Ethyl *trans*-3-Phenylglycidate.—A solution of 8.8 g (0.05 mole) of distilled ethyl cinnamate was mixed with a solution of 13 g (0.064 mole) of *m*-chloroperbenzoic acid in 100 ml of methylene chloride and treated as has already been described, except that the product was passed rapidly through a column of alumina, using benzene as solvent. The product from this treatment contained a trace of impurity, shown by thin layer chromatography, but after solvent was removed and the epoxide was subjected to short-path distillation, only the signals associated with the glycidic ester were observed in the nmr spectrum. Yield, after chromatography and distillation, was 4.5 g (47%), bp 115° at 1.1 torr, $n^{25}D$ 1.5159.

***t*-Butyl *trans*-3-Phenylglycidate.**—Cinnamoyl chloride was prepared as a slightly yellow solid having bp 130° at aspirator pressure (about 14 torr) in 82% yield from *trans*-cinnamic acid and thionyl chloride. The acid chloride was then converted to the *t*-butyl ester, bp 117–119° at 2.7–3.0 torr, in 59% yield by following a published procedure.[22]

To 50 ml of methylene chloride containing 6.14 g (0.030 mole) of *t*-butyl *trans*-cinnamate was added a solution of 7.60 g (0.037 mole) of 85% *m*-chloroperbenzoic acid in 60 ml of methylene chloride. An additional 10 ml of solvent was used for rinsing. After 48 hr at reflux, when about 90% of the initial amount of peracid had disappeared, the mixture was washed, successively, with dilute solutions of sodium sulfite and sodium bicarbonate, water, then with saturated sodium chloride to break an emulsion. After the organic layer was dried over sodium sulfate, it was stripped of solvent at aspirator pressure, and distilled in a simple Claissen-type apparatus, bp 135–138° at 3.5 torr. The distillate was barely colored and solidified shortly after distillation. Thin layer chromatography with 50% (vv) hexane–benzene, indicated complete conversion to glycidic ester (R_f values were 0.38 for the cinnamate, 0.15 for the glycidate). A satisfactorily sharp melting point has not been obtained. The distillate, air dried, melted at 64–66° on a Kofler block with prior sintering. A sample which had been chromatographed over neutral alumina, then air dried, melted at 65–66°, again with prior sintering. The product showed a tendency to sublime when it was distilled in a short-path distillation apparatus, and sublimed crystals used for elemental analysis did not have an improved melting point.

Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 70.77; H, 7.21, 7.36.

(22) B. Abramovitch, J. C. Shiver, B. E. Hudson, and C. R. Hauser, *J. Am. Chem. Soc.*, **65**, 986 (1943).

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Microbiological Dehydrogenation of Racemic 13β-Alkylgonanes[1a]

GEORGE GREENSPAN, LELAND L. SMITH,[1b] RICHARD REES, THEODORE FOELL, AND HARVEY E. ALBURN

Research Division, Wyeth Laboratories, Inc., Radnor, Pennsylvania

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Microbiological dehydrogenations of racemic 13β-ethyl- and 13β-propyl-17β-hydroxygon-4-en-3-one with *Corynebacterium simplex* and with *C. hoagii* and of racemic 13β-ethyl-3-methoxygona-1,3,5(10)-trien-17β-ol with *C. simplex* and with *Flavobacterium dehydrogenans* gave *d* and *l*, as well as racemic, transformation products.

In an earlier paper[1a] we detailed our experience in the preparation of some 13β-alkylgonane derivatives of unnatural configuration *via* microbiological means. The present report deals with an extension of this work in the preparation of 13β-alkylgonane derivatives primarily of natural *d* configuration.[2]

Microbiological oxidation–reduction reactions at C-17 have been successfully utilized in select cases for resolution of racemic steroids obtained by total synthesis,[3] and a variety of microorganisms are available for such transformations.[4]

Our study of oxidation–reduction reactions of the C-17 ketone–alcohol system generally did not afford satisfactory results. Reduction of the 17-ketone group of select racemic 13β-alkylgonan-17-ones (specifically IIa) by yeast did not occur, and bacterial oxidation of the 17β-hydroxy group of racemic IIIc was effected in poor yield. Although oxidation of IIIc by *Flavobacterium dehydrogenans* gave the 17β-ketone IIc, the product was racemic. The transformation with *Corynebacterium simplex* afforded in low yield a resolved 17-ketone IIc and unoxidized substrate. These poor results appeared to be a consequence of the additional steric hindrance of the 13β-ethyl group in comparison with estrane derivatives.

