methylphenol, 4-ethylphenol, and 3-ethyl-5-methylphenol were kindly furnished by Dr. R. A. Friedel, U. S. Bureau of Mines, Bruceton, Pa.

2,4,6-Tribromophenol and 2,4,6-tribromo-3-methylphenol were prepared as described by Shriner and Fuson.[5]

The remaining phenols were commercially available grades and were used without further purification, except phenol and 1-naphthol which were redistilled.

The solvents Skellysolve B and benzene were redistilled, and ethyl alcohol and ethyl acetate were used as purchased.

The adsorbents used in preparing the chromatographic columns were silicic acid (Mallinckrodt, prepared by the method of Ramsey and Patterson), alumina (Aluminum Company of America, Grade F-20) and celite-535 (Johns-Manville). *p*-Phenylazobenzoyl chloride is manufactured by Distillation Product Industries.

Preparation of aryl p-phenylazobenzoates. A mixture of acid chloride (approximately 0.1 g.), phenol (0.0003*M* excess), and 3 to 6 ml. of pyridine was refluxed gently for 4 hr. The reaction product was poured with stirring into ice and 50 ml. of 10% sodium carbonate solution. If the crude reaction product separated as a solid, it was filtered off and washed with water and dried. The crude product was dissolved in Skellysolve B or a mixture of Skellysolve B and benzene and chromatographed on a mixture of silicic acid–celite (2 to 1 by weight) on which the free acid was strongly adsorbed. The derivative was then recrystallized. Chromatography of the crude derivatives of the following phenols gave two colored bands with a colorless band in between: 2,3-dimethylphenol, 3,4-dimethylphenol, *o*-chlorophenol, *m*-chlorophenol, *p*-chlorophenol, *o*-bromophenol, *m*-bromophenol, *p*-bromophenol, *o*-nitrophenol, *m*-nitrophenol, and *p*-nitrophenol. Of these two bands the lower one gave sharp melting points and good analyses. When the reaction product separated out as an oil, it was extracted with ether. The ether extract was washed successively with water and a saturated sodium chloride solution, dried over sodium sulfate, and the ether was removed. The residue was chromatographed, then recrystallized. The red-colored esters crystallized from Skellysolve B or mixtures of Skellysolve B and benzene as crystalline solids or fine needles.

Chromatographic separations. A typical chromatographic separation of a mixture of two components was conducted as described below. A tube 20 mm. × 400 mm. was connected to a suction flask. A 50 to 50 mixture by volume of alumina and celite or a 2 to 1 mixture by weight of silicic acid and celite was prepared for use as the adsorbent. While tapping the sides of the tube with cork rings, the tube was filled with the adsorbent to a height of approximately 290 mm. Then full suction of the water aspirator was applied to the suction flask which caused the adsorption column to decrease to approximately 268 mm. in height. The adsorbent was then wetted with Skellysolve B. In order to obtain a suitable percolate rate it was necessary to apply full suction with silicic acid and celite but only partial suction was required for alumina and celite.

The mixture of esters (10 to 20 mg. of component) was dissolved in the minimum volume of warm Skellysolve B or solutions of benzene in Skellysolve B and was adsorbed on the column. The chromatogram was developed by passing Skellysolve B, then solutions of benzene in Skellysolve B, and finally solutions of ethyl acetate in Skellysolve B through the adsorbent. The adsorbent was dug out of the column by a long narrow spatula and eluted with absolute ethanol if the adsorbent was silicic acid–celite or 95% ethanol if it was alumina-celite. When a continuous band was obtained, the band was arbitrarily dug out in several sections. The pure components were obtained from the top and bottom sections whereas the intervening sections were mixtures. The eluents were concentrated, filtered into a tared flask, and the last traces of solvent removed *in vacuo* under a stream of nitrogen. Melting points of the residues were determined.

(5) R. C. Shriner and R. C. Fuson, *Identification of Organic Compounds*. John Wiley and Sons, Inc., New York, N. Y., 2nd ed., 1947, p. 174.

