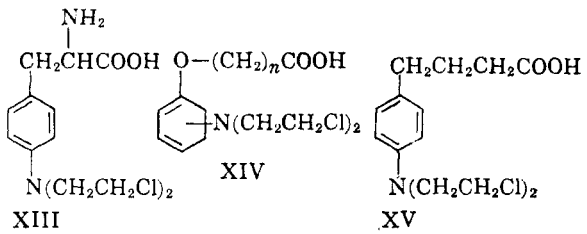
55–61% over-all yield from the bis(hydroxyethyl)-amino esters (IV–VI).

The *m*-mustard of cinamic acid (VIII) was also active against Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210; testing on the *p*-mustard (IX) is as yet incomplete.[3] It is of considerable theoretical interest that VII and VIII differ in tumor spectrum from Chloroambucil (XV) but are similar to phenylalanine mustard (XIII). In addition, mustards derived from phenoxyacetic acid and phenoxypropionic acid (XIV)[4] are more like phenylalanine mustard (XIII) in their tumor



spectra than like Chlorambucil (XV).[3] The cinnamic acid mustards (VII–IX), phenylalanine mustard (XIII), and the phenoxyalkanoic acid mustards (XIV) have in common a side chain functional group in addition to the usual carboxyl of Chlorambucil (XV); therefore, synthesis of other analogs of Chlorambucil (XV), and perhaps phenylalanine mustard (XIII), with other functional groups in the side chain for test evaluation would be warranted, since it would appear that the extra functional group causes a change in metabolism or tissue absorption of the candidate drug.

EXPERIMENTAL[7]

o-[*Bis*(*2-chloroethylamino*)]*cinnamic acid* (VII). *Method C.* Methyl *o*-[bis(2-hydroxyethyl)amino]cinnamate (IV)[2] was treated with phosphorus oxychloride as previously described for the preparation of *m*-[bis(2-chloroethyl)amino]hydrocinnamic acid,[2] except that the aqueous solution of decomposed phosphorus oxychloride was worked up by extraction without standing for 20 hr., since conversion of X to VII was slow under these conditions. The crude ester (X) was then refluxed with 12*N* hydrochloric acid for 15 min. and worked up as described for mustards of phenoxyacetic acid.[4] Evaporation of the benzene extract *in vacuo* gave a 55% yield of VII, m.p. 123–125°; $\lambda_{max(\mu)}^{Nujol}$ 3.50 (broad acidic OH); 5.90 (carboxyl C=O); 6.15, 6.25, 6.70 (C=C, aryl), 13.1 (*ortho*-disubstituted benzene). An analytical sample, m.p. 123–125°, was prepared by recrystallization from petroleum ether (b.p. 30–60°). See Table I for analytical data.

(3) The assays were performed at this Institute by Dr. J. Greenberg and staff under contract to the Cancer Chemotherapy National Service Center.

(4) W. A. Skinner, A. P. Martinez, and B. R. Baker, *J. Org. Chem.*, **26**, 152 (1961), Paper XLVI of this series.

(5) H. Salkowski, *Ber.*, **28**, 1917 (1895).

(6) F. Mayer, H. Philips, F. W. Ruppert, and A. T. Schmitt, *Ber.*, **61**, 1966 (1928).

(7) Melting points were taken on a Fisher-Johns block and are uncorrected.

Acknowledgment. The authors wish to thank Dr. Peter Lim for interpretation of the infrared spectra and his staff for spectrophotometry and paper chromatography. They also wish to thank Mr. O. P. Crews, Jr., and his staff for large-scale preparation of intermediates.

DEPARTMENT OF BIOLOGICAL SCIENCES
STANFORD RESEARCH INSTITUTE
MENLO PARK, CALIF.

Stereospecific Microbiological Reduction of 3-Keto-1,4-pregnadienes to 3-Keto-1-pregnenes

GEORGE GREENSPAN,[1] CARL P. SCHAFFNER, WILLIAM CHARNEY, MARGARET JEVNIK GENTLES, AND HERSHEL L. HERZOG

Received July 11, 1960

At the dawn of the application of microorganisms to the transformation of steroidal substrates, Mamoli and his collaborators[2] observed that testosterone and 4-androstene-3,17-dione were transformed by "putrefactive bacteria" into 4,5α-dihydro derivatives and by *B. putrificus* into 4,5β-dihydro derivatives. The application of these earlier discoveries in recent times has been hampered by the lack of availability and incomplete identification of the organisms involved.