Our attention was diverted to microbiological dehydrogenation of the A ring of 13β-alkylgon-4-en-3-one derivatives in anticipation of better transformations. Such dehydrogenations of 19-nor steroids with *C. simplex*[5] or other bacteria[6] are accompanied by aromatization, and indeed *C. simplex* attack on racemic Ia gave a product mixture of two phenols, recognized as the 17-ketone IIa and the 17β-alcohol IIIa. Attack by *C. simplex* on the 13β-propyl derivative Ib gave mainly the phenol IIb with traces of IIIb.

Methylation of the 17-ketone IIa gave the 3-methyl ether IIc, which was reduced by sodium borohydride to the 17β-alcohol IIIc, which in turn was reduced by lithium metal and liquid ammonia and hydrolyzed to the Δ^4-3-ketone Ia. A similar sequence employing the 13β-propyl derivative IIb led to Ib.

(1) (a) Paper IX in the series "Totally Synthetic Steroid Hormones." For Part VIII, see L. L. Smith, G. Greenspan, R. Rees, and T. Foell, *J. Am. Chem. Soc.*, **88**, 3120 (1966). (b) To whom correspondence should be addressed: Department of Biochemistry and Nutrition, University of Texas Medical Branch, Galveston, Texas 77550.

(2) The nomenclature convention of L. F. Fieser and M. Fieser ["Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 336] is used throughout.

(3) (a) E. Vischer, J. Schmidlin, and A. Wettstein, *Experientia*, **12**, 50 (1956); (b) A. Wettstein, E. Vischer, and C. Meystre, U. S. Patent 2,844,513 (July 22, 1958); (c) W. S. Johnson, W. A. Vredenburgh, and J. E. Pike, *J. Am. Chem. Soc.*, **82**, 3409 (1960); (d) K. V. Yorka, W. L. Truett, and W. S. Johnson, *J. Org. Chem.*, **27**, 4580 (1962).

(4) See the recent reviews by T. H. Stoudt, *Advan. Appl. Microbiol.*, **2**, 183 (1960); S. H. Eppstein, P. D. Meister, H. C. Murray, and D. H. Peterson, *Vitamins Hormones*, **14**, 359 (1956).

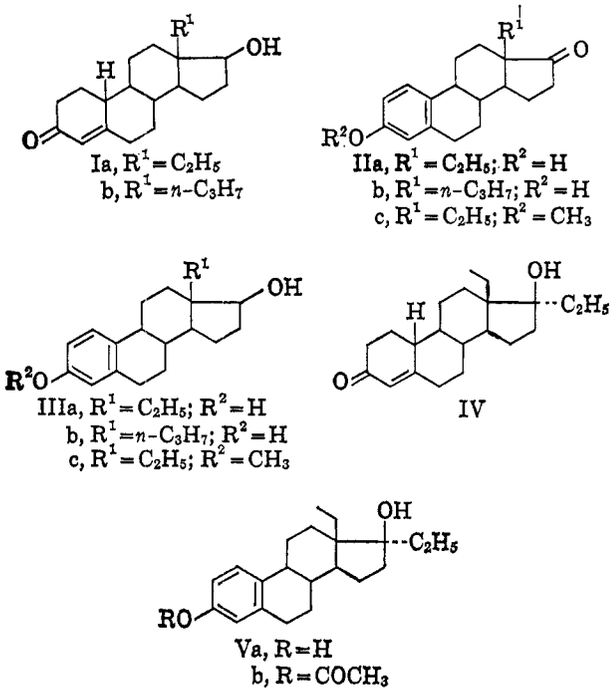
(5) S. Kushinsky, *J. Biol. Chem.*, **230**, 31 (1958); A. Bowers, C. Casas-Campillo, and C. Djerassi, *Tetrahedron*, **2**, 165 (1958); W. Charney, A. Nobile, C. Federbush, D. Sutter, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. J. Gentles, and E. B. Hershberg, *ibid.*, **18**, 591 (1962); A. Bowers, J. S. Mills, C. Casas-Campillo, and C. Djerzssi, *J. Org. Chem.*, **27**, 361 (1962).

(6) (a) H. R. Levy and P. Talalay, *J. Am. Chem. Soc.*, **79**, 2658 (1957); (b) C. Gual, R. I. Dorfman, and S. R. Stitch, *Biochim. Biophys. Acta*, **49**, 387 (1961); (c) D. H. Peterson, L. M. Reineke, H. C. Murray, and O. K. Sebek, *Chem. Ind.* (London), 1301 (1960).

