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Steroid Total Synthesis—Hydrochrysene Approach. XV.[1] Total Synthesis of Aldosterone

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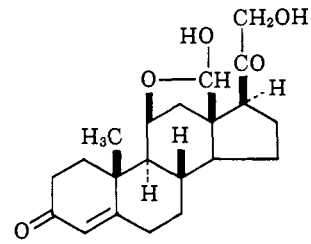
The starting point for the synthesis of aldosterone (I) was the dihydroxy ketone II, the preparation of which *via* the intermediates A → B → C → D has been described in previous work. The ketone II was converted into the furfurylidene derivative III which on treatment with methacrylonitrile in methanolic sodium methoxide was transformed into the adduct IV (R = H). Acetylation followed by ozonolysis, then saponification, gave on acidification the lactone dicarboxylic acid V (R = H) which was transformed by two alternative methods into the diketo lactone VII. Rearrangement with peracid afforded the triacetate VIII ($R^1 = R^2 = R^3 = Ac$) which on mild saponification followed by N-bromoacetamide oxidation and reacetylation gave the 3-keto diacetate XII ($R^1 = R^2 = H$). Bromination and dehydrobromination afforded the unsaturated ketone XIII which was converted into the ketal XV ($R^1 = R^2 = Ac$). Saponification gave the diol XV ($R^1 = R^2 = H$) which was transformed by selective reaction with *p*-toluenesulfonyl chloride in pyridine into the monoester XV ($R^1 = Ts$, $R^2 = H$). Oxidation with Sarett reagent converted the C-20 hydroxyl to ketone, yielding the substance XVI. This oxidation step eliminated the asymmetry at C-20 which had up to this point resulted in pairs of epimers for each of the substances in the synthetic sequence starting with compound IV. Treatment of the keto ester XVI with potassium *t*-butoxide effected cyclization to the keto lactol XVII which with 2 mole-equivalents of lithium aluminum hydride was selectively reduced to the lactol XIX. Acid hydrolysis afforded the unsaturated ketone XX (R = H) which on treatment with methanol and acid was converted into the lactol ether XX ($R = CH_3$). Oxidation of this substance with Sarett reagent afforded the ketone XXI which was converted by known procedures into the 21-acetoxy compound XXII ($R = CH_3$). Hydrolysis of the lactol ether XXII with 70% acetic acid gave DL-17α-aldosterone-21-acetate (XXII, R = H) which was identical with authentic material. Treatment of XXII with potassium carbonate in aqueous methanol gave the C-17 epimeric mixture from which DL-aldosterone was isolated and compared with authentic material. An alternative approach was also studied and carried to the point of the pentacyclic aldehyde XXXVI. Michael condensation of the furfurylidene ketone III with acrylonitrile afforded the adduct XXIII ($R^1 = R^2 = H$). The diacetate XXIII ($R^1 = R^2 = Ac$) was converted by treatment with ozone followed by sodium borohydride into the tetrahydroxy compound XXVI. This latter substance was transformed by the action of sodium metaperiodate, followed by treatment of the cleavage product with methanol and acid, into the cyanoacetal XXVII. Treatment with 70% acetic acid at room temperature selectively hydrolyzed the acetal group to give the cyanoaldehyde XXX which was converted into the diester XXXIII by the steps: oxidation with chromium trioxide in pyridine to the acid, saponification of the nitrile with potassium hydroxide, esterification with diazomethane, and acetylation at C-3 with pyridine and acetic anhydride. Treatment of the diester with phenyllithium followed by reacetylation at C-3, then dehydration, afforded the diene XXXIV which was transformed, by ozonization followed by treatment with piperidine and acetic acid in benzene, into the pentacyclic aldehyde XXXVI.

For many years after the elucidation of the structure of aldosterone (I), the only practical approach to this highly active hormone appeared to be *via* total synthesis; indeed for a considerable period, prior to the brilliant partial synthesis break-through by Barton and Beaton,[2] only the totally synthetic material was available for physiological study. This additional practical incentive to the study of the total synthesis has led to a prodigious amount of work in the field. The rather involved historical account of the development of various totally synthetic approaches to aldosterone has been well summarized elsewhere[3] and need not be repeated here. The present paper constitutes an account of the details of our work in the field, which was announced previously in a preliminary communication,[4] as well as some additional related studies.

The starting point was DL-3α,11β-dihydroxy-18-nor-D-homo-5β-androstane-17α-one (II), the stereoselective synthesis of which from 5-methoxy-2-tetralone (A) has already been described as summarized below with references to preferred procedures for each step. 5-Methoxy-2-tetralone, readily available from 2,5-dimethoxynaphthalene,[5] was converted by a succession of two Robinson annelation reactions into the tetracyclic ketone B,[6] which was in turn transformed into



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the hexahydro alcohol C by the steps: hydrogenation of the 4,5-(steroid numbering) double bond over palladium,[7] reduction of the keto group with sodium borohydride (preferred to lithium aluminum hydride as previously described[7]), and finally reduction of the 8,9-(styrene) double bond with potassium and alcohol in liquid ammonia.[8] The hexahydroalcohol C was in turn converted into the saturated ketone II by the sequence[9]: treatment of the 3-acetate of C with lead tetraacetate to effect selective acetoxylation at C-12; dehydroacetoxylation of the product, by warming in acetic acid solution, to produce the olefinic substance D; perbenzoic acid oxidation in the presence of excess benzoic acid to give the hydroxy benzoate which, without isolation, was submitted to Birch reduction under forcing conditions. The mixture of unsaturated

(1) Part XIV, K. V. Yorka, W. L. Truett and W. S. Johnson, *J. Org. Chem.*, **27**, 4580 (1962).

(2) D. H. R. Barton and J. M. Beaton, *J. Am. Chem. Soc.*, **82**, 2641 (1960); **83**, 4083 (1961). See also K. Schaffner, D. Arigoni and O. Jeger, *Experientia*, **16**, 169 (1960); L. Velluz, *et al.*, *Compt. rend.*, 725 (1960); and M. E. Wolff, *et al.*, *J. Am. Chem. Soc.*, **82**, 4117 (1960).

(3) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 713–720.

(4) W. S. Johnson, J. C. Collins, R. Pappo and M. B. Rubin, *J. Am. Chem. Soc.*, **80**, 2585 (1958).

(5) J. W. Cornforth and R. Robinson, *J. Chem. Soc.*, 1855 (1949).

