tween 200 ml each of water and chloroform. The layers were separated and the aqueous phase was extracted with three additional portions of chloroform. The chloroform layers were washed with water, dried, and evaporated to dryness *in vacuo* to yield 0.98 g of an oil which solidified. Thin layer chromatography in solvent D showed a number of spots with a major one at R_f 0.75.

Trituration of the oil with cold hexane gave 123 mg of product with mp 63–66°, $[\alpha]^{20}D$ −6° (*c* 0.26, chloroform). Thin layer chromatography in solvent D showed one spot at R_f 0.75: λ_{max}^{Nujol} 5.95 (carbonyl), 6.5 (amide II), 10.3 μ (weak, trans-disubstituted olefin).

Anal. Calcd for $C_{26}H_{43}NO_4$: C, 72.0; H, 10.0; N, 3.23. Found: C, 71.8; H, 10.2; N, 3.39.

2-Amino-D-*erythro*-octadecane-1,3-diol (12) (D-Dihydrosphingosine).—A solution of 105 mg (0.24 mmol) of 2-benzyloxycarbonylamino-D-*erythro*-Δ^4-octadecene-1,3-diol (**11**) in 2.7 ml of glacial acetic acid was hydrogenated at atmospheric pressure and room temperature using 68 mg of 10% palladium on charcoal for 20 hr. The hydrogenation mixture was filtered and the filtrate was evaporated to dryness *in vacuo* to yield 82 mg of product as a white solid which was characterized as the sulfate.[14] Recrystallization from glacial acetic acid gave crystals, mp 150° dec, λ_{max}^{Nujol} 6.5 μ (NH).

Anal. Calcd for $C_{36}H_{80}N_2O_8S$: C, 61.7; H, 11.5; N, 4.0. Found: C, 61.7; H, 11.3; N, 4.12.

Lesuk, *et al.*,[14] reported that the sulfate slowly darkened on heating and finally melted at 265° dec. Thus the melting point does not appear to be a reliable criterion for identification.

D-Dihydrosphinogosine Triacetate (13).—Acetylation of crude D-dihydrosphingosine using acetic anhydride in pyridine gave crystals, mp 90–93°, $[\alpha]^{19}D$ +16° (*c* 0.5 in chloroform), which had the same infrared spectrum as authentic dihydrosphingosine triacetate[8b] and which gave no melting point depression when a mixture melting point was determined with a sample of authentic dihydrosphingosine triacetate which had been prepared from commercially available D-sphingosine sulfate. D-dihydrosphingosine triacetate is reported to have mp 98°, $[\alpha]D$ +17° (*c* 1.4, chloroform).[15]

Registry No.—**2**, 25791-20-2; **4**, 25791-21-3; **6**, 25834-61-1; **7**, 25791-22-4; **8**-*cis*, 25791-23-5; **8**-*trans*, 25834-62-2; **11**, 25834-63-3; **12**, 764-22-7; **12** sulfate, 25791-25-7.

(14) A. Lesuk and R. J. Anderson, *J. Biol. Chem.*, **139**, 457 (1941).

(15) C. A. Grob, E. F. Jenny, and H. Utzinger, *Helv. Chim. Acta*, **34**, 2249 (1951).

12α-Etiojerva-1,4-diene-3,17-dione

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Microbiological oxidation of the 12α-pregnajervane **1f** produces the title compound **4a** whose structure was proven unambiguously by an alternate synthesis from the same starting material. This route involves successive Baeyer–Villiger oxidation, oxidation of the 3-hydroxyl group (→ **2e**, R′ = Ac), selenium dioxide oxidation of the A ring (→ **4e**, *R′* = Ac), saponification of the acetate, and oxidation of the resulting 17-hydroxyl group. The stereochemistry of the C-17 substituents is discussed.

Microbiological oxidation of saturated pregnanes has proved a useful method to prepare the corresponding A ring unsaturated derivatives.[1] Since analogous compounds in the 12α-etiojervane series were of interest for biological evaluation, the 12α-pregnajervane[2,3] (**1f**) (R = H) was submitted to bacterial oxidation. The chief product (~30%) was not the dienone **4f** although the desired A ring grouping was present (uv and nmr analysis). An overoxidation (Scheme I) of a type familiar in the pregnanes[1] had occurred, yielding a tetracycle in which the 17-acetyl group had been degraded to the 17 ketone (without epimerization at C-13). The gross structure **4a** was suggested by the presence of a saturated carbonyl band at 5.83 μ and the absence of the acetyl signal near 125 Hz.

Structural confirmation of the fermentation product was accomplished without difficulty since a similar compound, the 17β-hydroxy-1,4-diene (**4e**, R′ = H, 13α-CH_3) had been prepared earlier in the 12α,13α-etiojervane series by an unambiguous chemical synthesis.[3a] Oxidation of the hydroxyl group in the latter compound afforded a material spectrally very similar to the fermentation product, but differing in its optical rotation (+162° *vs.* −86°). The difference between the two compounds, a result of the stereochemistry at C-13, was resolved by treating the less stable 13β-methyl derivative (**4a**) with base, generating the more stable 13α-methyl compound **4b**.[4]

When the activity of the unstable dienone **4a** as an aldosterone-blocking agent was discovered,[5] additional supplies of this compound and its derivatives were required. The moderate yields of the dienedione **4a** from fermentation and the limited success of early attempts to utilize it chemically led to the exploration of its chemical synthesis.

Although the starting ketone **1c** has the 13-methyl in the desired configuration, side chain degradation by Beckmann rearrangement of its oxime, even under carefully controlled conditions, caused epimerization at C-13.[3a] Attempted utilization of this accessible 13α epimer **1b** by hydrogenation of its enol diacetate (Δ^{17}) yielded, after saponification, largely hydrogenolyzed materials containing little of the desired 17β-diol **1e** (R = R′ = H).

Conversion of the unsaturated ketone **1a** (Δ^{12})[3a] to the desired 13β-methyl compound was attempted by hydrogenation over several palladium catalysts; however, the preponderant product in each case was the stable 13α-methyl derivative.[6] Use of platinum catalysts, in an effort to reduce both the olefinic and the car-

(1) W. Charney and H. L. Herzog, "Microbial Transformations of Steroids," Academic Press, New York, N. Y., 1967.

