observation that the acetoxymercurial I can be regenerated by refluxing with mercuric acetate; it is known that "the general equilibrium between mixed and simple organometallic compounds is observable with mercurials: $2RHgX \rightleftarrows R_2Hg + HgX_2$."[5]

The scope and limitations of the addition of a mercurated olefin to another olefin has not been defined, except that no addition product was isolated in a room temperature reaction (dioxane, 2 days) of I with cholesteryl acetate or with cholestenone. However, mercuration of cholesterol with mercuric acetate itself proceeds in fairly low yield even at elevated temperatures, the product being isolated not as the acetoxymercurial, but as 6-chloromercuricholesterol.[6,7] No acetoxymercuri-compound was isolable when a dioxane solution of mercuric acetate was allowed to stand five days with cholesteryl acetate or cholestenone.

Experimental

Isolation of Bis-[5,6-bis-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-*p*-dioxanylmethyl]-mercury (IV) from Mercuration of 3-Allyl-1,2;5,6-diisopropylidene-D-mannitol.—Mercuration of 3-allyl-1,2;5,6-diisopropylidene-D-mannitol (II) with mercuric acetate under anhydrous conditions has been shown to give a crystalline mercurated derivative (I).[4] Evaporation of the mother liquors left a sirup which was partitioned between ether and water; the ether-soluble fraction deposited 2.0 g. (1%) of crude IV on very slow evaporation of an ether–methanol solution. Recrystallization from methanol afforded fine, white glistening needles, m.p. 152–152.2°, $[\alpha]^{25}D$ +27° (chf.).

Anal. Calcd. for $C_{30}H_{50}O_{12}Hg$ (803.31): Hg, 24.97. Found: Hg, 25.48, 24.75.

The infrared spectrum was consistent with the proposed structure and was very similar to that of the iodomercury compound[4] corresponding to I. Attempted molecular weight determination by the Rast method would not give consistent results with any solvent; the compound seemed to decompose.

Preparation of IV by Addition of I to II.—A solution of 2.98 g. (5.3 millimoles) of 2-acetoxymercurimethyl-5,6-bis-(2,2-dimethyl-1,3-dioxolan-4-yl)-*p*-dioxane (I), m.p. 110.5–111.5° and 1.60 g. (5.3 millimoles) of 3-allyl-1,2;5,6-diisopropylidene-D-mannitol (II), $n^{23}D$ 1.4577, in 30 cc. of dioxane was vigorously shaken at room temperature for 21 hours. After the solvent was removed at reduced pressure, the residue was taken up in ether, filtered and diluted with methanol. The concentrated solution deposited a small amount of low melting (51–56°) white solid before very slowly yielding the diorganomercury addition product IV. Spontaneous evaporation over several days afforded 0.55 g. (13%) of white needles, m.p. 142–147°, which melted at 150–152° after one crystallization from methanol; mixed melting point and comparison of infrared spectra established its identity with the diorganomercury compound isolated from the mercuration mother liquors. After an additional 0.31 g. (7.3%) of crude product was obtained from the mother liquors, the solvent was removed and the residue taken up in isopropyl ether. Seeding with I encouraged the crystallization of 0.69 g. of starting acetoxymercurial (I), m.p. 107–110°. The yield of addition product based on unrecovered I (conversion yield) is therefore 26% (17% pure and 9.5% crude). An added trace of acetic acid had no effect on the addition reaction.

When the same procedure was followed with larger quantities of I and II,[8] except that a small volume of ether was used on the crude residue, the more ether-soluble IV dissolved, whereas starting I did not dissolve and was recovered on filtration. By recycling recovered I four times with fresh II, a total yield based on I of 51% (69% conversion yield) of crude product was obtained; two crystallizations from methanol gave an 85% recovery of pure IV, m.p. 150–152°.

Conversion of IV to I.—When 1.00 g. of IV was refluxed for two hours with 0.40 g. (equimolar amount) of mercuric acetate in 15 ml. of methanol, 1.12 g. of pure I, m.p. 111–113° plus 0.14 g. of slightly impure I, m.p. 107–109°, was isolated. This represents an 80% yield of pure I (90% total yield) based on reaction of *both* halves of IV.