Other workers[3a,b] have since demonstrated the reduction of Δ^4-3-ketosteroids to 4,5α- and 4,5β-dihydroanalogs, incidental to hydroxylation elsewhere in the substrates employed.

We have now found that *Streptomyces sp.* W 3808 (Waksman Collection, Institute of Microbiology, Rutgers University) transforms selected 3-keto-1,4-pregnadienes into 3-keto-1-pregnenes. Incubation of prednisone for 112 hours with a 112-hour growth culture afforded, after chloroform extraction and silicic acid chromatography, a less polar product (I), $\lambda_{max}^{CH_3OH}$ 225 mμ (ε 6600). The infrared spectrum of I was essentially that of 1-pregnene-17α,21-diol-3,11,20-trione.[4] To complete the identification, I was acetylated with acetic anhydride in pyridine solution to yield a 21-acetate, whose infrared spectrum matched that of an authentic sample.[5]

(1) Present address, Wyeth Institute for Medical Research, Radnor, Pa.

(2) For the pertinent references see F. Fischer, *Newer Methods of Preparative Organic Chemistry*, Interscience, New York, 1948, p. 184–190.

(3a) D. Perlman, E. Titus, and J. Fried, *J. Am. Chem. Soc.*, **74**, 2126 (1952). (b) D. H. Peterson, H. C. Murray *et al.*, *J. Am. Chem. Soc.*, **74**, 5933 (1952); **75**, 412 (1953); **76**, 3174 (1954).

(4) Prepared by hydrolysis of authentic 21-acetate of I (Ref. 5).

(5) V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 287 (1951).

A similar transformation employing 16α-methylprednisone as the substrate gave a less polar product (II), $\lambda_{max}^{CH_3OH}$ 224 mμ (ε 8,650). The structure of II was assigned as 16α-methyl-1-pregnene-17α,21-diol-3,11,20-trione because the observed ultraviolet maximum was in good agreement with the predicted value[6] and by analogy with the proved structure of I.

Qualitative evidence for the same type of transformation was also obtained with 1,4-pregnadiene - 11α,17α,21 - triol - 3,20 - dione which was transformed into a less polar product with an ultraviolet maximum at 237 mμ (Δλ = −11 mμ[7]). Similar qualitative results were also obtained with 16α-methyl-1,4-pregnadiene-11α,17α,21-triol-3,20-dione.[8]

EXPERIMENTAL[9]

1-Pregnene-17α,21-diol-3,11,20-trione (I). To each of four 250-ml. Erlenmeyer flasks was added 50 ml. of 1% yeast extract-dextrose medium and an inoculum of spores of *Streptomyces sp.* W 3808 (Waksman collection). After a 64-hr. incubation with rotary shaking at 30° the cultures were each transferred to one of four 2-l. Erlenmeyers containing 400 ml. of the same medium. Following another 48 hr. of incubation, 0.130 g. of prednisone was added to each flask. After 112 hr. of additional incubation the contents of all flasks were pooled, the mycelium was removed by filtration, and a water wash of the mycelium was added to the filtrate. The combined filtrate was extracted with chloroform and the extracts were concentrated to a small volume. The resulting solution was chromatographed over silicic acid and the column was eluted with chloroform.

The combined crystalline fractions (I) from the chromatogram (0.070 g.) melted at 185–189°, $\lambda_{max}^{CH_3OH}$ 225 mμ (ε 6600) and gave a positive test with red tetrazolium reagent. In the Shull system[10] I migrated slightly faster than cortisone. The infrared spectrum of I was essentially the same as that of 1-pregnene-17α,21-diol-3,11,20-trione,[4] but I was clearly impure.

21-Acetate of I. A solution of 0.050 g. of I in 1.0 ml. of pyridine and 1.0 ml. of acetic anhydride was allowed to stand at room temperature overnight. Excess water was added and the resulting precipitate (0.048 g.) was collected by filtration. Recrystallization from aqueous methanol and from acetone-hexane gave 0.023 g. of the 21-acetate of I, m.p. 235–239°, $[\alpha]_D^{25}$ +131° (acetone), $\lambda_{max}^{CH_3OH}$ 225 mμ (ε 8400). Mattox and Kendall[5] report 1-pregnene-17α,21-diol-3,11,20-trione 21-acetate, m.p. 245–246°, $[\alpha]_D^{25}$ +138° (acetone), $\lambda_{max}^{CH_3OH}$ 225 mμ (ε 9100). The infrared spectrum of the 21-acetate of I matched that of an authentic sample.