(2) The term "etiojervane" and "pregnajervane" represent 17aβ-methyl-*C*-nor-D-homo-18-nor-5α,13β-androstane and its pregnane analog, respectively. See F. C. Chang and R. C. Ebersole, *Tetrahedron Lett.*, 3521 (1968).

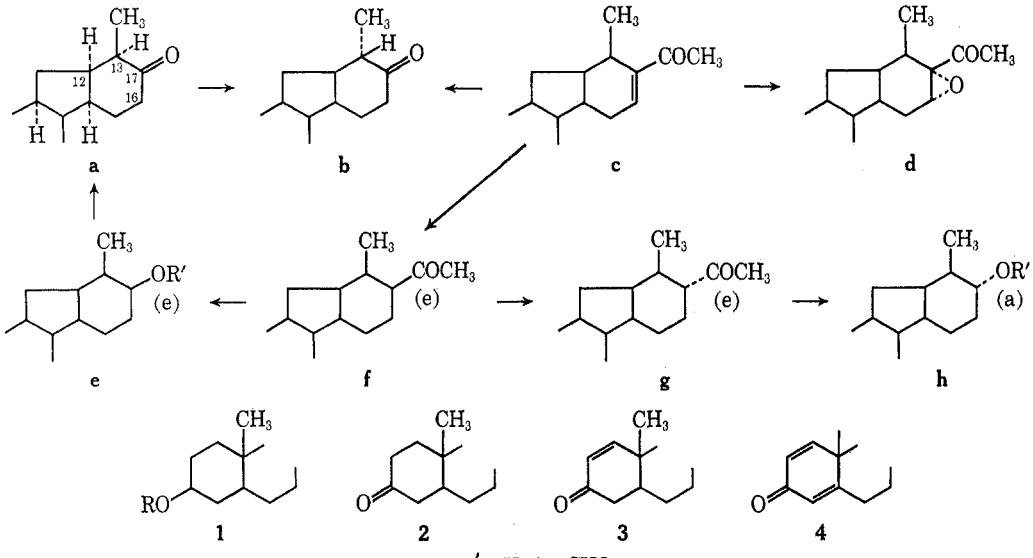
(3) (a) W. F. Johns and I. Laos, *J. Org. Chem.*, **30**, 123 (1965); (b) W. F. Johns, *ibid.*, **29**, 2545 (1964).

(4) A similar epimerization of the 13-methyl group is described in ref 3a.

(5) Private communication from Dr. L. Hofmann of these laboratories. The activities of this and related compounds will be included in a future communication.

(6) A recent communication reports formation of the 13β-methyl derivatives in this way, success apparently a result of the difference in catalysts employed: *cf.* H. Mitsuhashi and N. Kawahara, *Tetrahedron*, **21**, 1215 (1965).

SCHEME I



R, R′ = H, Ac, CHO

bonyl groups before C-13 epimerization occured, led mainly to hydrogenolyzed materials.

Reduction of the carbonyl group of **1a** (Δ^{12}) with lithium tri-*tert*-butoxyaluminohydride was a very slow reaction; subsequent catalytic reduction of the Δ^{12} double bond afforded the desired 13β-methyldiol **1h** (R′ = H) as well as hydrogenolysis products. The 17α configuration of the hydroxyl group in the final product (as well as in its unsaturated precursor) was demonstrated by its spectral identity with the known diol (see below). A successful concurrent attempt to produce diol **1h** was achieved by lithium aluminum hydride reduction of the 16,17 epoxide **1d**,[3a] periodate cleavage of the resulting 17,20-diol, and lithium alumium hydride reduction of the 17 ketone. Neither of these sequences proceeded in sufficiently high yields to warrant their further utilization.

Although initial studies[3a] of the Baeyer–Villiger oxidation of the 17β-pregnajervane **1f** to the 17β-acetate **1e** (R′ = Ac) with hot performic acid met with only moderate success, the yields were improved considerably by the use a large excess of *m*-chloroperbenzoic acid.[7] The 17α-pregnajervane **1g** was obtained from the β isomer by treatment with base and predominated (~20:1) in the resulting equilibrium mixture. This compound smoothly underwent *m*-chloroperbenzoic acid oxidation to yield a new acetate **1h** (R′ = Ac) clearly different from that derived from the β-acetyl derivative. The possibility of preoxidation isomerization of the β-acetyl side chain is thus ruled out.[8] Both the 17α- and 17β-acetates (**1e**, **1h**) from these two oxidations were hydrolyzed to their respective diols (**1e**, **1h**, R = R′ = H) and these in turn were oxidized to a single diketone **2a**, showing the pairs to be epimeric only at C-17.

A relatively unstrained D ring conformation, in which the 13-methyl group and the 17α substituent are equatorial, is postulated for both C-17 acetates **1e** and **1h**. This assignment is in agreement with the relative sharpness and position of the nmr signals for their respective C-17 protons. The ORD curve of the parent 17α-acetyl derivative **1g**[9] shows a weak positive Cotton effect; octant rule analysis indicated no change in D ring conformation from that in **1h**, if it is assumed that the 20-carbonyl group lies between C-18 and the 16β hydrogen. This assumption is reasonable both from inspection of the molecular model and by analogy to the normal steroids.[10] The strong positive Cotton effect seen in the ORD spectrum of the unstable 17β-acetyl compound **1f**[11] indicates a conformational change in which C-18 is now axial, again assuming that the carbonyl group lies between C-18 and the 16β hydrogen. This change results from an interaction of the axial 17β-acetyl group with C-19 which is stronger than that between the axial 13-methyl and C-19.