The specific rotations of the several products involved in these chemical transformations were all positive, thus establishing their natural *d* configuration. Furthermore, optical rotatory dispersion spectra on the resolved Δ^4-3-ketones Ia and b were essentially superimposable on rotatory dispersion spectra of *d*-19-nortestosterone.[7]

Residual substrate isolated from fermentations of racemic Ia and b with *C. simplex* in each case was of negative specific rotation, thus affording *l* Ia and *l* Ib in the expected manner according to Vischer, *et al.*[3a]

CHART I



Dehydrogenation of racemic 13β,17α-diethyl-17β-hydroxygon-4-en-3-one (IV) with *C. simplex* gave the resolved phenol Va, of positive specific rotation, thus of the *d* series. In this case the residual substrate IV was but partially resolved, with a low negative specific rotation.

Dehydrogenation of racemic Ia with *Corynebacterium hoagii* gave two phenolic products recognized as IIa and IIIa by paper and thin layer chromatography. However, the 17-ketone IIa could not be isolated in a pure form.

The 3,17β-diol IIIa isolated from the *C. hoagii* fermentation broth had a negative specific rotation and was identified as *l*-13β-ethylgona-1,3,5(10)-triene-3,17β-diol by comparison with an authentic sample.[1a] No unaltered substrate was detected in *C. hoagii* fermentations.

Dehydrogenation of racemic IV with *C. hoagii* led to the racemic phenol Va and to recovery of racemic substrate. This observation and those above indicate that *C. hoagii*, in contradistinction to *C. simplex* (which dehydrogenates the *d* enantiomer stereoselectively), has an enzyme system incapable of stereoselectively dehydrogenating the A ring. While both cultures dehydrogenated the *d*-17β-alcohol group, forming the *d*-17-ketone, *C. hoagii* alone dehydrogenated the A ring of both *d* and *l* enantiomers. Where the *d*-17β-alcohol dehydrogenase could operate, it converted the *dl*-phenol IIa, produced by the A-ring dehydrogenase(s), to a mixture of *d*-17-ketone and *l*-17β-alcohol. Where the 17β-alcohol could not be dehydrogenated, as in IV, the racemic phenol Va resulted. (A minor, more polar phenolic product also resulted but was not isolated.)

Several modes of metabolism of *d* and *l* enantiomers have been noted in mammalian systems,[8] and there is some evidence that enzyme preparations from both mammalian[9] and microbial[3d] sources may operate on either *d* or *l* enantiomers. The present work, together with that already reported,[1a] represents the first clear indication that microbiological agents exist which transform both antipodes of complex polycyclic compounds.

Experimental Section[10]

***d*-13β-Ethyl-3-hydroxygona-1,3,5(10)-trien-17-one (IIa).** A. *Via C. simplex.*—A cell suspension of *C. simplex* (*Arthrobacter simplex*) ATCC 6946, prepared from surface growth on agar slants, provided the inoculum for 1-l. flasks containing 200 ml of medium composed of yeast extract, 1%, glucose, 1%, and distilled water. The bacterial cells were incubated on a reciprocating shaker at 28° for 26 hr and transferred (9% broth) to 2-l. flasks, each with 400 ml of the above medium. Flasks were shaken for 23 hr, and *dl*-13β-ethyl-17β-hydroxygon-4-en-3-one (Ia) in an ethanolic solution of 30 mg/ml was added, giving a concentration of 0.4 g/l. in each flask.

Incubation of the flasks was carried out on rotary shakers at 26 or 28° for 27 hr before harvesting of the 7.0 g of fermentation. Paper chromatograms of samples extracted with methyl isobutyl ketone and developed in the toluene–propylene glycol system disclosed two phenolic products, as determined by Turnbull's reagent,[13] plus unconverted substrate. The latter had an R_f similar to that of the less polar of the two products. The pooled broth was extracted with 9 l. of ethyl acetate, and the combined extracts were evaporated under vacuum, yielding 2.08 g of crude solids which thin layer chromatography showed to contain both 13β-ethyl-3-hydroxygona-1,3,5(10)-trien-17-one and 13β-ethylgona-1,3,5(10)-triene-3,17β-diol. Chromatography on 150 g of silica gel (prepared in benzene) gave 1.1926 g of crude 17-ketone (eluted with 4% ethyl acetate in benzene), $[\alpha]_D$ +93.3° (0.5% in ethyl acetate).

Recrystallization from chloroform gave the analytical sample: 886 mg; mp 252–253.5°, with phase transition at *ca.* 210°; $[\alpha]_D$ +108.5° (0.5% in ethyl acetate); λ_{max} 282 mμ (ϵ 2170), 287 mμ (ϵ 2060); λ_{max}^{KBr} 3.08, 5.85, 6.20, 6.33, 6.67 μ, etc. An additional 117 mg of pure product was recovered from the chloroform mother liquor.