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Reduction with Hydroxylating the Organism, *Curvularia lunata*

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With the aim of obtaining a 14α-hydroxy derivative, 4,6-pregnadiene-17α,21-diol-3,11,20-trione[1] was incubated with *C. lunata*, an organism reported to hydroxylate certain steroids at C-7,[2] C-11[3] and C-14.[4] Paper chromatography showed two major spots absorbing ultraviolet light, of which only the less polar stained with triphenyltetrazolium reagent.[5] The more polar substance thus no longer had the dihydroxy acetone side chain.

Column chromatography permitted isolation of both compounds. The less polar was shown by infrared spectrum and melting point to be the starting material. The more polar substance, obtained in 20–25% yield was considered to be a 17,20,21-triol on the basis of the strong polarity and the altered staining chracteristics, since such reductions have been observed with other microorganisms.[6] The substance analyzed for 4,6-pregnadiene-17α,20ξ,21-triol-3,11-dione with methanol of crystallization, and had infrared and ultraviolet spectra and a molecular rotation change which agreed with the assigned structure.

Acetylation gave a diacetate confirming introduction of a new acylable hydroxyl group. The direction of the molecular rotation change, +556, supports the structure and demonstrates[6] that the transformation product is 4,6-pregnadiene-17α,-20β,21-triol-3,11-dione. The evidence thus confirms this conversion as a reduction by an organism

(1) V. R. Mattox, E. L. Woroch, G. A. Fleischer, and E. C. Kendall, *J. Biol. Chem.*, 197, 261 (1952).

(2) C. Meystre, E. Vischer, and A. Wettstein, *Helv. Chim. Acta*, **38**, 3816 (1955).

(3) G. M. Shull and D. A. Kita, *J. Am. Chem. Soc.*, **77**, 763 (1955).

(4) G. M. Shull, D. A. Kita, and J. W. Davisson, U. S. Patent 2,702,812 (1955).

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

(6) F. Carvajal, O. F. Vitale, M. J. Gentles, H. L. Herzog, and E. B. Hershberg, to be published.

known previously only to hydroxylate or epoxidize steroids.[7]

EXPERIMENTAL[8]

Preparation of 4,6-pregnadiene-17α,20β-21-triol-3,11-dione. The organism used in this study was a strain of *Curvularia lunata* (NRRL No. 2380), maintained in this laboratory for more than a year on Sabouraud agar slants at 28–30° for 14 to 20 days at each culture generation. In the conversion studies subcultures were prepared from well sporulated slant cultures, 7 to 14 days old. Such a culture was washed with 5–10 ml. of Sabouraud liquid medium and used for inoculation of Erlenmeyer flasks filled with 100 ml. of the following medium: proteose peptone No. 3 (Difco), 0.5 g.; cerelose, 2 g.; soybean oil meal, 0.5 g.; potassium dihydrogen phosphate, 0.5 g.; sodium chloride, 0.5 g.; yeast extract (Difco), 0.3 g.; tap water, 100 ml. The medium had *p*H 5.6 and was sterilized for 15 min. in an autoclave. The inoculated flasks were shaken on a rotary shaker for 48 hr. at 28°. Samples of 25 mg. (1.1 g. total) of 4,6-pregnadiene-17α, 21-diol-3,11,20-trione were dissolved in 2 ml. of ethanol and added to the heavy black 48-hr. culture. After additional shaking for 48 hr., the whole culture was removed, blended with a knife-blade mixer and extracted three times with equal volumes of chloroform. The chloroform extracts were pooled and evaporated on a steam bath. The residue was analyzed by paper chromatography.

Chromatography of the residue on activated Florosil gave a fraction eluted with 25–50% methylene chloride in ether (140 mg.), purified by crystallization from methanol, m.p. 238–240°; λ_{max}^{MeOH} 281 mμ (ε = 24,000); λ_{max}^{Nujol} 2.87 μ, 2.93 (OH), 5.87 (11,20 C=O), 6.10 (3 C=O), 6.17, 6.29 ($\Delta^{4,6}$), identical with starting material.

The major amount of substance (250 mg.) was obtained by elution with 1.5–2% methanol in methylene chloride. Crystallization from methanol gave a methanol solvate of 4.6-pregnadiene-17α,20β,21-triol-3,11-dione, sinters 125°, m.p. 208–209°, $[\alpha]_D^{25}$ 126° (dioxane), λ_{max}^{MeOH} 282 mμ (ε = 22,800), λ_{max}^{Nujol} 2.95 μ, 3.08 (OH), 5.86 (11 C=O), 6.11 (3 C=O), 6.19, 6.28 ($\Delta^{4,6}$).