The 17β-diacetate **1e** (R = R′ = Ac), the immediate product of the Baeyer–Villiger oxidation of **1f** (R = Ac), could not easily be hydrolyzed to the 17-monoacetate **1e** (R = H; R′ = Ac), a compound required for further A ring transformations. Selective oxidation of the corresponding 3,17β-diol **1e** (R = R′ = H) afforded the unwanted 3-hydroxy 17-ketone **1a** (although the 3,17α-diol **1h** did give the useful 3-keto-17α-ol **2h**). A more fruitful route entailed Baeyer–Villiger oxidation of the 3-formate derivatives. These were formed without epimerization at C-17 by a brief contact of either 3-hydroxy compound (**1f**, **1g**) in formic acid at room temperature, or alternatively, by hydrogenation of **1c** (R = CHO). The oxidation product from either formate, a mixed ester, was selectively hydrolyzed by a simple passage of the crude product over an alumina column[12] to yield the desired 3-hydroxy-17-acetates (**1e** or **1h**).

Oxidation of the 3-hydroxy group proceeded smoothly and each of the epimeric ketoacetates **2e**, **2h** (R′ = Ac) were characterized by saponification to the correspond-

(7) Dr. P. B. Sollman, of these laboratories, is to be thanked for having developed this procedure. See the Experimental Section for precautions necessary to ensure the safe isolation of the product from this mixture.

(8) These results amend the incorrect configurational designation of the Baeyer–Villiger product given in ref 3a.

(9) Professor W. Klyne, Westfield College, University of London, is to be acknowledged for providing the ORD measurements of these compounds as well as for a helpful discussion relating to their interpretation.

(10) N. L. Allinger, P. Crabbé, and G. Perez, *Tetrahedron*, **22**, 1615 (1966).

(11) A recent communication describes similar configurational assignments: H. Suginome, N. Sato, and T. Masamune, *Tetrahedron Lett.*, 2671 (1969).

(12) See W. F. Johns and D. M. Jerina, *J. Org. Chem.*, **28**, 2922 (1963), and references cited terein.

ing ketols **2e**, **2h** (R′ = H). The 17α derivative thus produced was identical with that obtained above. Oxidation of either hydroxy ketone afforded the 13β-methyl diketone **2a** in good yield.

Selenium dioxide oxidation of the 17β-acetate **2e** (R′ = Ac) effected introduction of the desired 1,4-diene system in only moderate yields. The chief by-products were the Δ^1 derivative **3e** (R′ = Ac) and intractable selenium-containing tars; none of the Δ^4 derivative was seen. Conversion of the Δ^1 derivative to additional dienone was possible in modest yields by further treatment with selenium dioxide. Dichlorodicyanoquinone oxidation of either the Δ^1 derivative **3e** (R′ = Ac) or the corresponding saturated ketone **2e** (R′ = Ac) yielded virtually none of the dienone **4e** (R′ = Ac). Removal of selenium from the intractable by-products was attempted with ammonium sulfide treatment[13] alone or preceded by saponification of the 17-acetate, but little improvement in yield resulted. The 17α-acetate **2h** (R′ = Ac) similarly provided a fair yield of the 1,4-dienone **4h** (R′ = Ac), accompanied by the Δ^1 ketone **3h** (R′ = Ac).

An alternative pathway for conversion of the Δ^1-etiojervane **3e** (R′ = Ac) to the corresponding 1,4-diene **4e** (R′ = Ac) was investigated by use of 17β-acetoxyandrost-1-en-3-one as a model compound. Cupric bromide bromination[14] of this compound followed by base catalyzed elimination of the resultant 4-bromine atom afforded 40% of the desired 17β-acetoxyandrosta-1,4-dien-3-one. The limited yields of this sequence coupled with the success of an alternative route led to abandonment of this scheme.

The 17β-acetate **4e** was saponified to provide the corresponding 17 alcohol. This compound in turn was oxidized with the chromium trioxide–pyridine to yield the dienedione **4a**, identical in all respects with the initially described fermentation product.

Experimental Section[15]

3,17-Diacetoxy-12α-etiojerv-13-ene (**1e**, Δ^{13}, **R = R′ = Ac**).—Aqueous perchloric acid (70%, 0.3 ml) was added to 3 ml of acetic anhydride at 5°. The resulting solution was added to a solution of 0.50 g 3β-hydroxy-12α,13α-etiojervan-17-one (**1b**, R = H)[3a] in 30 ml of benzene and 10 ml of carbon tetrachloride. The solution was allowed to come to room temperature. After 4 hr, ice was added and the product was isolated by extraction with carbon tetrachloride in a standard manner (washing consecutively with water and with aqueous potassium bicarbonate, drying over magnesium sulfate, and concentrating at a temperature below 50°). Recrystallization of the crude product from hexane gave 0.29 g of the enol diacetate: mp 101–102°; 5.74 μ; 45 (19-CH_3), 89 (C=CCH_3), 120 (3-OAc), 126 (17-OAc) Hz.

Anal. Calcd for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.96; H, 9.33.

Hydrogenation of this enol acetate over a variety of catalysts effected at best a slow reduction affording a preponderance of hydrogenolyzed product. After base hydrolysis of the crude hydrogenation products, no homogeneous sample of a diol could be obtained. Similarly, hydrogenolysis was seen when the unsaturated ketone **1a** (R = H, Δ^{12})[3a] was reduced over platinum catalyst.

Etiojerv-12-ene-3β,17α-diol 3-Acetate (**1h**, Δ^{12}, **R = Ac; R′ = H**).—A solution of 0.5 g of the unsaturated ketone **1a** (Δ^{12}, R = Ac) in 40 ml of tetrahydrofuran at 5° was treated with 1.5 g of lithium tri-*tert*-butoxyaluminohydride. After 18 hr the solution was poured into water and the product was extracted with methylene chloride. The crude product was purified by preparative thin layer chromatography yielding 355 mg of crystalline material which was recrystallized from acetone–hexane to give 180 mg of the unsaturated alcohol **1h** (R = Ac; R′ = H[12]): mp 133–135°; 2.75, 5.78 μ; 47 (19-CH_3), 102 (18-CH_3), 248 (m, 17-H) Hz.

Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.98; H, 9.68.

This compound was oxidized with manganese dioxide to the starting unsaturated ketone **1a** (Δ^{12}, R = Ac).