Anal. Calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 79.95; H, 8.33.

In another fermentation of 6.72 g of racemic substrate, 2.7 g of crude solids were recovered, from which 1.9 g of the *d*-17-ketone IIa, mp 249–250° (containing only a trace of the 3,17β-

(7) See Figure 2 in paper VIII.[1a]

(8) S. Ulick, *J. Biol. Chem.*, **236**, 680 (1961); J. T. August, *Biochim. Biophys. Acta*, **48**, 203 (1961); J. A. Luetscher, A. Dowdy, W. Lew, and A. M. Callaghan, *Endocrinology*, **70**, 445 (1962).

(9) V. P. Hollander, N. Hollander, and J. D. Brown, *ibid.*, **64**, 621 (1959).

(10) All melting points were taken under microscopic magnification on a calibrated Kofler block. Optical rotations were determined in chloroform unless otherwise noted. Optical rotatory dispersion data were obtained on dioxane solutions. Ultraviolet absorption spectra were recorded on 95% ethanol solutions. Infrared spectra were obtained on pressed potassium bromide disks using a Perkin-Elmer Model 21 instrument. Thin layer chromatography was conducted on silica gel chromatoplates prepared with rice starch.[11] All *d* derivatives described in the present report were compared with their respective *l* and *dl* analogs, wherever possible, by infrared spectroscopy and thin layer chromatography. A comparison of physical data for the *d*, *l*, and *dl* derivatives is given in paper II of this series.[12]

(11) L. L. Smith and T. Foell, *J. Chromatog.*, **9**, 339 (1962).

(12) H. Smith, *et al.*, *J. Chem. Soc.*, 4472 (1964).

(13) G. M. Barton, R. S. Evans, and J. A. F. Gardner, *Nature*, **170**, 249 (1952).

diol IIIa by thin layer chromatography), was obtained by recrystallization from methanol–chloroform. The mother liquors from the two fermentations were combined, partitioned between water and hexane, and the solids (5.4 g) obtained from chloroform extracts of the water layer were chromatographed on silica gel. Elution with 5% ethyl acetate in benzene gave 450 mg of *d* IIa, mp 252–253° from methanol: $[\alpha]_D$ +117.5° (0.5% in ethyl acetate); identical with the previously prepared sample by infrared spectral and thin layer chromatographic comparisons.

B. *Via C. hoagii*.—The fermentation medium and procedures employed with *C. hoagii* ATCC 7005 were similar to those described for *C. simplex*, except that racemic Ia was added at a concentration of 0.8 g/l. for the fermentation of 1.89 g of steroid substrate. The broth, harvested after 28 hr. of contact at 28° on a rotary shaker, was shown by paper chromatography to contain two phenolic products and no residual substrate. The broth, when processed in the usual manner, yielded 1.5 g of crude product. Chromatography on silica gel (elution with 5% ethyl acetate in benzene) afforded 200 mg of the 17-ketone IIa: mp 220–245°; $[\alpha]_D$ +92.6° (0.5% in ethyl acetate). The sublimed sample, mp 220–242°, $[\alpha]_D$ +98.8° (0.5% in ethyl acetate), λ_{max} 278 mμ (ε 2410), 287 mμ (ε 2026), analyzed satisfactorily, but the increased ultraviolet light absorption in the 270- to 280-mμ region (λ_{max} for the 17-ketone should be 282, not 278 mμ) and the minor differences in the infrared spectra, as well as the poor melting point, indicated the presence of a small amount of impurity not readily separated from the major product.

Anal. Calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.45; H, 8.52.

Reduction of 110 mg of the above product in 12 ml of methanol with 30 mg of sodium borohydride (36 hr) afforded the reduced product, which was sublimed under vacuum: mp 100° (phase change), 175–186°; λ_{max} 278 mμ (ε 2222), 287 mμ (ε 1825); identified as IIIa by infrared spectroscopy. Thin layer chromatography of this product showed clearly the presence of a minor, more polar component.

***d*-13β-Ethylgona-1,3,5(10)-triene-3,17β-diol (IIIa).**—Further elution of the silica gel column from which *d*-IIa had been removed (under A above) with 5% ethyl acetate in benzene gave 190 mg of IIIa, homogeneous by thin layer chromatography. Recrystallization from chloroform–ethyl acetate gave the product as an ethyl acetate solvate: mp 183–185°, λ_{max}^{KBr} 5.78 μ. Sublimation at 210° gave the unsolvated product: mp 106 and 187–189°; $[\alpha]_D$ +58.5°; λ_{max} 281 mμ (ε 2050), 288 mμ (ε 1885); λ_{max}^{KBr} 3.07, 6.21, 6.32, 6.67, 6.93, 8.01, 8.20, 9.53 μ, etc.

Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.50; H, 9.31.

***l*-13β-Ethylgona-1,3,5(10)-triene-3,17β-diol (IIIa).**—Further elution of the column from B above with 5% ethyl acetate in benzene gave 420 mg of mixed IIa and IIIa fractions (not further examined) and 167 mg of *l* IIIa. Vacuum sublimation gave pure *l* IIIa: mp 186–188°; $[\alpha]_D$ −55.2°; λ_{max} 280 mμ (ε 2120), 287 mμ (ε 1930); identified by infrared spectroscopy and thin layer chromatography as the 3,17β-diol IIIa.

Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.59; H, 8.95.

***l*-13β-Ethyl-17β-hydroxygon-4-en-3-one (Ia).**—Still further elution of the column from A above with 5% ethyl acetate in benzene afforded 1.6 g of unaltered substrate, which was recrystallized from chloroform–ethyl acetate, yielding 1.0 g of pure *l* Ia: mp 154–156°; $[\alpha]_D$ −55.2°; λ_{max} 240 mμ (ε 17,446); identical with the previously prepared *l* Ia by infrared spectral and thin layer chromatographic comparisons.

***d*-3-Hydroxy-13β-propylgona-1,3,5(10)-trien-17-one (IIb).**—Fermentation of racemic Ib by *C. simplex* was carried out under the conditions described above for this organism, with minor variations. After 19 hr of cell growth in the second stage, the steroid was added to the flasks as a 50 mg/ml of solution in ethanol, giving a concentration of 0.3 g/l. A total of 7.35 g was fermented for 26 hr. Paper chromatography revealed two phenolic products, the larger of which had an R_f similar to that of the remaining substrate. The broth was extracted three times with 10 l. of ethyl acetate, and the extracts were evaporated under reduced pressure to *ca.* 40 ml, affording 1.13 g of precipitate. Analysis by thin layer chromatography showed a mixture of IIIb and IIb. Column chromatography on 200 g of silica gel (elution with 1% ethyl acetate in benzene) gave 1.04 g of *d* IIb, which, after recrystallization from methanol–chloroform and sublimation afforded an analytical sample: mp 87–99°, resolidifying by 165°, remelting at 187–188°;[14] $[\alpha]_D$ +116.5°; λ_{max} 281 mμ (ε 1770), 287 mμ (ε 1630); λ_{max}^{KBr} 3.06, 5.80, 6.22, 6.66, 6.93, 8.48 μ, etc.

Anal. Calcd for $C_{20}H_{26}O_2$: C, 80.49; H, 8.78. Found: C, 80.41; H, 8.50.

Further elution of the column with 1% ethyl acetate in benzene gave mixed fractions of IIb and IIIb.

***l*-17β-Hydroxy-13β-propylgon-4-en-3-one (Ib).**—Still further elution of the column with 2.5–5% ethyl acetate in benzene gave 1.76 g of *l*-Ib, which was recrystallized from benzene–petroleum ether (bp 38–65°) and from acetone several times, yielding crystals with mp 140–142°, $[\alpha]_D$ −64.8°, identified by thin layer chromatographic and infrared spectral comparisons with an authentic sample.

***d*-13β,17α-Diethylgona-1,3,5(10)-triene-3,17β-diol (Va).**—The transformation of 5.0 g of racemic IV by *C. simplex* was effected in a medium (pH 6.4) composed of yeast extract, 0.05%; peptone, 0.05%; and distilled water, 100 ml. 19-Nortestosterone, 2.5 mg/ml of solution in ethanol, was added as enzyme inducer to flasks 18 hr old at a concentration of 10 mg/l. The 2-l. flasks were incubated for 2 hr on rotary shakers at 26 or 28°; then the steroid substrate, dissolved in ethanol at 40 mg/ml, was added to give a concentration of 0.35 g/l.

After 49 hr on the shakers, the broth was harvested and extracted with ethyl acetate. The extracts were concentrated, and the crystalline product thus obtained was filtered off, yielding 1.1 g of material shown to be a mixture of unaltered substrate and 13β,17α-diethylgona-1,3,5(10)-triene-3,17β-diol by thin layer chromatography. The material in the mother liquor was similarly shown to be a mixture of the two compounds. The solids and mother liquors were recombined and chromatographed on 400 g of silica gel. Elution with 10% ethyl acetate in benzene gave 650 mg of Va. Recrystallization from ether gave the analytical sample: mp 170–181°; $[\alpha]_D$ +27.4°; λ_{max} 281.5 mμ (ε 2000), 288.5 mμ (ε 1830); λ_{max}^{KBr} 2.81, 6.20, 6.66 μ, etc.