Anal. Calcd. for $C_{21}H_{28}O_5 \cdot CH_3OH$: C, 67.32; H, 8.22. Found: C, 67.58; H, 8.06.

The molecular rotation change from the starting material is −475°, agreeing in sign with changes which occur on reduction of a C-20 carbonyl to a hydroxyl group.[6] Analysis of the infrared spectrum showed the presence of three hydroxyl groups.

A separate run gave a polymorphic form, m.p. 204–205°, whose infrared spectrum in Nujol mull differed. When observed in bromoform solution, the spectrum was λ_{max} 2.72 μ, 2.81 (OH), 5.84 (11 C=O), 6.04 (3 C=O), 6.17, 6.25 ($\Delta^{4,6}$), identical with that of the other form.

4,6-Pregnadiene-17α,20β,21-triol-3,11-dione 20,21-diacetate. A sample of 100 mg. of the purified transformation product was treated with 3 ml. of acetic anhydride in 4 ml. of pyridine at room temperature for 18 hr. The solution was poured into dilute acid and extracted with methylene chloride. The residue from evaporation of the dried solution was crystallized from aqueous methanol to give a solvate, which, on drying *in vacuo*, had a melting point of 208.5–210°, $[\alpha]_D^{25}$ 237° (dioxane).

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.55; H, 7.26. Found: C, 67.34; H, 7.39.

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(7) G. M. Shull, 130th Meeting of the AMERICAN CHEMICAL SOCIETY, September, 1956, Atlantic City, N. J., reported that E. J. Agnello, *et al.*, had found a similar reduction as a by-product of the action of *C. lunata*.

(8) All melting points are corrected. Analyses and optical data were obtained by the Physical Chemistry and Microanalytical Departments of these laboratories. Interpretations of infrared spectra were performed by Dr. Jo-Yun Chen.

Synthesis of Spirolactams from Nitrocycloalkanes

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During an investigation of the Beckmann rearrangement of spiroketoximes,[1] it became necessary to prepare authentic samples of a series of spirolactams with the nitrogen atom bound directly to the spiro carbon. Two methods of preparation of such compounds have been reported; one is that of Lukes and Blaha,[2] who obtained 1-methyl-1-azaspiro[5,5]undecanone-2 in low yield by the action of the Grignard reagent of 1,5-dibromopentane on *N*-methylglutarimide. The other, which appeared to be more capable of extension to a variety of ring sizes, is the reduction of suitable nitroesters. Three foreign patents[3–5] and more recent work by Moffett[6] report the preparation of 1-azaspiro[4,5]decanone-2 by the hydrogenation of ethyl β-(1-nitrocyclohexyl)propionate.

In extending this second method, nitrocyclopentane and nitrocyclohexane were reacted with methyl or ethyl acrylate, using Triton B as the catalyst,[7] to yield the addition products I and V. Hydrogenation over Raney nickel at room temperature and subsequent cyclization by heating gave good yields of the spiropyrrolidones IX and XII.

To increase the size of the lactam ring, it was necessary to extend the length of the ester side chain. In one trial, the nitro-ester V was reduced with lithium aluminum hydride and the resulting amino alcohol converted to its *O*,*N*-di-*p*-toluenesulfonate. Heating this with potassium cyanide in an attempt to displace the *O*-tosylate gave no nitrile, however.

The chain lengthening was readily accomplished by the Arndt-Eistert homologation. Hydrolysis of the nitro-esters gave the corresponding acids, II and VI, which were converted successively to the

(1) R. K. Hill and R. T. Conley, *Chemistry and Industry*, 1314 (1956).
(2) R. Lukes and K. Blaha, *Chem. Listy*, 46, 726 (1952).
(3) Swiss patent 227,125 (1942).
(4) French patent 880,400 (1943); *Chem. Zentr.*, 114, 218 (1943 II).
(5) Dutch patent 57,433 (1946).
(6) R. B. Moffett, abstracts of papers, 130th Meeting, ACS, Atlantic City, N. J., September, 1956, 4N.
(7) H. A. Bruson, U. S. Patent 2,390,918; *Chem. Abstr.* 40, 2456 (1946).