12α-Pregnajervane-3β,17α,20-triol.—A solution of 1.0 g of the oxide **1d** (R = Ac) in 50 ml of tetrahydrofuran was added to a slurry of 0.7 g of lithium aluminum hydride in 100 ml of tetrahydrofuran. The mixture was stirred at room temperature for 18 hr and at reflux for 2 hr. The solution was cooled and diluted slowly and consecutively with 20 ml of ethyl acetate, 2 ml of water, and 2 ml of 10% aqueous potassium hydroxide. The mixture was filtered through Super-Cel and the resulting solution was concentrated to dryness. The residue was recrystallized from methylene chloride–ethyl acetate to yield 0.30 g of the triol, hydrated with 0.25 mol equiv of water: mp 200–205°; 2.75 μ; 48 (19-CH_3), 52 and 59 (18-CH_3) Hz.

Anal. Calcd for $C_{21}H_{36}O_3 \cdot 0.25\ H_2O$: C, 73.96; H, 10.79. Found: C, 73.99; H, 10.39.

Treatment of 65 mg of this triol in 4 ml of methanol and 0.2 ml of pyridine with 0.1 g of paraperiodic acid in 1 ml of water for 1.5 hr at room temperature provided, after dilution with water and filtration of the resulting precipitate, 32 mg of the pure 13β-methyl ketone **1a** (R = H) (by spectral comparison).[3a]

3β-Hydroxy-12α,17α-pregnajervan-20-one (**1g**, **R = H**).—A solution of 6 g of the saturated ketone **1f**(R = H) in 100 ml of methanol and 10 ml of 10% aqueous potassium hydroxide was heated at reflux in an atmosphere of nitrogen for 0.5 hr, and was cooled and diluted with excess 1% aqueous hydrochloric acid. The resulting precipitate was filtered, washed with water, and dried; it had $[\alpha]_D$ −46°, corresponding to 5% of the 17β derivative. Recrystallization from aqueous acetone gave 4.6 g of the pure 17α-acetyl derivative **1g** (R = H): mp 136–137°; 2.75, 5.84 μ; ORD (*c* 1.97 mg/ml, MeOH) $[\phi]^{tr}_{343}$ −415, $[\phi]^{pk}_{300}$ +300°; inflection 250 mμ (ε 3195); α −35°; $[\alpha]_D$ −52°.[9,16]

Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.35; H, 10.57.

Separation of this material from its epimer by thin layer, paper, or gas chromatography in a number of systems was not possible. Its ir and nmr spectra were essentially indistinguishable from those of the unstable β epimer **1f** (R = H). Attempted formation of the enol acetate of the β-acetyl derivative **1f** (R = H) with iso-propenyl acetate and *p*-toluenesulfonic acid led to mixtures containing minor amounts of enol acetate mixed with starting material and dark polymers.

The 3-acetate of the 17α-acetyl derivative **1g**, prepared with acetic anhydride and pyridine, failed to crystallize but exhibited the proper nmr absorption (3-OAc, 122; 17-$COCH_3$, 128 Hz). Again the nmr and ir spectra were very similar to those of the β epimer.

3β-Formyloxy-12α,17α-pregnajervan-20-one (**1g, R = CHO**).—The alcohol **1f** (34.5 g) was dissolved in 300 ml of formic acid. After 1 hr the stirred solution was slowly diluted with water. The resulting precipitate was recrystallized from aqueous methanol to yield 35.0 g of the formate **1g** (R = CHO): mp 95–96°; 5.80 μ; 45 and 52 (18-CH_3), 50 (19-CH_3), 483 (HCO_2) Hz; $[\alpha]_D$ − 53°.

Anal. Calcd for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 75.93; H, 9.81.

3β-Formyloxy-12α-pregnajervan-20-one (**1f, R = CHO**).—The alcohol **1f** (R = H, 26 g) dissolved in 150 ml of formic acid for 1 hr and diluted with water, afforded 26 g of the formate, mp 92 − 94°, after recrystallization from aqueous acetone: 5.81 μ; 45 and 51 (18-CH_3), 50 (19-CH_3), 483 (HCO_2) Hz; $[\alpha]_D$ +44°.

(13) A successful utilization of this procedure in the normal pregnanes has been described: M. Kocor and M. Maczka, *Bull. Acad. Pol. Sci.*, **14**, 347 (1966); *Chem. Abstr.*, **65**, 18651c (1966).

(14) See, *e.g.*, P. B. Sollman and R. M. Dodson, *J. Org. Chem.*, **26**, 4180 (1961); L. C. King and G. K. Ostrum, *ibid.*, **29**, 3459 (1964).

(15) We wish to thank Dr. J. W. Ahlberg and staff for the analyses and spectra reported. The infrared spectra were determined in chloroform, ultraviolet spectra in methanol, and rotations in chloroform (1%). Nmr spectra were determined in deuteriochloroform on a Model A-60 spectrometer, Varian Associates, Inc, at 60 Hz, using tetramethylsilane as an internal standard ($\Delta\nu$ = 0). $W^1/_2$ denotes peak width at half-height.

(16) This compound was first prepared by Mr. I. Loas, of these laboratories. The 17β-acetyl derivative displayed a = +116; see ref 3a.

Anal. Found: C, 76.40; H, 9.91.

3β-Formyloxy-12α-pregnajerv-16-en-20-one (1c, R = CHO), prepared by dissolving **1c** (R = H) in formic acid for 1 hr, was recrystallized from aqueous acetone: mp 158–163°; 5.80, 6.00 μ; 235 mμ (10,600).

Anal. Calcd for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.60; H, 9.62.

12α-Etiojervane-3β,17α-diol 3-Formate 17-Acetate (1h, R = CHO; R′ = Ac). Procedure A.—The formate **1g** (R = CHO, 4.8 g) in 80 ml of methylene chloride was treated with 3 g of *m*-chloroperbenzoic acid. After 6 days, 10 g of calcium hydroxide[17] was added to the solution and the mixture was stirred for 0.5 hr. The mixture was filtered and the solvent distilled. The residue was crystallized from pentane and recrystallized from aqueous acetone to yield 1.63 g of the formate **1h** (R = CHO, R′ = Ac): mp 102–106°; 5.78 μ; $[\alpha]_D$ −66°.

Anal. Calcd for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 73.21; H, 9.65.