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Microbiological Converison of Steroids. I. Introduction of the 11β-Hydroxyl Group into C_{21} Steroids

BY G. M. SHULL AND D. A. KITA

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The introduction of hydroxyl groups into the steroid nucleus by microörganisms has been reported in several papers; however, only two examples of 11β-hydroxylation have appeared.[1] This type of hydroxylation is of particular interest in the synthesis of adrenal cortical hormones such as 17α-hydroxycorticosterone (hydrocortisone) and corticosterone. We wish to report the introduction of the 11β-hydroxyl group into several C_{21} steroids by means of the fungus *Curvularia lunata*.[2]

Experimental

Curvularia lunata[3] was grown for two days in shake flasks at 28° on a malt extract–sucrose–salts medium.[4] One hundred ml. of the resulting growth was used to inoculate 2000 ml. of the same medium contained in a fermenter equipped with a submerged aerator. The inoculated medium was agitated at 1700 r.p.m. and aerated at the rate of 0.5 volume of air per volume of medium per minute for 22 hours in a 28° water-bath. Fifty grams of damp mycelium (12.5 g. dry weight), obtained by filtration of the resulting broth, was added to 2000 ml. of water and 0.5 g. of steroid contained in a similar fermenter. After the mycelium-steroid suspension had been agitated (in the manner described above) for the period indicated, it was extracted with chloroform. The extract was qualitatively examined by means of paper chromatography to determine the number of transformation products. The solvent system described by Zaffaroni and Burton[5] and modifications of those described by Bush[6] were used in the paper chromatography. The compounds discussed below were isolated from the extract by silica gel partition chromatography in which ethanol was the stationary phase and methylene chloride the mobile phase.

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17α-Hydroxycorticosterone.—One gram of 11-desoxy-17α-hydroxycorticosterone (Reichstein's Compound S) was incubated with a mycelium suspension for 16 hours. The effluent fractions from the silica gel column, which contained a compound with the same mobility on paper chromatograms as 17α-hydroxycorticosterone were combined and evaporated to dryness. From a solution of the residue in ethyl acetate there were obtained 410 mg. of crystals, m.p. 208.5–209.5°, $[\alpha]^{25}D$ +165.7° (95% ethanol), $\lambda_{max.}^{KBr}$ 2.95 μ (OH), 5.85 μ (20-ketone); 6.08 and 6.20 μ (Δ⁴-3-ketone). (*Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.42; H, 8.49.) The m.p. and optical rotation are in good agreement with those reported in the literature for 17α-hydroxycorticosterone.[7] In addition to 17α-hydroxycorticosterone, paper chromatograms indicated the presence of three less polar and four more polar transformation products in the extract.

11β,17α-Dihydroxyprogesterone.—Incubation of 1.0 g. of 17α-hydroxyprogesterone for 6 hours with a suspension of *C. lunata* mycelium provided a chloroform extract which yielded, after silica gel partition chromatography, 340 mg. of crystals, m.p. 226–228°, $[\alpha]^{25}D$ +135.5° (acetone), $\lambda_{max.}^{KBr}$ 2.95 and 3.02 μ (OH), 5.94 μ (20-ketone), 6.09 and 6.20 μ (Δ⁴-3-ketone). (*Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 72.80; H, 8.73. Found: C, 73.25; H, 8.59.) The m.p. and optical rotation agree with those published for 11β,17α-dihydroxy-Δ⁴-pregnene-3,20-dione (21-desoxy compound F).[8] Additional evidence for the presence of an 11β-hydroxy group in the isolated steroid was provided by (1) the failure of the compound to form an acetate with acetic anhydride–pyridine under the usual conditions, and (2) the oxidation of the compound with chromic acid to yield a product,[9] m.p. 231–232.6°, $[\alpha]^{25}D$ +194° ($CHCl_3$). The properties of the compound agree with those reported for 17α-hydroxy-Δ⁴-pregnene-3,11,20-trione.[10] Paper chromatograms indicated that there were four products more polar and one product less polar than 11β,17α-dihydroxyprogesterone also present in the extract.

