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.

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(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]

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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).

TABLE I

ULTRAVIOLET ABSORPTION MAXIMA OF SUBSTITUTED 2-NAPHTHOLS

Compound	mμ (εmax)[a]					
2-Naphthol	226 (91,194)	265 (3911)	275 (4559)	286 (3301)	320 (1861)	331 (2163)
1-Morpholinomethyl-2-naphthol	230 (61,044)	269 (3890)	279 (4890)	290 (3850)	324 (2150)	335 (2480)
Equilenin	231 (65,476)	270 (4428)	281 (5047)	292 (3571)	328 (1752)	341 (641)
I	236 (57,320)	274 (4510)	284 (5510)	296 (4510)	333 (2690)	345 (3120)
II	237 (67,330)	274 (4400)	285 (5480)	297 (4360)	333 (2560)	345 (2880)

[a] The solvent was 95% ethyl alcohol.

the Mannich reaction specifically at position-1;[8,9] therefore, by comparison, equilenin should react specifically at position-4, the counterpart of position-1 in 2-naphthol. In addition, the introduction of the morpholinomethyl group into equilenin causes a bathochromic shift of the ultraviolet spectrum which is almost identical to the spectral shift observed when the same group is introduced into position 1 of 2-naphthol (see first four lines of Table I).

The hydrogenolysis of I with Raney nickel by a known procedure[1,5] removed the morpholine part of the molecule to give 4-methylequilenin (II). The ultraviolet absorption spectrum of II is essentially parallel to that of I (see Table I); therefore, the aromatic structure remained unchanged throughout the hydrogenolysis reaction. Evidence that the carbonyl group at C-17 was not reduced during the hydrogenolysis reaction was provided by the infrared spectrum of II which showed a strong absorption peak at 5.77μ. In addition, II exhibited absorption at 2.99μ; this is characteristic of the phenolic hydroxyl group. The infrared absorption spectrum of I, like the spectra of 2-dialkylaminomethylestrogens,[1] did not show absorption in the 3μ region.

EXPERIMENTAL[10]

4-Morpholinomethylequilenin (I). Equilenin (266 mg. 0.001 mole) was dissolved in a solution of ethyl alcohol (12 ml.) and morpholine (0.3 ml.) by heating; then 37% formaldehyde (0.1 ml.) was added. After the solution had refluxed for 30 min. an additional 0.2 ml. of formaldehyde was added, and the solution was heated at reflux temperature for 2 hr. It then remained at room temperature overnight. The solvent was removed at water pump pressure, and the viscous residue was made to solidify by the addition of a few drops of ethyl alcohol. The crude product was taken up in ether and washed with 10% hydrochloric acid to remove the amino steroid. The acid wash was cooled and made alkaline with ammonium hydroxide. The product was extracted with several portions of ether. After washing the combined extracts with water and drying them over anhydrous sodium sulfate the ether was evaporated at reduced pressure. The residue was recrystallized from ethyl alcohol to give 235 mg. of pink colored crystals. The second recrystallization gave colorless crystals of analytic purity, m.p. 167–168°; λ_{max}^{KBr} 5.75 μ (carbonyl at C_{17}) and 7.14 μ (free methylene at C_{16}).

Anal. Calcd. for $C_{23}H_{27}O_3N$. C, 75.58; H, 7.44; N, 3.83. Found. C, 75.61; H, 7.39; N, 3.91.

4-Methylequilenin (II). A suspension of Raney nickel (1 g.) in a solution of I (121 mg., 0.00033 mole) in 95% ethyl alcohol (11 ml.) was heated at reflux temperature for 15 hr. During this time (overnight) the suspension apparently bumped rather vigorously, because a large amount of the material had been lost through the top of the condenser and was on the outside of the condenser, flask, and heating mantle. The product which remained inside the apparatus was isolated as follows. The suspension was diluted with ethyl alcohol and filtered hot to remove the catalyst. The filtrate was concentrated to a volume of less than 1 ml. and set aside to allow crystallization to occur. The product weighed 20 mg. (7.1%); m.p. 244–246° (red melt). Normally, the yield of II would be expected to be 60–65% since this yield of 2-methylestradiol is obtained by the hydrogenolysis of 2-diethylaminomethylestradiol under the same reaction conditions.[1] However, this expectation remains unconfirmed, because the rarity of the starting material prevented the repetition of this experiment. Further recrystallization from ethyl alcohol gave 11 mg. of analytically pure II, m.p. 249–251° (red melt); λ_{max}^{KBr} 2.99 μ (OH at C_3), 5.77 μ (carbonyl at C_{17}), and 7.15 μ (free methylene at C_{16}).

Anal. Calcd. for $C_{19}H_{20}O_2$: C, 81.40; H, 7.19. Found: C, 81.62; H, 7.30.

1-Morpholinomethyl-2-naphthol. This was synthesized by a known procedure to give a product melting at 115° (lit.[11] m.p. 115–116°).

Acknowledgment. The author wishes to acknowledge a gift of equilenin generously supplied by Dr. W. L. Glen of Ayerst, McKenna & Harrison, Ltd., Montreal, Canada.

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(8) J. Decombe, *Compt. rend.*, **197**, 258 (1933).

(9) F. F. Blicke, *Org. Reactions*, **1**, 303 (1942).

(10) Melting points were taken on a Fisher-Johns block and are uncorrected. The microanalyses were done by Dr. Carl Tie'cke, Laboratory of Microchemistry, Teaneck, N. J. A Beckman Model DU spectrophotometer with a photomultiplier attachment was used to obtain the ultraviolet spectra. The infrared spectra were recorded by a Perkin-Elmer Model 21 infrared spectrophotometer using a sodium chloride prism.

(11) R. L. Shriner, G. F. Grillot, and W. O. Teeters, *J. Am. Chem. Soc.*, **68**, 946 (1946).