The mother liquors of this reaction on chromatography[18] showed some of the starting material to be present. To effect complete reaction and circumvent the prolonged reaction times a modified procedure ("B") was developed.

B.—The formate **1g** (43 g, R = CHO) and 100 g of *m*-chloroperbenzoic (70% pure) were dissolved in 0.5 l. of methylene chloride with stirring. After 22 hr the mixture was diluted with 3 l. of methylene chloride and added to a well-stirred slurry of 200 g of calcium hydroxide in 1 l. of methylene chloride over a 30-min period. (This method of work-up is required to avoid a very vigorous decomposition of the excess peracid). After 1 hr the mixture was filtered and the product isolated as above yielding a comparable yield of the 17α-acetate **1h** (R = CHO, R′ = Ac) with none of the starting material in evidence.

Baeyer–Villiger Oxidation of the 17β-Acetyl 3-Formate 1f.—Both procedures A and B were successfully employed on the 17β-acetyl derivative **1f** (R = CHO) but the product failed to crystallize, even after chromatography. In procedure A, appreciable amounts of starting material were found after 14 days of reaction time. Boiling the reaction mixture was relatively ineffective in increasing the rate of reaction.

An earlier method used performic acid at 75° for 2 hr. The material balance was low and, under these conditions, the reaction incomplete. Basic hydrolysis of the product yielded the known 17β-diol **1e**[19] in good yield.

12α-Etiojervane-3β,17β-diol Diacetate (1e, R = Ac; R′ = Ac).—The *m*-chloroperbenzoic acid oxidation (procedure A) yielded after 3 days, from 3.2 g of the starting acetate **1f** (R = Ac), a product which crystallized from pentane to yield 1.6 g of the diacetate **1e** (R = R′ = Ac): mp 107–111°; 5.78 μ; 50 (19-CH_3), 50 and 57 (18-CH_3), 122 (OAc), 280 (m, 3β-H), 298 (m, 17α-H) Hz; $[\alpha]_D$ 0°.

Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.15; H, 9.41.

Saponification of this compound with base afforded the known 17β-diol **1e** in good yield.

12α-Etiojervane-3β,17α-diol Diacetate (1h, R = R′ = Ac).—The 3-acetate **1g** (2.0 g) was treated with *m*-chloroperbenzoic acid (procedure A) and after 2 days yielded a product crystallized from ether and recrystallized from methylene chloride–hexane to give 0.18 g of the 17α-diacetate **1h** (R = R′ = Ac): mp 107–109°; 5.78 μ; 48 (19-CH_3), 52 and 57 (18-CH_3), 122 (OAc), 270–290 (3α,17β-H's) Hz; $[\alpha]_D$ −67°.

Anal. Found: C, 73.63; H, 9.58.

12α-Etiojervane-3β,17α-diol (1h, R = R′ = H). A. Hydrolysis of the Diacetate 1h. Procedure C.—A solution of 0.12 g of the diacetate **1h** (R = R′ = Ac) in 10 ml of methanol and 1 ml of 10% aqueous potassium hydroxide was heated at reflux for 1 hr, the methanol was distilled, and the reaction mixture was diluted with water. The resulting precipitate was collected on a filter and washed with water. Recrystallization from ether give the pure 17α-diol **1h**: mp 178–181°; 2.75 μ; 47 (19-CH_3), 56 and 63 (18-CH_3), 198–230 (17β-H, 3α-H) Hz.

Anal. Calcd for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03. Found: C, 77.92; H, 10.94.

For comparison, the 3β,17β-diol **1e**[19] had the following nmr signals: 48 (19-CH_3), 56 and 63 (18-CH_3), 200–225 (3α-H), 225–235 (17α-H) Hz.

B. Hydrogenation of the Unsaturated Alcohol 1h (Δ¹², R = Ac; R′ = H).—The acetate **1h** (Δ¹², R = Ac; R′ = H; 0.11 g) in 50 ml of acetic acid containing 0.1 g of 5% rhodium–alumina was shaken with hydrogen at 40 psi for 6 hr. The mixture was filtered and the filtrate concentrated to dryness. The residue was saponified (procedure C) to afford a semicrystalline product which was recrystallized to yield 41 mg of the 3β,17α-diol **1h**, mp 172–176°, identical by ir, nmr, and tlc comparisons with the above sample.

Selective Oxidation of the 3β,17α-diol 1h.—Jones reagent[20] (0.25 ml, 4 *N* chromic acid solution) was added to a solution of 300 mg of the 17α-diol **1h** in 50 ml of acetone at 10°. After 10 min the product was extracted with methylene chloride, yielding as the principal product (nmr, ir, and tlc analysis), the 3β-hydroxy 17-ketone **1a**. With excess oxidizing reagent the known 3,17-diketone **2a**[3a] was formed.

12α-Etiojervane-3β,17β-diol 17-Acetate (1e, R = H; R′ = Ac). Procedure D. A. Alumina-Catalyzed Hydrolysis.—The 3-formate 17β-acetate **1e** (43 g) was chromatographed on 3 kg of Merck Alumina. Early eluates afforded 0.5 g of a crude ester mixture. Elution with 10% ethyl acetate–benzene afforded 34.4 g of material which was recrystallized from acetone–hexane to give 24.6 g of the 3-alcohol **1e** (R = H, R′ = Ac): mp 92–94°; 2.75, 5.79 μ; 48 (19-CH_3), 51 and 58 (18-CH_3), 214 (m, 3α-H), 298 (m, $W_{1/2}$ = 10 Hz, 17α-H) Hz.

Anal. Calcd for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.28; H, 10.04.

B. Potassium Carbonate Hydrolysis.—A solution of 1.82 g of the diacetate **1e** (R = R′ = Ac) in 20 ml of methanol and 10 ml of water containing 0.75 g of potassium carbonate were mixed at 15°. After 7 hr, the solution was diluted with water. The product was extracted with methylene chloride and purified by chromatography yielding material which was crystallized from ether–hexane to afford 0.65 g of the 17β-acetate **1h** (R = H, R′ = Ac), mp 92–94°, described above.