Corticosterone.—A four-hour fermentation of 11-desoxycorticosterone according to the procedure outlined above produced a conversion product with the same mobility on paper chromatograms as corticosterone. In addition, five other oxygenated products were in evidence on the chromatograms of the broth extract. After silica gel fractionation as in the previous experiments, the effluent fractions containing the compound with a mobility similar to that of corticosterone were combined to yield crystals, m.p. 178–180°, $[\alpha]^{25}D$ +210.5° (95% ethanol), $\lambda_{max.}^{KBr}$ 2.96 μ (OH), 5.93 μ (20-ketone), 6.10 and 6.20 μ (Δ⁴-3-ketone). (*Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.34; H, 8.61.) The properties of the isolated compound agree with those reported for corticosterone.[11]

11β-Hydroxyprogesterone.—Paper chromatography of a six-hour fermentation of progesterone indicated the formation of 11β-hydroxyprogesterone and four other progesterone transformation products. Fractionation of the broth extract provided crystals, m.p. 185–187°, $[\alpha]^{25}D$ +213.6° (acetone), $\lambda_{max.}^{KBr}$ 2.94 μ (OH), 5.92 μ (20-ketone), 6.08 and 6.20 μ (Δ⁴-3-ketone). (*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.5. Found: C, 76.95; H, 9.13.) The analytical data on the isolated compound are in agreement with those published for 11β-hydroxyprogesterone.[12]

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The Electric Moments and Configurations of Some *cis–trans* Isomers

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Dipole moment studies have been used to prove the configurations of the *cis–trans* isomers of 11,12-dichloro-9,10-dihydro-9,10-ethanoanthracene, of 1,-5-dichloro-9,10-dihydro-9,10-anthradiol diacetate and of 1,2-dichloroacenaphthene. The electric moments of 1,8,10-trichloroanthracene and of *trans*-1,2-dibromoacenaphthene also have been determined to establish their structures.

In the course of a study of the mechanisms of elimination reactions, the *cis* and *trans* isomers of 11,12-dichloro-9,10-dihydro-9,10-ethanoanthracene were prepared by the Diels–Alder addition of anthracene and the isomeric 1,2-dichloroethylenes.[2] The structures of the products may be presumed from the Alder rule of *cis* addition.[3] However, the lower melting *trans* compound, which has the hydrogen and chlorine atoms *cis*, was found to react with sodium hydroxide in ethanol–dioxane to give the chloroölefin at a rate approximately eight times as fast as the higher melting *cis* isomer. In most other cases of second-order elimination which have been studied,[2,4] *trans* elimination is significantly[1] faster than *cis* elimination; as these compounds disobey the normal rule, it seemed desirable to have some physical data to substantiate the presumed structures. Therefore, the electric dipole moments of the isomers have been measured in benzene solution at 25°.

If the carbon–chlorine bond moment in aliphatic halides is taken to be 2.0 Debye units, and the bond angles for the bonds involving the bridge carbon atoms are assumed to have normal tetrahedral values, then the electric moments calculated for the *cis* and *trans* isomers are 3.77 and 1.89 *D*, respectively. The observed moments are 3.16 ± 0.12 and 2.17 ± 0.12 *D*, in fair agreement with the calculated values; the isomer with the higher dipole moment is the one assigned the *cis* configuration on the basis of the Alder rule. It is evident that the Alder rule leads to the correct assignment of configuration and the rates of elimination are anomalous.[2] The moment observed for the *cis* isomer is lower by 0.6 *D* than the calculated value—a lowering comparable to the difference between observed and calculated values for *o*-dichlorobenzene. This may indicate that there is some twisting about the 11–12 carbon–carbon bond so that the chlorine atoms are not directly opposed or it may result from interaction of the carbon–chlorine dipoles as in *o*-dichlorobenzene. A twist of 60° about

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