16α-Methyl-1-pregnene-17α,21-diol-3,11,20-trione (II). Under essentially the conditions employed in the preparation of I, with the exception that a 2% yeast extract–dextrose medium and a 55-hr. incubation time with steroid were used, 1.95 g. of 16α-methylprednisone was transformed. Chromatography of the concentrated chloroform extract over silicic acid afforded by chloroform elution 0.114 g. of tan powder which ran as a single compound, slightly faster moving than 16α-methylprednisone, in the Shull system.[10] The powder was rechromatographed over Florisil and eluted with 1% methanol and 2% methanol in methylene chloride. The combined crystalline fractions on recrystallization from acetone-hexane gave 0.083 g. of II, m.p. 206–212°, $[\alpha]_D^{25}$ +116° (dioxane), $\lambda_{max}^{CH_3OH}$ 224 mμ (ε 8650), λ_{max}^{Nujol} 2.88 μ (OH), 5.88 μ (11- and 20-carbonyl), 6.00 μ (3-carbonyl), 6.22 μ (Δ^1).

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.83; H, 8.03.

INSTITUTE OF MICROBIOLOGY
RUTGERS UNIVERSITY
NEW BRUNSWICK, N. J.

SCHERING CORP.
BLOOMFIELD, N. J.

(6) Calculated for the shift in u.v. Δλ = λ (1-pregnene-17α,21-diol-3,11,20-trione 21-acetate)-λ (prednisone) = 225 mμ − 238 mμ = −13 mμ; observed λ (II) − λ (16α-methylprednisone) = 225 mμ − 238 mμ = −13 mμ.

(7) The preparation of 1,4-pregnadiene-11α,17α,21-triol-3,20-dione is given in U. S. Patent **2,837,464**. The ultraviolet maximum (methanol) is at 248 mμ (ε 17,800).

(8) E. P. Oliveto *et al.*, *J. Am. Chem. Soc.*, **80**, 4431 (1958).

(9) All melting points are corrected. Analyses and optical data were obtained by the Physical Chemistry Departments of these laboratories. The infrared spectra were interpreted by Mr. Richard Wayne.

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

Estrogens. III. Synthesis of 4-Methylequilenin[1,2]

TAD L. PATTON

Received July 28, 1960

Procedures for the introduction of a methyl group at positions-1, 2, 4, and 6 of estrone and estradiol and at position-1 of equilenin have been reported.[1,3–7] It is the purpose of this note to report a procedure for the introduction of a methyl group at position-4 of equilenin.

Since the phenolic rings of estrone and estradiol condense with formaldehyde and secondary amines to yield 2-dialkylaminomethylestrogens,[1,5] the phenolic ring of equilenin would be expected to react similarly. In fact, it was found that formaldehyde and morpholine condense with equilenin to give one compound, a monosubstituted product, which was assigned the structure of 4-morpholinomethylequilenin (I). This assignment was made because equilenin is a 5,6-disubstituted 2-naphthol, and the reaction would be expected to occur at the position equivalent to the one at which it occurs in 2-naphthol. The latter has been shown to undergo

(1) The preceding paper in this series is T. L. Patton, *J. Org. Chem.*, **25**, 2148 (1960).

(2) This investigation was supported by a grant, CY-2873, from the National Cancer Institute, U. S. Public Health Service.

(3) C. Djerassi, G. Rosenkranz, J. Romo, J. Pataki, and S. Kaufmann, *J. Am. Chem. Soc.*, **72**, 4540 (1950).

(4) J. Iriarte and H. J. Ringold, *Tetrahedron*, **3**, 28 (1958).

(5) T. L. Patton, *Chem. & Ind. (London)*, 923 (1959).

(6) H. Dannenberg, C. H. Deering, and D. Donnenberg-von Dresler, *Hoppe-Seyler's Z. physiol. Chem.*, **317**, 174 (1959).

(7) E. Velarde, J. Iriarte, H. J. Ringold, and C. Djerassi, *J. Org. Chem.*, **24**, 311 (1959).