12α-Etiojervane-3β,17α-diol 17-Acetate (1h, R = H; R′ = Ac).—A brief alumina chromatograph (procedure D) afforded, from 31 g of the pure formate **1h** (R = CHO, R′ = Ac), 21.5 g of the recrystallized (acetone–hexane) 3-alcohol 17-acetate **1h** (R = H, R′ = Ac): mp 129–131°; 2.72, 5.76 μ; 48 (19-CH_3), 51 and 58 (18-CH_3), 214 (m, 3α-H), 280 (m, $W_{1/2}$ = 19 Hz, 17β-H) Hz.

Anal. Found: C, 75.69; H, 10.23.

17β-Acetoxy-12α-etiojervan-3-one (2e, R′ = Ac). Procedure E.—A solution of 0.63 g of the monoacetate **1e** (R = H, R′ = Ac) in 20 ml of acetone at 5° was treated with 1 ml of 4 *N* chromic acid solution (Jones reagent).[20] After 0.5 hr at ambient temperature the solution was diluted with water and 1 ml of 2-propanol. The product was extracted with methylene chloride and chromatographed. The material eluted with 2% ethyl acetate–benzene consisted of 0.56 g of the 17β-acetate **2e** which solidified slowly (recrystallization failed): 5.82 μ; 53 and 59 (18-CH_3), 61 (19-CH_3), 301 (m, 17α-H, $W_{1/2}$ = 11 Hz) Hz.

Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.01; H, 9.88.

17α-Acetoxy-12α-etiojervan-3-one (2h, R′ = Ac).—Oxidation of the alcohol **1h** (R = H, R′ = Ac) by use of procedure E yielded a crystalline product on dilution of the reaction mixture with water. From 15.5 g of the starting alcohol, there was obtained 14.6 g of the pure ketone **2h** (R′ = Ac): mp 104–105°; 5.78 μ; 52 (19-CH_3), 52 and 59 (18-CH_3), 280 (m, $W_{1/2}$ = 18 Hz, 17β—H) Hz.

Anal. Found: C, 75.57; H, 9.62.

17β-Hydroxy-12α-etiojervan-3-one (2e, R′ = H).—Saponification of 0.35 g of the 17β-acetate **2e** (procedure C) afforded directly a crystalline product, recrystallized from acetone–hexane to yield 0.19 g of the 17β-alcohol **2e**: mp 121-124°; 2.73, 5.84 μ; 58 and 64 (18-CH_3), 60 (19-CH_3), 232 (m, $W_{1/2}$ = 10 Hz, 17α-H) Hz; $[\alpha]_D$ +31°.

Anal. Calcd for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.52; H, 10.41.

Reacetylation of the hydroxy compound afforded the 17β-acetate **2e** in good yield.

(17) H. B. Henbest and B. Nicholls, *J. Chem. Soc.*, 4608 (1957).

(18) The chromatographies described in this paper were routinely run on a weight of silica gel 60 times that of the weight of the product being separated. We thank Mr. R. T. Nicholson and staff for their competent execution of these chromatograms.

(19) This compound, with the C-17 configuration incorrectly assigned, appears in ref 3a: *cf.* the discussion above.

(20) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

17α-Hydroxy-12α-etiojervan-3-one (2h, R′ = H).—Saponification of 0.85 g of the 17α-acetate **2h** (procedure C) afforded an amorphous product which was crystallized from ether and recrystallized from acetone–cyclohexane to yield the 17α alcohol **2h**: mp 150–153°; 2.72, 5.82 μ; 58 (19-CH_3), 58 and 64 (18-CH_3) Hz; $[\alpha]D$ −10°.

Anal. Found: C, 78.50; H, 10.43.

17β-Acetoxy-12α-etiojervane-1,4-dien-3-one (4e, R′ = Ac). Procedure F.—Selenium dioxide (0.3 g) was added to a solution of 0.9 g of the ketone **2e** (R′ = Ac) in 20 ml of *tert*-butyl alcohol and 0.1 ml of pyridine. The solution was boiled under an atmosphere of nitrogen. The same quantities of pyridine and selenium dioxide were added after 24 and 48 hr. After a total of 4 days, the solution was cooled and filtered, using methylene chloride to wash the insoluble precipitate. The combined solutions were concentrated to near dryness and then diluted with water and extracted with ethyl acetate. The extract was washed with water, potassium bicarbonate, water, cold ammonium sulfide solution, cold ammonium hydroxide, and again with water. The product obtained (0.90 g) was chromatographed. Elution with 5% ethyl acetate–benzene afforded 0.10 g of amorphous 17β-acetoxy-12α-etiojervan-1-en-3-one (**3e**, R′ = Ac): 5.78, 5.97 μ; 230 mμ (9900); 53 and 60 (18-CH_3), 62 (19-CH_3) 347 and 357 (C_1 H), 418 and 428 (C_2 H) Hz.

Anal. Calcd for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.06; H, 8.95.

Attempts to convert the ketone **3e** (R′ = Ac) to 1,4-diene by retreatment with selenium dioxide under similar conditions or by use of dichlorodicyanoquinone in refluxing benzene led to additional dienone **4e** (R′ = Ac), but the yields were erratic and generally low.

Continued elution of the above chromatographic column gave crude fractions (0.51 g) recrystallized from acetone–hexane to afford 0.34 g of the dienone **4e** (R′ = Ac): mp 135–137° as a hemiacetonate; 5.78, 6.00, 6.16 μ; 243 mμ (16,600); 53 and 59 (18-CH_3), 73 (19-CH_3), and C=CH signals at 365, 376, 378, 412, and 422 Hz.

Anal. Calcd for $C_{21}H_{28}O_3 \cdot {}^1/_2C_3H_6O$: C, 75.59; H, 8.74. Found: C, 75.75; H, 8.47.

In other runs, removal of selenium from the product was attempted by boiling the dark product in ethanol containing aqueous ammonium sulfide.[18] The product was isolated, reacetylated with pyridine–acetic anhydride, and rechromatographed. The yields after such treatment (or retreatment of chromatographed portions) were not noticeably improved. Also, saponification of the entire product followed by chromatography gave no increase in yield.

