been observed.[1] In the preceding communication[2] we have described the first 2β-hydroxylation of Compound S with *Streptomyces* sp. We wish now to report the microbiological hydroxylation of 4-pregnene-17α,21-diol-3,20-dione (I, Reichstein's Compound S) in the 1ξ and 2β positions by *Rhizoctonia ferrugena* (CBS, Holland). The fermentation of I (3.0 g.) by *R. ferrugena* was carried out in a medium containing corn steep liquor, ammonium dihydrogen phosphate, yeast extract, dextrose, soybean oil and calcium carbonate. In the fermentation stage a forty-eight hour culture was incubated with the steroid substrate (introduced in ethanolic solution) on a rotary shaker for five days at 23°. The culture filtrate was extracted with chloroform and chloroform–methanol (4:1), and the extracts were concentrated to dryness in vacuo below room temperature. The residue was redissolved in dry, acid and alcohol-free chloroform and the solution was chromatographed on a silicic acid adsorption column. A methanol–chloroform gradient elution[3] was utilized to separate the two steroidal transformation products and residual substrate. The steroid substrate, I, was collected in the first fractions, the 2β-hydroxylated product, II, in the middle fractions and the 1-hydroxylated product, III, in the last fractions. Homogeneity was indicated by paper chromatography.

Crystallization of the middle fractions from chloroform–ether mixture afforded 101 mg. of 4-pregnene-2β,17α,21-triol-3,20-dione (II), m.p. 214–220° dec. Further recrystallization from acetone–hexane raised the m.p. to 220–222° dec. The infrared spectrum of II was identical with that of an authentic sample,[2] and the mixed m.p. showed no depression.

The crystallization of the last group of fractions (Product III) afforded 163 mg. of crystalline solid, m.p. 180–200° dec. Cautious recrystallization from acetone–hexane without application of heat, raised the m.p. to 193–207° dec. (with a transition of about 170°), $[\alpha]^{25}\mathrm{D}$ +89 (dioxane) (corrected for acetone of solvation), $\lambda_{\max}^{\mathrm{MeOH}}$ 241 mμ (ϵ = 16,500 —corrected for acetone of solvation), $\lambda_{\max}^{\mathrm{Nujol}}$ 2.83 μ (OH), 5.81 μ (20-carbonyl), 6.04 μ (3-carbonyl), 6.18 μ (Δ^4) (one additional polymorphic variety has been observed.) Calcd. for $C_{21}H_{30}O_5 \cdot C_3H_6O$: C, 68.54; H, 8.63. Found: C, 68.41; H, 8.66.

The identity of III was established in the following manner. Integration of the hydroxyl bands of the infrared spectrum confirmed the presence of three hydroxyl groups. Measurement of the ultraviolet spectrum of III in alkaline solution[4] showed a shift in the maximum to 245 mμ but no peak in the 380 mμ region after two hours at 60°. This is consistent with the conversion of a 1-hydroxy-3-keto-Δ^4-steroid into a $\Delta^{1,4}$-diene-3-ketosteroid, which transformation would be expected to occur under these conditions. Also the ultraviolet spectrum of III was strikingly different from that of a 2-hydroxy-3-keto-Δ^4-steroid in alkaline solution.[2,4] The presence of the dienone among the products from the alkaline treatment of III was further substantiated by the measurement of the polarographic reduction potential of the solution.[5] A shift in the half-wave potential from 1.46 volts (III) to 1.31 volts (after alkaline treatment) corresponds well to the shifts observed for cortisone *vs.* prednisone (0.16 volt) and cortisol *vs.* prednisolone (0.17 volt). Furthermore, pyrolysis of a sample of III at the melting point for ten minutes afforded a mixture of products whose infrared spectrum contained the characteristic $\Delta^{1,4}$ diene-3-one bands.

Finally, a solution of III in glacial acetic acid was refluxed for one hour. Upon concentration of the solution *in vacuo*, chromatography of the residue on Florisil and recrystallization from acetone-hexane there was obtained 1,4-pregnadiene-17α,21-diol-3,20-dione, m.p. 237–240°, whose infrared spectrum was identical with that of an authentic sample.[6] From this evidence we conclude that the structure of III is clearly 4-pregnene-1ξ,17α,21-diol-3,20-dione. The configuration of the 1-hydroxyl group remains to be fixed.

With cortisol, cortisone, corticosterone, desoxycorticosterone, progesterone and testosterone transformations occur upon incubation in the presence of *R. ferrugena* as determined by paper chromatographic examination of the product. The structure of the transformation products in these instances remains to be elucidated.

Other species of *Rhizoctonia* have effected the transformation of I into cortisol, cortisone and 11-epicortisol; a more detailed account of these findings will be published subsequently. Additional conversion products obtained with organisms of this genus are now under investigation.

We wish to make the following acknowledgments. From the Schering Corporation we acknowledge the helpful advice of Drs. André Meyer and Alexander Nussbaum, the assistance of Dr. P. Kabasakalian and Mr. J. McGlotten who carried out the polarographic reductions, of Dr. J. Y. Chen who interpreted the infrared spectra, and of Mrs. M. J. Gentles. From the Institute of Microbiology we acknowledge the technical assistance of Mr. A. Chernov. We also wish to thank Dr. C. M. Haenseler for his helpful advice and for various cultures of *Rhizoctonia*.