Anal. Calcd for $C_{21}H_{30}O_2 \cdot {}^1/_3H_2O$: C, 78.72; H, 9.65. Found: C, 78.38; H, 9.55.

Further elution of the column with the same solvent gave 1.0 g of mixed fractions and finally 990 mg of partially resolved IV, which, after recrystallization from ethyl acetate–hexane had mp 140–162°, $[\alpha]_D$ −6.5° (50% methanol in chloroform).

***d*-3-Acetoxy-13β,17α-diethylgona-1,3,5(10)-trien-17β-ol (Vb).**—A solution of 35 mg of *d* Va in 0.35 ml of pyridine and 0.30 ml of acetic anhydride was maintained at room temperature for 15 hr, after which the solvents were removed under vacuum. The residue was crystallized from acetone–hexane, yielding 28 mg of the 3-acetate Vb. Recrystallization from ether–pentane yielded the pure acetate: mp 129–131°; λ_{max} 217 mμ (ε 8390, sh), 269 (ε 785), 275.5 (ε 785); λ_{max}^{KBr} 2.80, 5.72, 6.23, 6.32, 6.70, 6.85 μ, etc.

Anal. Calcd for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.77; H, 9.05.

***dl*-13β,17α-Diethylgona-1,3,5(10)-triene-3,17β-diol (Va).**—A 7.177-g fermentation of racemic IV by *C. hoagii* in 1% yeast extract–dextrose medium was run in 1- and 2-l. flasks. A reciprocating shaker was utilized for the growth stages and rotary shakers for the conversion phase. Racemic IV was added to the flasks as previously described, and the mixtures were incubated at 26 or 28° for 47 hr. Papergrams developed in the toluene–propylene glycol system showed two phenolic components and three ultraviolet light-absorbing zones all more polar than the remaining substrate. The fermentation broth was extracted and processed in the usual manner, yielding a dark residue, which was chromatographed on 400 g of silica gel. Elution with 3% ethyl acetate in benzene gave 700 mg of a crystalline phenol, which was recrystallized three times from chloroform–methanol, yielding racemic Va: mp 170–193°; $[\alpha]_D$ ±0° (50% methanol in chloroform); λ_{max} 281 mμ (ε 2240), 287.5 mμ (ε 2040); λ_{max}^{KBr} 3.00, 3.20, 6.20, 6.29, 6.66, 6.90 μ, etc.

Anal. Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 79.22; H, 9.46.

Continued elution of the column with 4% ethyl acetate in benzene gave 400 mg of substrate, which after three recrystallizations from acetone–hexane yielded the purified racemic substrate: mp 143–145°; $[\alpha]_D$ ±0° (50% methanol in chloroform);

(14) G. Nominé, R. Bucourt, and C. Snozzi [U. S. Patent 3,075,970 (Jan 29, 1963)] described *d* IIb, mp 187°.

λ_{max} 242 mμ (ε 16,433); identified as racemic IV by infrared spectroscopy.

d-**13β-Ethyl-3-methoxygona-1,3,5(10)-trien-17-one (IIc).**—Flasks (12, 1 l.) containing 200 ml of a yeast extract (1%)–glucose (1%)–aqueous medium were each inoculated with washings from *C. simplex* growth on agar slants. The cells were incubated for 24 hr on a reciprocating shaker at 28°, transferred (10%) to 2-l. flasks containing 400 ml of the same medium, and incubated a further 24 hr. Racemic IIIc (4.28 g) was added as a 40 mg/ml of ethanol solution to each flask, making the final concentration 0.2 g./l. Fermentation progress was followed by paper chromatography of broth extracts on Whatman No. 1 filter paper irrigated with the heptane–formamide system. Phenolic zones were located by Turnbull's blue. After 120 hr the harvested broth was extracted with ethyl acetate in the usual manner. The concentrated extracts yielded 1.20 g of solids which were shown to be a mixture of IIc and IIIc by thin layer chromatography. Chromatography of the mixture on silica gel in benzene afforded the 17-ketone IIc. Recrystallization several times from acetone–hexane yielded the analytical sample: mp 147–149°; $[\alpha]_D$ +110.9°; λ_{max} 278 mμ (ε 2100), 286 mμ (ε 1990); $\lambda_{max}^{CHCl_3}$ 3.55, 5.80, 6.23, 6.37, 6.71 μ, etc. Further elution of the column gave mixed fractions of IIc and IIIc, 868 mg, not worked further.