17β-Acetoxyandrosta-1,4-dien-3-one from 17β-Acetoxyandrostan-1-en-3-one.—A solution of 0.75 g of 17β-acetoxyandrost-1-en-3-one and 2 g of cupric bromide in 200 ml of tetrahydrofuran was heated at reflux under nitrogen for 20 hr. The colorless solution was consecutively distilled to half volume, diluted with water, and extracted with methylene chloride. The resulting unstable bromide in 20 ml of dimethylformamide was added to 40 ml of boiling dimethylformamide containing 2 g of magnesium oxide. After 2 hr the mixture was cooled and filtered. The solvent was removed *in vacuo* and the residue extracted with methylene chloride. The resulting dark oil was chromatographed on silica and yielded first 90 mg of starting material followed by 0.29 g (40%) of the 1,4-diene, spectrally identical with an authentic sample.

17α-Acetoxy-12α-etiojerva-1,4-dien-3-one (4h, R′ = Ac).—The acetate **2h** (R′ = Ac, 0.63 g) was oxidized with selenium dioxide (procedure F). After 20 hr the product was isolated and chromatographed on silica, yielding, besides some starting material, 0.20 g of crude 1,4-diene, which was recrystallized from acetone–hexane to yield the pure compound: mp 112–114°; 5.78, 6.01 μ; 242 mμ (14,100).

Anal. Calcd for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.42; H, 8.48.

17β-Hydroxy-12α-etiojerv-1-en-3-one (3e, R′ = H). Procedure G.—A solution of 0.8 g of the ester **3e** (R′ = Ac) in 30 ml of *tert*-butyl alcohol containing 5 ml of 10% aqueous potassium hydroxide was heated at reflux with stirring under an atmosphere of nitrogen for 1 day. The solvent was distilled and the product isolated by methylene chloride extraction. Chromatography yielded fractions (0.18 g) eluted with 15% ethyl acetate–benzene which were recrystallized from acetone–hexane to yield 0.10 g of the 17β-alcohol **3e** as a hemiacetonate: mp 79–87°; 2.72, 5.95 μ; 230 mμ (8900); 59 and 66 (18-CH_3), 62 (19-CH_3), 345 and 355 (C_1 H), 417 and 422 (C_2 H) Hz.

Anal. Calcd for $C_{19}H_{28}O_2 \cdot {}^1/_2C_3H_6O$: C, 77.56; H, 9.84. Found: C, 77.71; H, 9.81.

17β-Hydroxy-12α-etiojervane-1,4-dien-3-one (4e, R′ = H).—The acetate (**4e**, R′ = Ac, 0.9 3 g) was saponified (procedure G) in 3 hr yielding, after recrystallization from methylene chloride–hexane, 0.44 g of the dienone: mp 125–127°; 2.75, 6.07, 6.18 μ; 244 mμ (16,400); 58 and 66 (18-CH_3), 73 (19-CH_3) Hz; $[\alpha]D$ +41°.

Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.77; H, 9.26.

Acetylation of this material afforded in good yield the acetate **4e** (R′ = Ac).

12α-Etiojerva-1,4-diene-3,17-dione (4a). A. Fermentation.[21]—*Nocardia sp.* ATCC No. 19534 was innoculated into a sterile nutrient mixture of 6 g of beef extract and 10 g of peptone in 2 l. of distilled water, and the mixture was incubated with agitation and aeration for 25 hr at room temperature. A solution of 1.37 g of the acetate **1f** (R = Ac) in 32 ml of acetone and 32 ml of methanol was added and the mixture was incubated for 40 hr. The mixture was then extracted with methylene chloride and the product (0.80 g) was chromatographed. Starting material (0.18 g) was eluted with 1% ethyl acetate–benzene. Elution with 30% ethyl acetate–benzene afforded 0.32 g of material, recrystallized from methylene chloride–hexane, to yield 0.16 g of the dienedione **4a**: mp 176–180°; 5.82, 5.99 μ; 242 mμ (16,800); 58 and 63 (18-CH_3), 72 (19-CH_3), 364 (C_4 H), 373 and 374 (C_1 H), 406 and 411 (C_2 H) Hz; $[\phi]^{pk}_{255}$ +17,800; $[\phi]^{tr}_{309}$ 5680; α +235°;[9] $[\alpha]D$ −86°.

Anal. Calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.57. Found: C, 79.99; H, 8.36.

B. Oxidation of the 17β-Alcohol (4e, R′ = H).—A solution of 0.14 g of the 17β alcohol **4e** in 5 m of pyridine was added to a slurry of 0.2 g of the Sarett reagent[22] prepared from 0.2 g of chromium trioxide. After 10 min at 5° the solution was allowed to warm to room temperature. After 1.5 hr the mixture was diluted with water and the product extracted with ether. The material was recrystallized from methylene chloride–cyclohexane to yield 95 mg of the dione **4a**, mp 169–176°, identical in the ir and nmr with the above material.

12α,13α-Etiojervane-1,4-diene-3,17-dione (4b). A. Base-Catalyzed Epimerization.—The dione (**4a**, 40 mg) was treated with base (procedure C) for 3 hr. The solvent was blown off in a stream of nitrogen and the product isolated by methylene chloride extraction. The material was recrystallized from ether to yield the 13α-dione **4b**, mp 122–124°, mmp (with starting material) 100–110°; both ir and nmr spectra were very similar with those of **4a**; $[\alpha]D$ +162°.

Anal. Found: C, 80.00; H, 8.38.

B. Chromic Acid Oxidation.—The alcohol **4e** (R′ = H, 13α-CH_3) was oxidized with chromic acid (procedure E). The mixture was diluted with water and the product extracted with methylene chloride. The material crystallized and was recrystallized to afford the diketone **4b** identical with that produced above.