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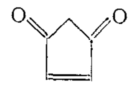
RECEIVED JUNE 3, 1957

CYCLOPENTENE-3,5-DIONE

Sir:

The synthesis of many substituted and unsaturated cyclopentane derivatives of theoretical and practical importance is hindered by the lack of readily available intermediates. With few exceptions, substituted cyclopentadienes are too unstable to be isolated, and these exceptions have not proved useful for further synthetic work.[1,2] We wish to report the synthesis of cyclopentene-3,5-dione (I),


I

a yellow, stable, crystalline, highly reactive molecule which promises to provide an entry into many new synthetic fields.

Cyclopentene-3,5-dione can be prepared easily from cyclopentadiene by bromination,[3] and treatment of the resultant 3,5-dibromocyclopentene with tetraethylammonium acetate in dry acetone.[4] Hydrolysis affords cyclopentene-3,5-diol which can be oxidized to I in 50% yield with chromic acid in acetic acid. After crystallization from ether–pentane and two sublimations it showed m.p. 36–37°. *Anal.* Calcd.: mol. wt., 96. Found: C, 62.3; H, 4.0; mol. wt., 96; ultraviolet: λ_{max} 222 (4.16), 322 (1.30), 367 (1.30); infrared: 5.73, 5.83.

We shall report on the details of several of its reactions later, but the following will indicate their scope. (1) It is readily reduced to 1,3-cyclopentanedione (m.p. 150–151°, reported[5] 151–152°) and this sequence may provide the best synthesis of this useful compound. (2) The enedione is highly reactive in the Diels–Alder reaction, and readily gives adducts with cyclopentadiene, anthracene and other dienes. (However, it is *less* reactive than maleic anhydride. We consider this difference significant, and will discuss it in due course.) (3) Cyclopentene-3,5-dione condenses rapidly with aldehydes and ketones in the presence of weak bases. These and other reactions are being explored.

The dione is an acid (*p*H of a 0.1 *N* aqueous solution, 4.5) which is rapidly destroyed by base. Despite its acidity, the molecule appears to be completely ketonic. It does not give a ferric chloride test. In its enolic form it would be a derivative of cyclopentadienone and as such perhaps partake of the unfavorable electronics which have been suggested to explain the lack of existence of that molecule. In addition to its interest for its own sake, we believe that this unique molecule, containing as it does three different types of functional groups and with each of its carbon atoms potentially reactive, will be a useful intermediate in the synthesis of many hitherto unavailable molecules.

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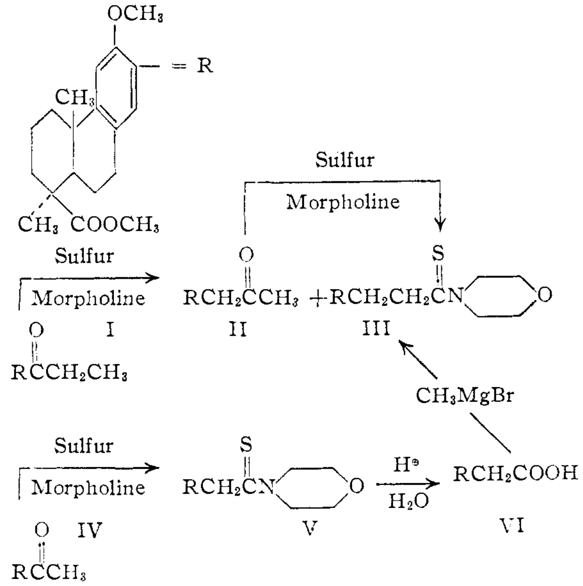
A NEW TYPE OF PRODUCT FROM THE WILLGERODT REACTION

Sir:

We wish to report the migration of a keto group to an adjacent carbon atom during a Willgerodt reaction. Methyl O-methyl-7-propionylpodocarpate (I) under Willgerodt conditions is converted (10% yield) to methyl O-methyl-7-acetonylpodocarpate (II). Since this new ketone is also converted to the thiomorpholide (III) under Willgerodt conditions, it is probable that at least part of the material goes through II as an intermediate.



The reaction of methyl O-methylpodocarpate[1] and propionyl chloride in the presence of aluminum chloride gave methyl O-methyl-7-propionylpodocarpate [I, m.p. 103.5–105°, λ max 255 mμ (ϵ = 10,500)[2] and 315 mμ (ϵ = 4,090), 5.82 and 5.98 μ μ($CHCl_3$); $[\alpha]^{25}_D$ +134° (ethanol); calcd. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 74.04; H, 8.54]. Chromatography of I on silica under conditions which were shown to separate I and II failed to reveal the presence of any II.

A mixture of methyl O-methyl-7-propionylpodocarpate (107.5 g.), sulfur (14.4 g.), and morpholine (39.2 g.) was heated at reflux for 10 hr. The reaction mixture was dissolved in benzene, filtered, and the solvent was removed. The expected thiomorpholide, (III, 85.6 g., m.p. 172–177°) crystallized when an ether solution was concentrated. Further recrystallization from a mixture of chloroform and methanol gave the pure thiomorpholide [m.p. 177–178.5, λ max 281 μ (ϵ = 18,000), $[\alpha]^{25}_D$ +110° (chloroform); calcd. for $C_{26}H_{37}NO_4S$: N, 3.05; S, 6.98; OCH_3, 13.50. Found: N, 3.08; S, 6.94; OCH_3 13.02]. The material in the mother liquors from this reaction was chromatographed over silica (1700 g.). Among the compounds eluted with 2% ethyl acetate in benzene was methyl O-methyl-7-acetonylpodocarpate (II) which crystallized from aqueous methanol as

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