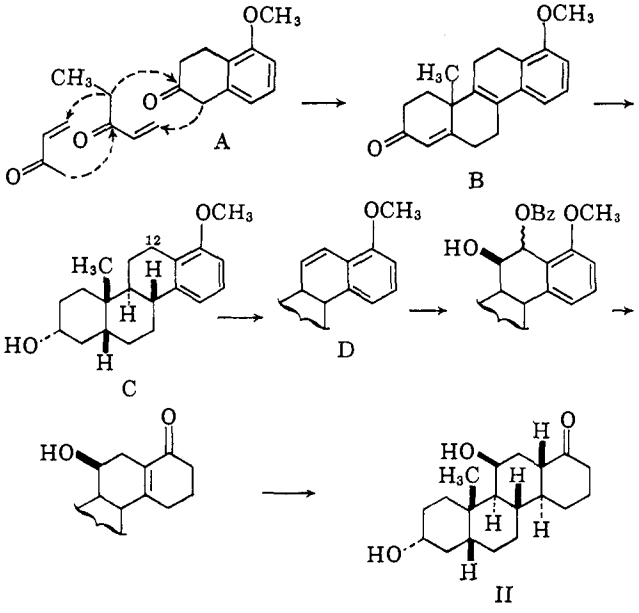
(6) W. S. Johnson, J. Szmuszkovicz, E. R. Rogier, H. I. Hadler and H. Wynberg, *J. Am. Chem. Soc.*, **78**, 6285 (1956).

(7) W. S. Johnson, E. R. Rogier, J. Szmuszkovicz, H. I. Hadler, J. Ackerman, B. K. Bhattacharyya, B. M. Bloom, L. Stalmann, R. A. Clement, B. Bannister and H. Wynberg, *ibid.*, **78**, 6289 (1956).

(8) W. S. Johnson, A. D. Kemp, R. Pappo, J. Ackerman and W. F. Johns, *ibid.*, **78**, 6312 (1956).

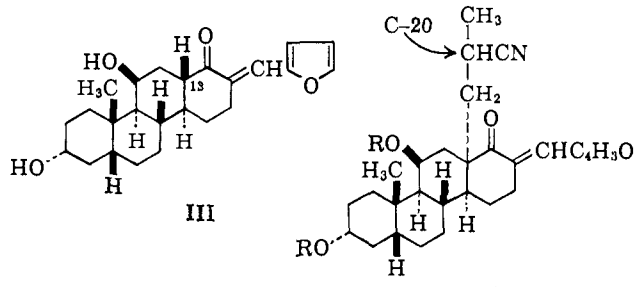
(9) W. S. Johnson, R. Pappo and W. F. Johns, *ibid.*, **78**, 6339 (1956).

ketones (mainly the $\Delta^{13,14}$- plus the $\Delta^{16,17}$-isomer) resulting from acid hydrolysis was hydrogenated over palladium in the presence of sodium hydroxide to yield the dihydroxy ketone II.[10] This substance was most conveniently purified as the furfurylidene derivative III, m.p. 193–194°, which was required for the continuation of the synthesis.



The next stage involved modification of the ketonic ring of substance II so as to provide appropriate substituents with functional groups juxtaposed for the elaboration of rings D and E of aldosterone. Previous studies[11] have demonstrated that the methylation of systems like the enolate of III invariably involves preferential attack (at C-13) on the alpha face of the molecule to yield the C/D *cis* ring fusion. Hence the direct introduction of a β-oriented group at this site, which would be required for the attachment of ring E, was obviously unpromising *a priori*. We elected, therefore, to take advantage of the tendency for this alkylation reaction to produce a *cis* product by thus introducing an angular substituent which could be used instead in the elaboration of ring D. The carbon atom (of the alkylating group) that was to be attached in the α-orientation to C-13 of substance III was envisaged as becoming C-17, while the β-oriented carbonyl carbon of III was destined to become C-18 of aldosterone.

With this plan in view, the Michael condensation of the furfurylidene ketone III with methacrylonitrile was examined. In the first experiments the hydroxyl groups of III were protected against reaction with the methacrylonitrile by using the ditetrahydropyranyl ether of III. The Michael reaction on this substance appeared to proceed satisfactorily, but the acidic conditions required for hydrolysis of the hindered 11β-tetrahydropyranyl ether group also attacked the acid-

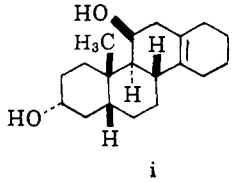


sensitive furan ring; hence this approach was abandoned. A satisfactory solution to the problem was provided by carrying out the Michael reaction in methanol solution with sodium methoxide catalyst. The solvent thus provided a competitive source of hydroxyl groups in high concentration so that reaction with the hydroxyl groups in the substrate was minimized. In this way condensation occurred almost exclusively at the angular position of III to give (as shown above) the C/D *cis* product IV (R = H) as the only isolable species. The problem was complicated at this stage by the fact that a new asymmetric center was introduced non-stereoselectively at that carbon (C-20) carrying the cyano group; thus a mixture of two epimeric nitriles IV (R = H) was produced which could be partially separated into its two components through the diacetates IV (R = Ac), m.p. 234° and 210–213.5°. For the purpose of the over-all synthesis, the separation of the C-20 epimers was unnecessary because this asymmetric center was eliminated at a later stage when it was converted into the C-20 ketone group (see below). For preliminary study, however, it proved expedient to examine a number of the reactions with the pure epimers.

The epimeric mixture of cyano diacetates IV (R = Ac) was converted, by ozonolysis followed by oxidation with hydrogen peroxide and then prolonged alkaline hydrolysis, into a crystalline mixture of lactonic dibasic acids V (R = H). The over-all yield of this product from the furfurylidene ketone III was 64%. Evidence for the structure was provided by the neutral equivalent (found 208–210; calcd. for V (R = H), 211) and by the infrared spectrum which exhibited a maximum at 5.70 μ characteristic of a γ-lactone. In addition, treatment of the epimeric mixture of acids with diazomethane afforded the dimethyl esters of V (R = H) which could be separated into the pure epimers, m.p. 120–121° and 171–172°. The diacid mixture V (R = H) was also readily converted by direct (hydrogen chloride-catalyzed) esterification with acetic acid,[12] into a crystalline mixture of 3-acetates V (R = Ac) from which one isomer, m.p. 251–254°, could be separated.