Registry No.—**1c** (R = CHO), 26019-85-2; **1e** (Δ^{13}, R, R′ = Ac), 26019-86-3; **1e** (R = R′ = Ac), 26019-87-4; **1f** (R = CHO), 26019-88-5; **1g** (R = H), 26094-16-6; **1g** (R = CHO), 26019-89-6; **1h** (Δ^{12}, R = Ac; R′ = H), 26019-90-9; **1h** (R = CHO, R′ = Ac), 26019-91-0; **1h** (R, R′ = Ac), 26019-92-1; **1h** (R, R′ = H), 26019-93-2; **1e** (R = H, R′ = Ac), 26019-94-3; **2e** (R′ = Ac), 26019-95-4; **2e** (R′ = H), 26019-96-5; **2h** (R′ = Ac), 26019-97-6; **2h** (R′ = H), 26019-98-7; **3e** (R′ = Ac), 26019-99-8; **3e** (R′ = H), 22785-13-3; **4a**, 22785-14-4; **4b**, 26020-02-0; **4e** (R′ = Ac), 22785-06-4; **4e** (R′ = H), 22785-11-1; **4b** (R′ = Ac), 26020-05-3; 12α-pregnajervane-3β,17α,20-triol, 26020-06-4.

(21) We are indebted to Dr. R. D. Muir and staff, of these laboratories, who ran this fermentation.

(22) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Amer. Chem. Soc.*, **75**, 422 (1953).

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The Synthesis of Model Compounds for Maleylacetoacetic Acid. Maleylacetone[1]

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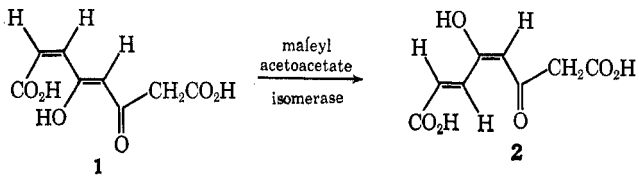
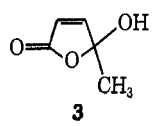
Configurationally labile maleylacetone has been prepared by the careful hydrolysis of 4-acetonylidenebut-2-ene-4-olide (*Z*) and shown to exist as a mixture of closed "pseudo" acid **4a** and open enol acid **4b** in chloroform and benzene and as "pseudo" acid in water. Ultraviolet and nmr spectra of the acids and anions are reported along with dissociation constants of the acid.

In the course of our study of *cis* to *trans* isomerizations we became interested in the mechanism of the enzymatic conversion of maleylacetoacetic acid (**1**) to fumarylacetoacetic acid (**2**). This *cis* to *trans* isomerization occurs in nature in the metabolism of aromatic amino acids.[2]



1 **2**

The study of this mechanism involves the synthesis and investigation of model compounds of **1** having similar but simpler structures. The first and simplest model compound prepared and its mechanism of isomerization studied was *cis*-β-acetylacrylic acid (**3**) which exists as the cyclic "pseudo" acid but as an

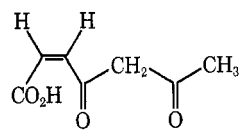


3

acyclic carboxylate anion.[3] The geometrical isomerization is catalyzed by thiocyanate ion which we consider to be a model for glutathione, the coenzyme needed for the enzyme-catalyzed isomerization of maleylacetoacetate. The role of the enzyme in this reaction is more obscure. The enzyme, we have suggested,[4] might catalyze the formation of a Schiff base between maleylacetoacetate and itself. The Schiff base, being more basic, would have a larger fraction protonated than the substrate and this in turn would enhance nucleophilic attack by glutathione thereby to catalyze isomerization.

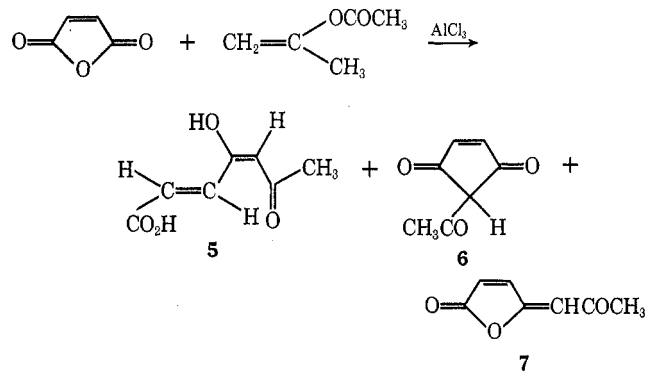
The anion of **3** was examined as to its ability to form Schiff bases with amines.[4] The predominant reaction, unfortunately, appears to be conjugate addition. Those amines possessing an α-nucleophilic atom such as hydroxylamine or semicarbazide, however, do form Schiff bases and the reactivity of the α,β-unsaturated semicarbazone towards thiocyanate was studied. Conjugate addition of amines to the γ,δ double bond in **1** might be less favorable because of the greater delocalization of positive charge and so it was of interest to see the effect of the introduction of an additional keto group, β to the original α′,β′-unsaturated carbonyl group of our first model compound. Moreover, such a molecule would be closer in structure to the natural substrate (**1**) and would be a more accurate model for studying other aspects of the chemistry of **1**.

A compound having the β-diketone moiety α to the *cis* double bond yet being simpler than maleylacetoacetic acid is maleylacetone (**4**). Maleylacetone has



4

previously been reported to have been isolated in 87% purity as a product of the enzymatic oxidation of homogentisic acid.[5] In addition, **4** has been suggested by Nilsson as a probable intermediate in the formation of fumarylacetone (**5**), 2-acetylcyclopentene-1,3-dione (**6**), and 4-acetonylidenebut-2-ene-4-olide (**7**), from the



5 **6** **7**

(1) Research carried out at Brookhaven National Laboratory under contract with the U. S. Atomic Energy Commission.

(2) W. E. Knox in "The Enzymes," Vol. 2, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1960, pp 282–289.

(3) (a) S. Seltzer and K. D. Stevens, *J. Org. Chem.*, **33**, 2708 (1968); (b) K. D. Stevens and S. Seltzer, *ibid.*, **33**, 3922 (1968).

(4) C. Santiago and S. Seltzer, in preparation.

(5) D. I. Crandall, *et al.*, *J. Biol. Chem.*, **235**, 3011 (1960), reported that in the isolation of maleylacetoacetic acid from the enzymatic oxidation of homogentisic acid nonenzymatic decarboxylation occurred to give maleylacetone.