Anal. Calcd for $C_{20}H_{26}O_2$: C, 80.49; H, 8.78. Found: C, 80.68; H, 8.67.

dl-**13β-Ethyl-3-methoxygona-1,3,5(10)-trien-17-one (IIc).**—Fermentation of racemic IIIc (4.7 g) by *Flavobacterium dehydrogenans* ATCC 13930 was carried out in 2-l. flasks with 400 ml of an aqueous medium containing yeast extract and dextrose, 1% each. Cells were first inoculated into 1-l. flasks containing 200 ml of the medium and incubated on a reciprocating shaker for 46 hr at 28°. Transfers (8%) were made to 2-l. flasks, which were shaken for 24 hr, and the steroid was added as an ethanolic solution, 40 mg/ml, to give a concentration of 0.4 g/l. The conversion phase (on rotary shakers at 26 or 28°) was discontinued at 96 hr. A single product, less polar than the substrate and positive to Turnbull's blue, was noted on papergrams; some substrate was also found.

Eight liters of broth was extracted five times with equal volumes of ethyl acetate, and the combined extracts were evaporated under vacuum, yielding a brown oil which crystallized from methanol. The crystals, 650 mg, were recrystallized several times from methanol, yielding 470 mg of colorless needles: mp 124–126° (mp of racemic IIc, 126–127.5°; mmp, 124–125°); $[\alpha]_D$ ±0°; λ_{max} 278 mμ (ε 1623), 287.5 mμ (ε 1597); further identified as the racemic steroid by infrared spectroscopy using potassium bromide disks. The mother liquor yielded additional 17-ketone by chromatography on alumina (Woelm activity I), elution with benzene and with 30% chloroform in benzene giving 570 mg of colorless needles, mp 127–128.5°, not depressed on admixture with the known racemic IIc. An additional 360 mg of material, mp 121–123°, was also recovered from later fractions.

d-**13β-Ethyl-17β-hydroxygon-4-en-3-one (Ia).**—A solution of 856 mg of *d*-IIa in 40 ml of boiling ethanol was treated with 7.2 ml of 60% sodium hydroxide solution and then with 10 ml of dimethyl sulfate. The addition sequence of alkali and dimethyl sulfate was repeated three times, after which the mixture was cooled, 100 ml of water added, and the mixture chilled in ice. The solids were filtered off and dried, affording 881 mg of *d* IIc. The product was dissolved in 10 ml of boiling ethanol and 100 mg of sodium borohydride was added. The reaction mixture was refluxed for 2 hr, an additional 20 mg of sodium borohydride was added, and reflux was continued for 1 hr. The solution was cooled and 1.0 ml of 50% aqueous acetic acid was added, followed by 8 ml of water. Ethanol was removed under vacuum and the crystals were filtered off, yielding 852 mg of IIIc, which was dissolved in 21.5 ml of 1-methoxy-2-propanol and added to 135 ml of liquid ammonia (distilled from lithium metal) and 34 ml of 1-methoxy-2-propanol. Lithium metal (0.87 g) was added in small pieces, with vigorous stirring. After 30 min the ammonia was allowed to evaporate and 108 ml of water was added. The mixture was cooled and filtered, yielding 900 mg of product. Chromatography on silica gel (elution with 5% ethyl acetate in benzene) gave the intermediate 13β-ethyl-3-methoxygona-2,5(10)-dien-17β-ol, which was slurried in 15 ml of methanol and 0.65 ml of water. Concentrated hydrochloric acid (1 ml) was added (under nitrogen), and the mixture was stirred for 1 hr and neutralized with sodium bicarbonate. The solids were filtered off and chromatographed on silica gel (elution with 10% ethyl acetate in benzene), yielding 148 mg of crystalline Ia. The product was recrystallized twice from ether–hexane, giving the pure Ia: mp 151–158°; $[\alpha]_D$ +52.4°; λ_{max} 240.5 mμ (ε 17,200); λ_{max}^{KBr} 3.01, 6.02, 6.17 μ, etc.

Anal. Calcd for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 78.82; H, 9.61.