The production of the lactonic dibasic acid V (R = H) from IV (R = Ac) undoubtedly proceeds through the steps indicated in the accompanying flow sheet. The primary product of the saponification step, namely the dihydroxy triacid E, was not isolated because it underwent spontaneous lactonization on liberation from its sodium salt. This behavior demonstrated that the Michael condensation had indeed proceeded in the expected manner to give predominantly, if not exclusively, the C/D *cis* substance IV. The C-13 epimer obviously would not have undergone such ready conversion to a γ-lactonic product.

(10) In the present work a hydrogenolysis product was isolated at the Birch reduction stage. From spectral considerations (see Experimental) this material, which melted at 156.2–157.3°, has been assigned the structure DL-3α,11β-dihydroxy-13,14-dehydro-18-nor-D-homo-5β-androstane (i).



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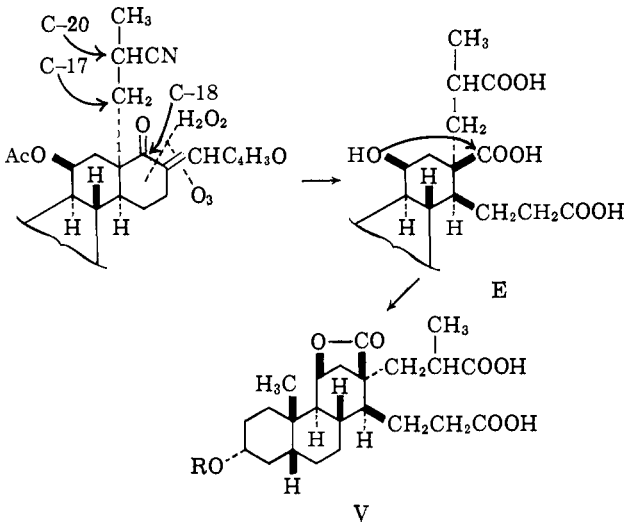
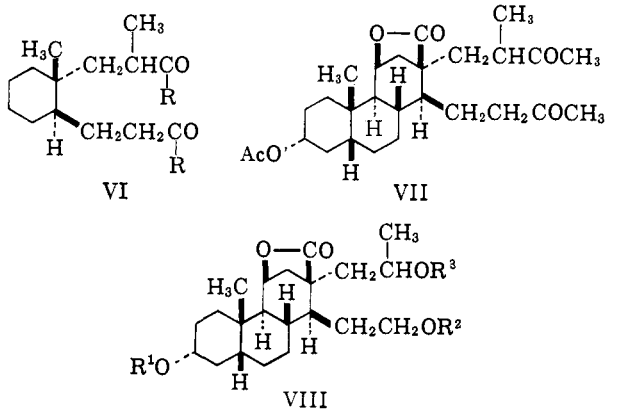
(11) W. S. Johnson, D. S. Allen, Jr., R. R. Hindersinn, G. N. Sausen and R. Pappo, *J. Am. Chem. Soc.*, **84**, 2181 (1962).

(12) Acetylation with acetic anhydride and pyridine yielded mixed anhydrides which could not be selectively hydrolyzed without also cleaving the 3-acetate. Poor yields of the desired acetates were also realized when the sodium hydride-catalyzed *trans*-acetylation with phenyl acetate was used. See R. Pappo, B. M. Bloom and W. S. Johnson, *ibid.*, **78**, 6347 (1956).



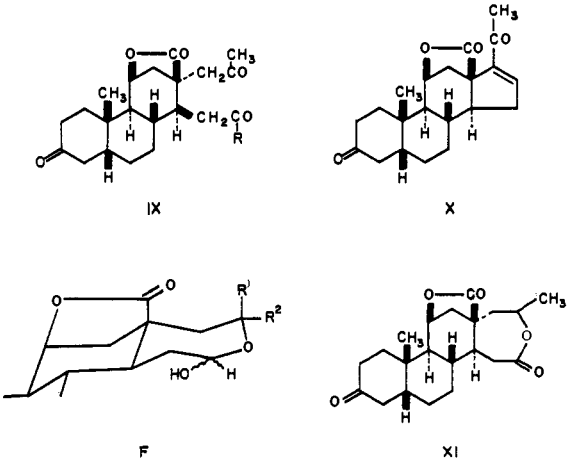
The next problem involved a degradation to eliminate both of the carboxyl carbons in order to produce side chains of appropriate length for the elaboration of ring D. Exploratory experiments on application of the Hunsdiecker degradation to the diacid V (R = Ac) gave unpromising results (yields 10–15%). Another degradative study was made with the collaboration of R. A. Clement and S. D. Darling, using a model system VI (R = OH). The steps involved production of the dialdehyde VI (R = H) by the Rosenmund method with the view to converting it into the bisenamine which was to be degraded to the ketoaldehyde by ozonolysis.[13] This approach, however, failed because the dialdehyde VI (R = H) apparently underwent an intramolecular aldol condensation under the conditions for enamine formation.



The Baeyer–Villiger degradation of the diketone VII provided a satisfactory solution to the problem at hand. Two methods were examined for the preparation of the diketone. When the ingenious method of McElvain and McKay[14] was applied to the diacid V (R = Ac), a crystalline epimeric (at C-20) mixture of ketones VII was produced in 69% yield or in 60% over-all yield from V (R = H). Because ketene dimethylacetal is not readily accessible and because of its tendency to undergo almost explosive polymerization in the presence of acid, another approach was developed for use in larger scale preparations (see below).

The first alternative chosen was the di-*t*-butyl malonate method[15] which had been studied by Martin[16] in the model series VI (R = OH) → VI (R = CH_3), but this gave capricious results. However, the method of Bowman,[17] utilizing dibenzyl malonate, proceeded well[16] and was then applied to the tetracyclic series. Thus a solution of the diacid V (R = Ac) in tetrahydrofuran was treated with sodium hydride to form the sodium salt, then with oxalyl chloride to produce the acid chloride[18] which was isolated only in a crude form before condensation with sodio dibenzyl malonate. After hydrogenolysis and decarboxylation, the crystalline epimeric mixture of diketones VII was obtained in 54% yield. Chromatography and fractional crystallization of this mixture effected separation into isomer A, m.p. 172–173°, and isomer B, m.p. 145–146°. Treatment of the latter isomer with sodium acetate in methanol, followed by reacetylation and then by fractional crystallization, afforded two isomers that were isolated in approximately equal amounts. Evidence for the interconvertible, epimeric nature of the 173° and 146° isomers was thus provided. The degradation of the side chains was now effected *via* the Baeyer–Villiger oxidation of the diketone VII. The crude epimeric diketone mixture was thus converted, by the use of trifluoroperacetic acid in the presence of disodium hydrogen phosphate,[19] into a crystalline epimeric mixture of triacetates VIII (R^1 = R^2 = R^3 = Ac) in yields as high as 83%. Since the Baeyer–Villiger rearrangement is known to proceed with retention of configuration,[20] it was not surprising to find that diketone A afforded a single form of triacetate A, m.p. 140–141°; and that diketone B gave triacetate B, m.p. 142–143°.

