of crude solid, m.p. 168–190°. The latter was recrystallized from acetone-hexane affording 540 mg., m.p. 199–209°. The m.p. of this mixture could not be raised significantly by further recrystallization, so it was acetylated with 5 ml. of acetic anhydride and 5 ml. of pyridine. Upon dilution of the reaction mixture with water, a precipitate formed which was removed by filtration and melted 225–240°. Chromatography on 30 g. of Florisil and elution with ether gave 360 mg. of crude solid, m.p. 242–252°. Recrystallization from acetone-hexane yielded 230 mg. of shiny needles of 20,21-diacetate of XI, m.p. 251–253°, $\lambda_{max}^{methanol}$ 242 mμ (ε = 16,100), $[\alpha]_D^{25}$ + 35° ($CHCl_3$), + 19° (dioxane), $\lambda_{max}^{Nujol}$ 2.90 μ (OH), 5.78 μ (acetate), 5.93 and 6.14 μ (Δ⁴-3-one), 7.94 and 8.09 μ (C—O—C of acetate). Julian[3b] gives m.p. 251–253°, $[\alpha]_D^{32}$ + 31.5° ($CHCl_3$).

*Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.05; H, 8.43.

From another run (3 g. of I) the crude, extracted steroids were washed with cold hexane and crystallized from acetone-hexane. There resulted 1.6 g. of solid, which was then acetylated. There was isolated 2.29 g. of crude acetate, m.p. < 190°, which was chromatographed on 125 g. of Florisil. From 25% ether-hexane eluates there was obtained 20 mg. of needles, m.p. 187–189°, $\lambda_{max}^{Nujol}$ 2.89 μ (OH), 5.69, 5.73, and 5.80 μ (acetate carbonyl), 7.98 and 8.23 μ (C—O—C of acetate). (Hydrolysis of this product afforded a compound, m.p. 230–232° which had no carbonyl bands in its infrared spectrum.) From 100% ether and from 25% methylene chloride–ether there resulted a total of 740 mg. of XI diacetate, m.p. 245–250° dec.

Hydrolysis of 150 mg. of purified 20,21-diacetate was accomplished by solution in 10 ml. of 0.5*N* methanolic sodium hydroxide and standing at room temperature overnight. The solution was concentrated *in vacuo*, and the residue was made acid and extracted with methylene chloride. Chromatography of the extracts on Florisil and elution with 5% methanol in methylene chloride afforded, after crystallization from acetone-hexane, 40 mg. of XI, m.p. 221–225°, $[\alpha]_D^{25}$ + 55° (dioxane), $\lambda_{max}^{Nujol}$ 2.88, 2.93, and 3.01 μ (OH), 6.04 and 6.19 μ (Δ⁴-3-one). Julian[3b] gives m.p. 225–227.5°, $[\alpha]_D^{25}$ + 76° (chloroform), $\Delta M^{Ac-H}$ −129.

*4-Androstene-3,17-dione from* XI. A solution of 65 mg. of XI was dissolved in 8 ml. of acetic acid and diluted to 16 ml. with water. One gram of sodium bismuthate was added, and the mixture was stirred overnight at room temperature, whereupon the solids were removed by filtration and leached with methylene chloride. The filtrate was diluted with water and extracted with methylene chloride. The combined extracts were washed with water, dried, and

(16) L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, 3rd ed., p. 426.

concentrated to a crystalline residue. Recrystallization from ether-hexane gave 18 mg. of 4-androstene-3,17-dione, m.p. 157–165°, with an infrared spectrum identical with that of an authentic sample.

*4-Pregnene-17α,20α,21-triol-3,11-dione* (XII) *from cortisone.* From the action of *R. longissima* (OFV No. 2) on 2 g. of cortisone (final concentration of steroid in medium 0.5 g./l.; added in 40 ml. of ethanol; transformation in 5 days) there was obtained, after crystallization from acetone-hexane, 0.65 g. of XII, m.p. 229–231° dec. Several recrystallizations from the same solvent mixture raised the m.p. to 240–242° (dec.), $[\alpha]_D^{25}$ + 158° (dioxane), $\lambda_{max}^{Nujol}$ 2.89, 2.96, and 3.03 μ (OH), 5.87 μ (11-carbonyl), 6.02 and 6.19 μ ($\Delta^4$-3-one).

*Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.97; H, 7.83. Found: C, 69.69; H, 8.01.

*4-Pregnene-17α,20α,21-triol-3,11-dione 20,21-diacetate.* Acetylation in the usual way afforded a 20,21-diacetate m.p. 273–275° (dec.) after recrystallization from acetone, $[\alpha]_D^{25}$ + 107°, $\lambda_{max}^{methanol}$ 238 mμ (ε = 15,500), $\lambda_{max}^{Nujol}$ 2.91 μ (OH), 5.76 and 5.80 μ (acetate carbonyls), 5.85 μ (11-carbonyl), 5.94 and 6.16 μ ($\Delta^4$-3-one), 8.00 and 8.11 μ (C—O—C of acetate).

*Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 67.09; H, 7.87.

*1,4-Pregnadiene-17α,20α,21-triol-3,11-dione* (XIII) *from 1-dehydrocortisone.* From the action of *R. longissima* (OFV No. 2) on 1.875 g. of VII (final concentration of steroid in medium 0.25 g./l. added in 40 ml. of methanol; transformation in 6 days) there was isolated after recrystallization from acetone-hexane 0.41 g. of XIII, m.p. 233–235° dec. Additional recrystallization raised the m.p. to 238–240° dec. with a phase change at 225° (polymorphic samples of XIII which melted at 225–228° have also been obtained), $[\alpha]_D^{25}$ + 117° (dioxane), $\lambda_{max}^{methanol}$ 239 mμ (ε = 15,400), $\lambda_{max}^{Nujol}$ 2.95 μ (OH), 5.85 μ (11-carbonyl), 6.01, 6.19, and 6.23 μ ($\Delta^{1,4}$-diene-3-one).