Optical rotatory dispersion spectra of Ia in dioxane over the range 265–400 mμ are $[\alpha]_{400}$ −17.3°, $[\alpha]_{363}$ −462°, $[\alpha]_{350}$ −505°, $[\alpha]_{335}$ +220°, $[\alpha]_{325}$ +1135°, $[\alpha]_{313}$ +1750°, $[\alpha]_{300}$ +1970°, $[\alpha]_{265}$ +2760°.

d-**13β-Propylgona-1,3,5(10)-triene-3,17β-diol (IIIb).**—A solution of 200 mg of *d* IIb in 2.3 ml of absolute ethanol containing 23 mg of sodium borohydride was refluxed for 1 hr, cooled, and 0.3 ml of 50% aqueous acetic acid was added. Water (2 ml) was added and the solvents were removed under vacuum. The aqueous mixture was extracted with chloroform concentrated under vacuum, and chilled. Filtration yielded 135 mg of crystalline product, which was recrystallized from acetone, giving an acetone solvate: mp 109–113°; $[\alpha]_D$ +58.1° (ethanol);[15] λ_{max} 281 mμ (ε 2280), 288 mμ (ε 2060); λ_{max}^{KBr} 2.95, 3.12, 5.85, 6.22, 6.66, 6.90 μ, etc.

Anal. Calcd for $C_{20}H_{28}O_2 \cdot C_3H_6O$: C, 77.05; H, 9.56. Found: C, 77.36, 77.43; H, 9.37, 9.40.

d-**17β-Hydroxy-13β-propylgon-4-en-3-one (Ib).**—A solution of 1.62 g of *d*-IIb in 52 ml of refluxing ethanol was treated with 7.2 ml of 60% sodium hydroxide solution, then with 10 ml of dimethyl sulfate. The alkali–dimethyl sulfate addition sequence was repeated four times, after which the solution was cooled and 162 ml of water added. The mixture was extracted with chloroform, the chloroform extracts were evaporated, and the residue was crystallized from methanol, yielding 1.51 g of the 3-methyl ether of IIb, homogeneous on thin layer chromatograms. The methyl ether product was dissolved in 18 ml of ethanol, 180 mg of sodium borohydride was added, and the mixture was refluxed for 2 hr. An additional 40 mg of sodium borohydride was added and reflux was continued for 1 hr. To the cooled reaction mixture was added 2 ml of 50% aqueous acetic acid and 15 ml of water. The solvents were removed under vacuum, affording the 3-methyl ether of IIIb as a gum, homogeneous on thin layer chromatograms. The gum was dissolved in 21 ml of redistilled tetrahydrofuran and added to 36 ml of liquid ammonia (distilled over lithium metal). Lithium metal (0.65 g) was added in small pieces over 30 min, the reaction mixture was stirred for 4 hr, and 7.7 ml of absolute ethanol added (dropwise), followed by 14.4 g of solid ammonium chloride. The ammonia was boiled off, the product was extracted from the residue with chloroform, and the chloroform was evaporated yielding 1.51 g of the crude intermediate 13β-propyl-3-methoxygona-2,5(10)-dien-17β-ol. This product was slurried in 29 ml of methanol, and (under nitrogen) a mixture of 1.3 ml of water and 1.98 ml of concentrated hydrochloric acid was added. The mixture was stirred under nitrogen for 2 hr, neutralized with sodium bicarbonate, and filtered. Chromatography on silica gel (elution with 5% ethyl acetate in benzene) afforded 405 mg of Ib: mp 162–165°; $[\alpha]_D$ +69.0°; λ_{max} 240.5 mμ (ε 17,150);[16] λ_{max}^{KBr} 2.92, 6.01, 6.19 μ, etc.

Anal. Calcd for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.43; H, 9.85.

Optical rotatory dispersion spectra of Ib in dioxane over the range 265–400 mμ are $[\alpha]_{400}$ +68°, $[\alpha]_{363}$ −345°, $[\alpha]_{357}$ −304°, $[\alpha]_{349}$ −413°, $[\alpha]_{335}$ +268°, $[\alpha]_{325}$ +1115°, $[\alpha]_{315}$ +1680°, $[\alpha]_{300}$ +2000°, $[\alpha]_{265}$ +3000°.

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(15) L. Velluz, G. Nominé, R. Bucourt, A. Pierdet, and P. Dufay [*Tetrahedron Letters*, **No. 8**, 127 (1961)] described IIIb as a methylene chloride solvate, mp 110°, $[\alpha]_D$ +57° (0.2% ethanol).

(16) G. Nominé, and R. Bucourt [in U. S. Patent 3,061,617 (Oct 30, 1962) and 3,074,978 (Jan 22, 1963)] described *d* Ib, mp 163°, $[\alpha]_D$ +64° (0.5% in methanol), λ_{max} 240–241 mμ (ε 16,100).