Completion of Ring D.—At this point in the discussion a digression is made in order to describe work on a method of forming ring D which, although abandoned because of low yields, nevertheless paved the way to a successful approach. This preliminary study, moreover, afforded some evidence for the configuration at C-20 in the A and B series. The approach envisaged hydrolysis of the triacetate VIII (R^1 = R^2 = R^3 = Ac) to the trihydroxy compound VIII (R^1 = R^2 = R^3 = H), oxidation to the diketoaldehyde IX (R = H), followed by cyclization to yield the desired pentacyclic substance X.



The hydrolysis of triacetate VIII (R^1 = R^2 = R^3 = Ac) posed a new problem in that, if the lactone ring

(13) *Cf.* M. E. Herr and F. W. Heyl, *J. Am. Chem. Soc.*, **74**, 3627 (1952); **75**, 1918 (1953).

(14) S. M. McElvain and G. R. McKay, Jr., *ibid.*, **78**, 6086 (1956). This method involves reaction of the acid chloride with ketene dimethylacetal to give the acylketene acetal, $RCOCH{=}C(OCH_3)_2$, which is hydrolyzed first to the β-keto ester, then to the β-keto acid which is decarboxylated.

(15) G. S. Fonken and W. S. Johnson, *ibid.*, **74**, 831 (1952).

(16) D. G. Martin, Ph.D. Thesis, University of Wisconsin, 1957.

(17) R. E. Bowman, *J. Chem. Soc.*, 325 (1950).

(18) A. L. Wilds and C. H. Shunk, *J. Am. Chem. Soc.*, **72**, 2388 (1950).

(19) W. D. Emmons and G. B. Lucas, *ibid.*, **77**, 2287 (1955).

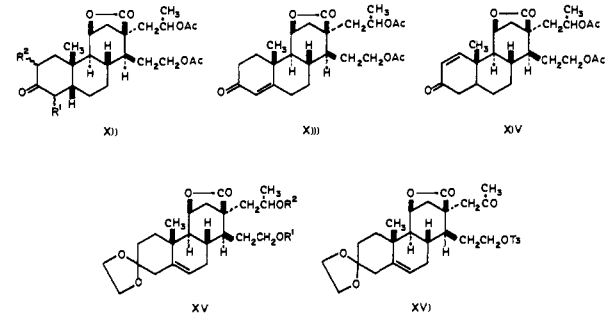
(20) See C. H. Hassal, *Org. Reactions*, **9**, 73 (1957).

were opened, on reclosure three lactones (two γ and one δ) could theoretically be formed. In an effort to obviate this anticipated difficulty, conditions were sought for the hydrolysis which would not be severe enough to open the lactone ring. With triacetate A, methanolic potassium hydroxide at room temperature effected hydrolysis without the formation of appreciable acidic material. Thus the triol A (VIII, $R^1 = R^2 = R^3 = H$) was produced in 73% yield, m.p. 175–176°, after purification. Acetylation regenerated triacetate A, showing that no lactonic disproportionation had occurred. Similar treatment of triacetate B gave less promising results; however, acid-catalyzed methanolysis gave triol B in 80% yield, m.p. 184–185°, after purification. Acetylation of this triol regenerated the triacetate B. The acid-catalyzed methanolysis also converted triacetate A into triol A in good (86%) yield, so that this was the preferred method for hydrolyzing the mixture of epimers. It is noteworthy that when the triacetate B was saponified under conditions which gave considerable opening of the lactone ring (see above) and the alkali-soluble fraction was treated with acid in order to effect relactonization, the product of this treatment was mainly, but not exclusively, triol B, showing that the 11→18-lactone is the most stable of the three possibilities.

Oxidation of triol A (VIII, $R^1 = R^2 = R^3 = H$) with Sarett reagent (chromium trioxide in pyridine) under anhydrous conditions gave a 28% yield of acidic material IX (R = OH) and a non-crystalline neutral product which was clearly aldehydic as was shown by the absorption at 3.68 μ in the infrared spectrum. Treatment of this neutral fraction with sodium acetate in acetic acid effected ring closure to produce in 29% over-all yield the pentacyclic compound X, m.p. 256–259°, after purification, λ_{max} 236.6 mμ (ϵ 7,947). From the extinction coefficient of the ultraviolet spectrum of the crude cyclized product, it was estimated that the substance X was produced in about 41% yield. In contrast with the behavior in the A-series, the corresponding neutral fraction obtained in about 80% yield on oxidation of triol B gave, after similar sodium acetate treatment, a mixture which contained a maximum of only 29% of cyclized material X. The major product, which was isolated in about 30% yield, melted after purification at 305–315° and exhibited no appreciable absorption in the ultraviolet region. Alkaline hydrolysis of this product afforded an acidic material which still retained the γ-lactone residue as shown by the absorption at 5.71 μ in the infrared. On heating, this acidic material re-formed the high-melting neutral product which was therefore assigned the dilactonic structure XI.

The isolation of the dilactone in the B- but not the A-series provides a clue to the configuration at C-20. Qualitative rate studies on model compounds indicated that Sarett oxidation of primary alcohols proceeds much more rapidly than of secondary. In the oxidation of the triols, therefore, the diol aldehyde would be the primary product. This substance would be expected to be in equilibrium with the lactol F. The evidence set forth above suggests that this equilibrium is more in favor of lactol F in the B- than in the A-series. If the reasonable assumption is made that the geometry shown in expression F represents a major contributing conformation of the lactol, then it follows that the isomer where $R^1 = CH_3$ and $R^2 = H$ will be less stable than its epimer where $R^1 = H$ and $R^2 = CH_3$ because of the destabilizing 1,3-diaxial interaction between C-21 and C-18 in the former epimer. On the basis of this presumptive argument, the relative configuration at C-20 in the epimeric series described above may be tentatively assigned as being related to the configuration shown in formula F with $R^1 = CH_3$ and $R^2 = H$ for the A- and $R^1 = H$ and $R^2 = CH_3$ for the B-series. When the oxidation–cyclization sequence was carried out on the mixture of epimeric triols, it proved to be extremely difficult to separate the desired substance X from the dilactone XI. Some attention was given to the possibility of minimizing dilactone formation (in the B-series) by treating the triol first with N-bromosuccinimide to oxidize preferentially the secondary alcoholic groups[21] before treating with Sarett reagent. In experiments with triol B it was thus possible to raise the yield significantly. Although ultraviolet spectral analysis indicated a maximum yield of 49%, the product was still contaminated with enough of the dilactone to render isolation of the desired product X difficult. The two-step oxidation procedure also resulted in an increase in yield from 29 to 38% of X from triol A. This result suggests that some of the dilactone, although not isolated, may have been formed in the direct oxidation of triol A. The approach to aldosterone *via* the substance X thus clearly has possibilities, but was not pursued further in view of more promising results in the scheme described below.