*Anal.* Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found: C, 70.06; H, 7.71.

*1,4-Pregnadiene-17α,20α,21-triol-3,11-dione 20,21-diacetate.* Preparation of the 20,21-diacetate in the usual way gave a compound, m.p. 250–251° dec. (samples have also been obtained m.p. 267–270° dec.) $[\alpha]_D^{25}$ + 75° (dioxane), $\lambda_{max}^{methanol}$ 239 mμ (ε = 15,100), $\lambda_{max}^{Nujol}$ 2.92 μ (OH), 5.74 and 5.81 μ (split acetate carbonyls), 5.86 μ (11-carbonyl), 5.97, 6.11, and 6.21 μ ($\Delta^{1,4}$-diene-3-one) and 8.05 μ (C—O—C of acetate).

*Anal.* Calcd. for $C_{25}H_{32}O_7$: C, 67.55; H, 7.26. Found: C, 67.67; H, 7.13.

SCHERING CORP.
BLOOMFIELD, N. J.

## The Pyrolysis of Perfluoroethyl Ether[1]

R. D. DRESDNER, T. J. MAO,[2] AND J. A. YOUNG

*Received October 14, 1958*

In this work perfluoroethyl ether, $C_2F_5OC_2F_5$, was pyrolyzed by passing it slowly over a bed of sodium fluoride pellets in a nickel reactor heated to 800°. The isolable and identified products were $C_2F_6$, $COF_2$, $C_3F_8$, $CF_3CF{=}CF_2$, $(CF_3)_2C{=}CF_2$ and carbon. In the main this ether shows a pyrolytic stability comparable to most fluorocarbons. Except for the $COF_2$ evolved the ether yields products similar to many of the fluorocarbons pyrolyzed by the hot filament technique.[3] At least one report[4] shows that under pyrolytic conditions in a static system at pressures less than an atmosphere $CF_2{=}CF_2$ corrodes nickel less than it does stainless steel at temperatures between 600–700°.

In general past work tends to show that nature of the pyrolysis reaction of a simple molecule such as $CF_2{=}CF_2$ depends at least upon the variables of temperature, pressure, the contact time, and the geometry and composition of the reaction vessel.[4–7] A correlation of the results is further complicated by whether the pyrolysis is performed by a static or flow method.

### EXPERIMENTAL

The perfluoroethyl ether was prepared by the electrochemical (Simons) process[8] in a nominally 50-ampere cell not unlike that described by Hoffmann, Simmons, *et al.*[9] Seven hundred and four g. of ether (11 moles) produced 690 g. of product condensable at −80° of which 450 g. (1.77 moles) was the fluorocarbon ether, b.p. 2.5°, mol. wt. 254.

The pyrolysis equipment was simple. The ether was allowed to escape from a cylinder through a needle valve, its flow being observed with a flow meter. It was then passed through a 1/2-in. i.d. nickel tube, packed with 1/8-in. sodium fluoride pellets, which was heated in a Hoskins furnace. Products were collected in cold traps. A pressure of about 1 atm. in the system was controlled by a valve before the traps and was observed on a manometer. Air was never in contact with the system. Temperatures were measured with a thermocouple placed in a well welded to the top the reactor.

Several small trial pyrolyses established that at contact times as high as 3.5 min. there was no reaction at 650°, 3% conversion to products at 700° and 30–50% conversion to products at 800°. It was also established that the number of equivalents of $COF_2$ formed was always equal to the number of equivalents of fluorocarbon ether used.

Finally, in order to effect a more complete study of the reaction 102 g. (0.402 mole) of $C_2F_5OC_2F_5$ were pyrolyzed at a flow rate of 0.03 g./min., equivalent to a theoretical contact time of not less than 6.3 min. The reaction products were collected in a liquid air-cooled trap, transferred to the pot of a low temperature microcolumn and allowed to reflux from the head cooled with a mixture of Dry Ice and acetone. A liquid air-cooled trap was attached to the head outlet in which the uncondensed gases that escaped overhead were collected. The column equilibrated at a head

(1) This work was supported by the Chemistry Branch, Office of Naval Research. Reproduction of all or any part of this paper is permitted for purposes of the United States Government.

(2) Graduate assistant in chemistry.

(3) (a) G. C. Rogers and G. H. Cady, *J. Am. Chem. Soc.*, **73**, 2523 (1951). (b) R. K. Steunenberg and G. H. Cady, *J. Am. Chem. Soc.*, **74**, 4165 (1951).

(4) B. Atkinson and V. A. Atkinson, *J. Chem. Soc.*, 2086 (1957).

(5) E. E. Lewis and H. A. Naylor, *J. Am. Chem. Soc.*, **69**, 1967 (1947).

(6) B. Atkinson and A. B. Trenwith, *J. Chem. Soc.*, 2082 (1953).

(7) J. R. Lacher, G. W. Tompkins, and J. D. Park, *J. Am. Chem. Soc.*, **74**, 1693 (1952).

(8) J. H. Simons, U. S. Patent **2,500,388** (1950).

(9) F. W. Hoffmann, T. C. Simmons, R. B. Beck, H. V. Holler, T. Katz, R. V. Koshar, E. R. Larsen, J. E. Mulvaney, F. E. Rogers, B. Singleton, and R. S. Sparks, *J. Am. Chem. Soc.*, **79**, 3424 (1957).