The discussion now reverts to the major synthetic sequence. Before completing ring D it was considered desirable to introduce the unsaturated ketone system in ring A. Mild saponification of the triacetate VIII ($R^1 = R^2 = R^3 = Ac$) with aqueous methanolic potassium carbonate—presumably to give mainly the dihydroxy acetate VIII ($R^1 = R^2 = H$, $R^3 = Ac$)—followed by treatment of the crude hydrolysate with N-bromoacetamide gave, after reacetylation, the keto diacetate XII ($R^1 = R^2 = H$). Thus in the A-series an isomer was produced in 50% yield, m.p. 135–136°, after purification; and in the B-series the epimer was obtained in 61% yield, m.p. 147–148.5°, after purification. From the mixture of triacetates, yields of 55–62% of the mixture of epimeric keto diacetates XII ($R^1 = R^2 = H$) were obtained.



The next step involved conversion of the keto diacetate XII ($R^1 = R^2 = H$) into the unsaturated ketone XIII. In the A-series, bromination in acetic acid followed by dehydrobromination with lithium chloride in dimethylformamide[22] afforded the unsaturated ketone A (XIII) in 64% yield, m.p. 182.5–183.5°, after purification. In addition to the unsaturated ketone, a chloro ketone XII ($R^1 = H$, $R^2 = Cl$) was isolated in 14% yield, m.p. 200–208°. That the chloro group was probably located at C-2 follows from the fact that further treatment under dehydrohalogenation conditions of a fraction containing this chloro ketone yielded a product which absorbed at 230 mμ in the ultraviolet region, indicative of the Δ^1-(XIV) rather than the Δ^4-

(21) R. E. Jones and F. W. Kocher, *J. Am. Chem. Soc.*, **76**, 3682 (1954); T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

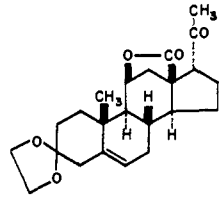
(22) R. P. Holysz, *ibid.*, **75**, 4432 (1953).

unsaturated ketone. In the B-series better results were obtained when lithium bromide was employed in place of the chloride. Thus the Δ^4-unsaturated ketone XIII was obtained in 52% yield, m.p. 154–155°, after purification. The Δ^1-unsaturated ketone XIV was also isolated in 28% yield, m.p. 167–168°, after purification. From the mixture of ketones XII-A and XII-B ($R^1 = R^2 = H$) (lithium bromide method), the epimeric mixture of Δ^4-unsaturated ketones XIII was produced in 53% yield along with the Δ^1-unsaturated ketone XIV in 34% yield. Catalytic hydrogenation of the crude Δ^1-unsaturated ketone regenerated the epimeric mixture of keto diacetates XII ($R^1 = R^2 = H$) in 73–87% yield, which was thus available for reconversion to XIII.

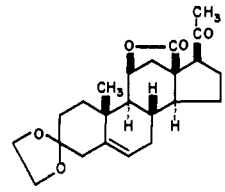
The unsaturated ketone XIII was next transformed into the ketal XV ($R^1 = R^2 = Ac$) by the exchange method with 2-methyl-2-ethyl-1,3-dioxolane.[23] The A-ketal was thus formed in 62% yield (26% of starting material being recovered) and melted at 155–156°, after purification. The B-ketal was formed in 58% yield (21% of starting material being recovered) and melted at 174.5–175°, after purification. From the epimeric mixture of unsaturated ketones, a crude crystalline ketal mixture was produced in 72% yield, the balance of the material representing crude recovered unsaturated ketone.

Saponification of the ketal diacetate XV ($R^1 = R^2 = Ac$) with methanolic potassium hydroxide appeared to be attended by some lactonic disproportionation, possibly to form the 18→20-lactone. However, representative specimens of the A-isomer of XV ($R^1 = R^2 = H$), m.p. 185–185.5°, and of the B-isomer, m.p. 206–208.5°, were thus obtained. In the B-series this treatment gave about 25% of base-soluble material which relactonized only after acidification of the solution. On the other hand, in the A-series very little alkali-soluble material was produced. The production of acidic material could largely be eliminated by conducting the saponification with potassium carbonate in aqueous methanol. The mixture of A and B isomeric ketal diacetates was thus converted in 94% yield into neutral material consisting largely of the epimeric diols XV ($R^1 = R^2 = H$).

The plan was to close ring D by the method of Johns, Lukes and Sarett,[24] *i.e.*, cyclization of the keto *p*-toluenesulfonate XVI to give XVII. In order to prepare the former compound we investigated selective tosylation of the ketal diol XV ($R^1 = R^2 = H$) at the primary hydroxyl group followed directly by oxidation with Sarett reagent. After extensive study of the esterification step with different arylsulfonyl chlorides, it was shown that mesitylenesulfonyl chloride, which yielded a keto ester, m.p. 161–162°, offered no significant advantage over *p*-toluenesulfonyl chloride and that good yields of selectively esterified material were realized with the latter reagent if the reaction mixture was fairly dilute and the reaction temperature was low (8°). In order to prevent some hydrolysis of the ketal residue, it was also found necessary to utilize low temperatures so long as water was present during the isolation of the product and to maintain very anhydrous conditions during the esterification and oxidation steps. Under the most favorable conditions found, crystalline keto *p*-toluenesulfonate XVI was obtained in yields as high as 86% over-all from the ketal–diol mixture. A purified specimen melted at 113–114° dec. It is noteworthy that at this stage of the synthesis the asymmetric carbon atom at C-20 is eliminated and the A- and B-series therefore converge.



XVII



XVIII

Of the various conditions for cyclization of the keto tosylate XVI which were examined, potassium *t*-butoxide in *t*-butyl alcohol[24] proved to be best. The yields, however, were not high and varied from 30–54%, apparently depending upon the purity of the keto tosylate. The pentacyclic product XVII was thus obtained in yields up to 43% over-all in three steps from the ketal–diol mixture. This method of cyclization has been shown[24] to proceed stereospecifically to give the 17α-acetyl compound XVII even though the 17β-epimer is probably the more stable form. In our work the 17α-isomer was clearly the major product of cyclization under the preferred conditions, but with prolonged treatment or the use of enolizing media such as dimethyl sulfoxide[25] the 17β-epimer is produced also. Both of these epimers were previously known from the work of Wettstein[26] and have also been isolated in the present study. The 17α-epimer melted at 195–199° with transition points at 133–139° and 150–155°, which is in agreement with the reported properties.[26] The 17β-epimer was obtained in a form melting at 199–207°. On admixture with the Wettstein specimen[27] the melting point was not depressed. The infrared spectra of the two specimens were identical. Confirmation of the identity of our material with that of the Swiss workers was afforded by acid-catalyzed hydrolysis of the 17β-epimer to yield the unsaturated diketone, m.p. 215–218°, undepressed on admixture with a sample[27] (m.p. 211.5–217°) from the Wettstein synthesis.[26] The infrared spectra of these two specimens of the unsaturated diketone were identical. Similar hydrolysis of the 17α-epimer yielded the corresponding unsaturated diketone, m.p. 224.5–228° (reported[26] 224–227°).

We established that the 17α-epimer XVII can be transformed in part into the 17β-epimer by methoxide-catalyzed equilibration.[28] With this conversion, our synthesis completes a pathway to aldosterone *via* the concluding stages of the Wettstein scheme which utilizes the 17β-isomer XVIII. This approach, however, was attended by one serious drawback due to the β-orientation of the 17-substituent which at the last step in the synthesis became involved in an irreversible cyclization involving C-18.[26,29] We therefore elected to take advantage of the stereoselective production of the 17α-isomer XVII in our synthesis and to proceed with the groups so oriented that the undesired cyclization involving C-18 would be precluded. We thus envisaged producing 17α-aldosterone which we ex-

(23) H. J. Dauben, Jr., B. Löken and H. J. Ringold, *J. Am. Chem. Soc.*, **76**, 1359 (1954).

(24) W. F. Johns, R. M. Lukes and L. H. Sarett, *ibid.*, **76**, 5026 (1954).

(25) *Cf.* D. J. Cram, B. Rickborn and G. R. Knox, *ibid.*, **82**, 6412 (1960).

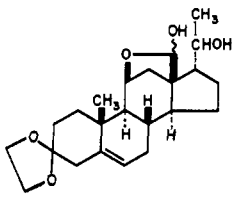
(26) (a) J. Schmidlin, G. Anner, J.-R. Billeter and A. Wettstein, *Experientia*, 11, 365 (1955); (b) J. Schmidlin, G. Anner, J.-R. Billeter, K. Heusler, H. Ueberwasser, P. Wieland and A. Wettstein, *Helv. Chim. Acta*, **40**, 1438 (1957).

(27) We wish to thank Dr. Wettstein for supplying us with comparison specimens of the materials from his laboratory.

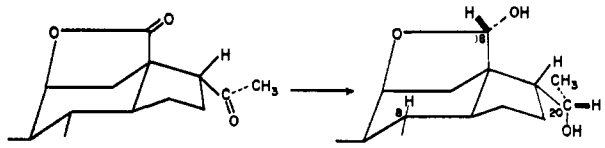
(28) These are the same conditions that were employed by the Swiss workers (see ref. 26) to convert the 17β- into the 17α-isomer.

(29) Since the completion of our alternative approach (see below), J. Schmidlin, G. Anner, J.-R. Billeter, K. Heusler, H. Ueberwasser, P. Wieland and A. Wettstein, *Helv. Chim. Acta*, **40**, 2291 (1957), have found conditions whereby this undesired cyclization can be essentially eliminated.

pected to be readily isomerized to aldosterone. A description of this study follows.

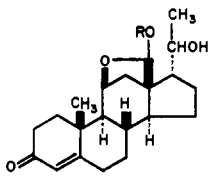


XIX

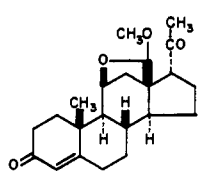


G H

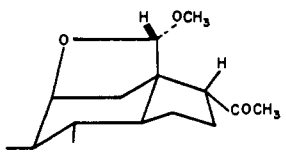
Treatment of the 17α-acetyl ketal XVII with 2 mole-equivalents of lithium aluminum hydride in tetrahydrofuran at room temperature afforded the lactol ketal XIX, m.p. 190–194°, in 82% yield. Since the pure material melted at 193–194°, the reduction, which introduces two new asymmetric centers, was evidently highly stereoselective. It is tentatively proposed that the configuration of this product is that represented by formula H. The preferred conformation of the lactone (see formula G) suggests that the approach of hydride toward the acetyl group would be from the less hindered (right) side, resulting in the configuration shown in formula H. The preferred direction of hydride approach to the C-18 carbonyl group would be from the outside (*exo*), and the initially formed *endo*-hydroxy substance would be expected to isomerize readily *via* the open-chain (aldehyde) form into the *exo*-hydroxy isomer H which is more stable because the hydroxyl at C-18 is directed away from the axial 8β-hydrogen atom.



XX

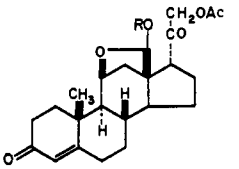


XXI



J

Treatment of the lactol ketal XIX with aqueous acetone and *p*-toluenesulfonic acid effected hydrolysis of ketal residue to give the lactol unsaturated ketone XX (R = H) in high yield. The purified material, m.p. 247–248.5° dec., evidently contained water of crystallization as shown by carbon–hydrogen analysis. In order to protect the lactol residue during subsequent steps, the substance XX (R = H) was converted by treatment with methanol and *p*-toluenesulfonic acid into the lactol methyl ether XX (R = CH_3), m.p. 168–169.5°. Oxidation of this substance with Sarett reagent provided in 76% yield the lactol methyl ether XXI of DL-21-desoxy-17α-aldosterone, m.p. 166.5–168°, after purification. As to the question of the configuration of the diketo lactol ether XXI at C-18, the same stability considerations set forth above for the lactol XIX suggest that the methoxy group is in the more stable *exo* configuration as depicted in formula J. Some evidence supporting this view was afforded by preliminary equilibration studies under basic conditions which indicated that the 17α- was more stable than the 17β-epimer (ratio about 3:1 at equilibrium). Inspection of molecular models suggests that the 17β-epimer is hindered relative to the 17α-form only when the methoxyl at C-18 is in the *exo* configuration.



XXII

The 21-acetoxy group was introduced into the diketo lactol ether XXI by a procedure especially developed for performing this conversion in the presence of the sensitive 3-keto-Δ^4-unsaturated system.[30] Pilot experiments on the conversion of progesterone to desoxycorticosterone acetate were carried out to demonstrate that the method could be adapted to a smaller (21-mg.) scale than reported.[30] Application to the diketo lactol ether XXI afforded the 21-acetoxy compound XXII (R = CH_3) in about 20% yield, and about 30% of the starting material was recovered.[31] An analytical specimen of this material was not obtained; instead the crude product was hydrolyzed under mild conditions, *i.e.*, warm 70% aqueous acetic acid, to give DL-17α-aldosterone acetate (XXII, R = H), m.p. 166–169°. Although the reported[32] melting point for this material is 158.5–162°, an infrared spectrum of the latter product kindly provided by Dr. Wettstein was identical with that of our own material.

With DL-17α-aldosterone acetate in hand, there remained only the problem of epimerizing the C-17 side chain in order to complete the synthesis. Wettstein and his collaborators[32] had already described conditions for epimerizing aldosterone partially into the 17α-isomer by equilibration with 0.1 *N* potassium carbonate at room temperature. This procedure was therefore applied to our synthetic material. With 100-γ quantities it was possible to ascertain by paper chromatography that the acetate was undergoing hydrolysis and that an equilibrium between the C-17 epimers was also being established. The treatment was then repeated on a 1.1-mg. scale, and the products were separated by paper chromatography. Elution of the aldosterone fraction gave DL-aldosterone. The melting point of this material is not characteristic,[32] but our material was identified unequivocally by comparison of the infrared spectrum with that of authentic aldosterone,[33] by its identical R_f value on paper chromatography and by its mineralocorticoid activity which proved to be the same as that of the Wettstein racemic aldosterone.[34]

(30) J. A. Hogg, P. F. Beal, A. H. Nathan, F. H. Lincoln, W. P. Schneider, B. J. Magerlein, A. R. Hanze and R. W. Jackson, *J. Am. Chem. Soc.*, **77**, 4436 (1955).

(31) Several unsuccessful attempts were made to apply the attractive and more direct C-21 acetoxylation method of H. J. Ringold and G. Stork *ibid.*, **80**, 250 (1958)), and also as modified by O. Halpern and C. Djerassi (*ibid.*, **81**, 439 (1959)) and by E. S. Rothman, T. Perlstein and M. E. Wall (*J. Org. Chem.*, **25**, 1966 (1960)).

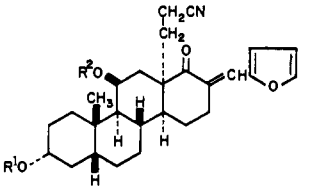
(32) J. Schmidlin, G. Anner, J.-R. Billeter, K. Heusler, H. Ueberwasser, P. Wieland and A. Wettstein, *Helv. Chim. Acta*, **40**, 2291 (1957).

(33) We are indebted to Dr. R. N. Jones of the National Research Council of Canada for performing the solution infrared comparison on our limited supply of material.

(34) We wish to thank Professor E. Gordon of the University of Wisconsin Medical School for performing the physiological tests.

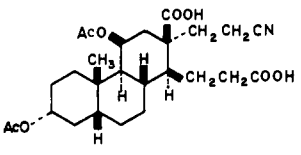
Investigation of an Alternative Approach

This part of our report contains a description of a study of another pathway for forming ring D. This phase of our work was terminated with the production of the pentacyclic aldehyde XXXVI which has obvious advantages for the completion of the synthesis of aldosterone. Even though we were thus very close to the conclusion of an alternative approach to the hormone, this study was discontinued because of the announcement of the partial synthesis.[2]

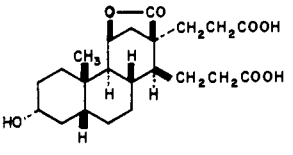


XXIII

The furfurylidene derivative III was allowed to participate in the Michael condensation with β-methoxypropionitrile under conditions described above for the higher homolog. The major product, which was isolated in 63% yield by chromatography, was shown (see below) to be the adduct XXIII ($R^1 = R^2 = H$), m.p. 192–193°, after purification. When acrylonitrile was used directly, without pretreatment to form β-methoxypropionitrile, the yield by direct crystallization was 40%. The remainder of the material was acetylated with pyridine and acetic anhydride, and on chromatography an additional 9% of the adduct was isolated as the diacetate XXIII ($R^1 = R^2 = Ac$), m.p. 236–236.5°, after purification. This same material could be produced in 85% yield directly from the dihydroxy adduct. A second product was isolated, also in 9% yield, from the chromatography of the acetylated residues, and compositional analysis showed that this material, m.p. 218–219°, was the product of the addition of 2 mole-equivalents of acrylonitrile. Since this substance was stable to boiling methanolic sodium bicarbonate—conditions which are known to effect hydrolysis of a 3-acetate group—the structure is undoubtedly correctly represented by formula XXIII ($R^1 = CH_2CH_2CN$, $R^2 = Ac$).

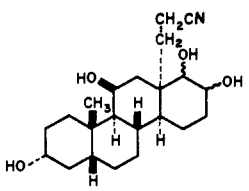


XXIV

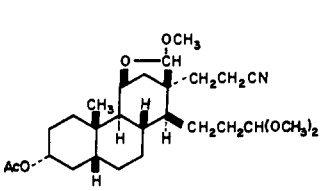


XXV

Evidence for the configuration of the Michael adduct was provided by ozonolyzation of the diacetate XXIII ($R^1 = R^2 = Ac$) followed by treatment with hydrogen peroxide which afforded, in 77% yield, a diacid (XXIV), m.p. 201–204°, after purification. The configuration of this diacid and in turn of its precursor was shown by hydrolysis with aqueous potassium hydroxide. On acidification of the alkaline solution, a lactonic diacid, m.p. 268–269°, was produced, thus proving that the cyanoethylation process occurred on the α-side of the nucleus to produce XXIII ($R^1 = R^2 = H$).



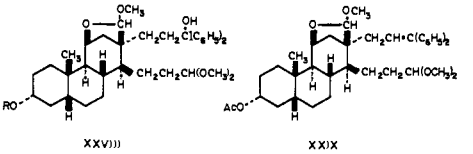
XXVI



XXVII

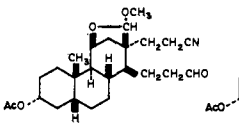
As contrasted with the approach described in the first part of this paper, the present scheme envisaged the cleavage of ring D in such a way as to produce an intermediate having the C-18 carbon in the desired aldehydic (rather than the carboxylic acid) state of oxidation. To this end the Michael adduct was reduced with sodium borohydride to the 17a-hydroxy compound, m.p. 200–200.5°, with the view to converting it or its acetate, by ozonolysis, into the 17-keto-17a-hydroxy substance which was to be submitted to periodate cleavage. Unfortunately the ozonolysis experiments gave unpromising results so we turned our attention to producing the ring D glycol XXVI. This last substance was readily prepared by prolonged treatment of the ozonide of XXIII ($R^1 = R^2 = Ac$) with sodium borohydride. The product evidently was a mixture of stereoisomers from which one pure form was isolated as the hydrate, m.p. 137–140°. The tetrol mixture XXVI was converted by treatment with sodium metaperiodate, then with methanol and acid, followed by acetic anhydride and pyridine into the acetal lactol ether XXVII, m.p. 126–127°. In the first experiments considerable carbonyl-containing material (as shown by infrared spectroscopy) remained after the borohydride treatment and, in addition, significant amounts of γ-lactone were found after the periodate treatment. By appropriate modification of conditions, a procedure was finally developed which minimized these effects and afforded the desired substance XXVII in 60% over-all yield from XXIII ($R^1 = R^2 = Ac$).

With the lactol ring in hand in the form of the ether XXVII, it remained only to degrade the side chains appropriately to a substance such as the dialdehyde XXXV which would then be expected to undergo cyclization[35] to complete ring D and to produce the substance XXXVI. An attempt to form a piperidine enamine of the aldehydic precursor of XXVII resulted in what appeared to be an aldol cyclization between the C-18 aldehyde (after liberation by opening of the lactol ring) and the methylene group alpha to the side-chain aldehyde. Also attempts to form an enol acetate of the dialdehyde did not show promise. It therefore appeared preferable to work with the more stable acetal lactol ether XXVII. The experiments described directly below constitute some attempts, only partially successful, to degrade this substance.

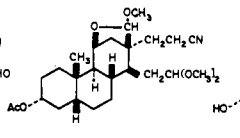


XXVIII

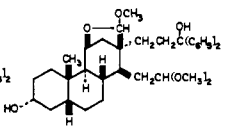
XXIX



XXX



XXXI

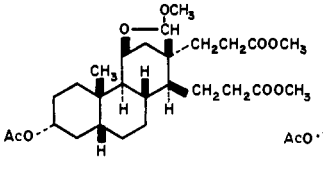


XXXII

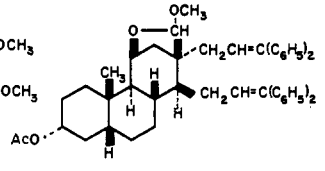
With the view to degrading the cyanoethyl group of acetal XXVII by the Barbier–Wieland method, this substance was treated with aqueous potassium hydroxide to hydrolyze the nitrile, followed by diazomethane to yield the ester which, without isolation, was submitted to reaction with phenyllithium. The carbinol XXVIII (R = H) was thus obtained in 63% over-all yield from XXVII and could be obtained in a form, m.p. 101–103°, containing acetone of crystallization. After numerous unsuccessful attempts to

(35) *Cf.* R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, *J. Am. Chem. Soc.*, **74**, 4223 (1952).

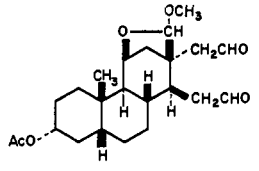
dehydrate the carbinol, success was finally realized by applying a modification of the procedure of Roberts, Shoppee and Stephenson.[36] The 3-acetate XXVIII (R = Ac) was thus converted by treatment with thionyl chloride in pyridine into the corresponding chloro compound which, on heating in pyridine, underwent dehydrohalogenation to produce, in 88% yield, the olefin XXIX, m.p. 159–160°. It was planned next to liberate the aldehyde group by selective hydrolysis of the acetal without attacking the lactol ether. Using the more readily available cyano compound XXVII, we found conditions, namely 70% acetic acid for 16 hr. at room temperature, for effecting this selective hydrolysis to give the aldehyde XXX in 80% yield, m.p. 83–85°, after purification. Unfortunately, however, attempts to apply this procedure to the hydrolysis of the olefin XXIX gave unpromising results. Since the aldehyde XXX was readily available, we turned our attention to a study which envisaged initial degradation of the aldehydo side chain by the method of Herr and Heyl,[13] followed by application of the Barbier–Wieland method as above. This aldehyde was converted into the piperidine enamine which was treated with ozone followed by zinc in acetic acid. The noraldehyde was not isolated but was converted directly with methanol and acid into the acetal XXXI, m.p. 150–152°. The over-all yield from XXX was 34%. The cyanoacetal XXXI was then saponified, converted into the ester, and treated with phenyllithium to give, in 91% yield, the carbinol XXXII, m.p. 116–119°, after purification. This last substance was acetylated to protect the 3-position and then submitted to the dehydration conditions described above. The oxidation of this olefin to the desired dialdehyde XXXV has not been properly studied. Although this approach still has promise, it was discontinued because of the low yields in the enamine degradation and because the simultaneous degradation of the two side chains (see below) promised to be more efficient.



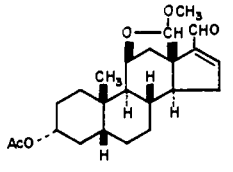
XXXIII



XXXIV



XXXV



XXXVI

In the successful approach, a double Barbier–Wieland degradation was carried out on the diester XXXIII (m.p. 117–118.5°, after purification) which was produced in 60% yield from the cyanoaldehyde XXX by the steps: oxidation, with chromium trioxide in pyridine containing a small amount of water, to the acid; saponification of the nitrile with aqueous potassium hydroxide; esterification with diazomethane; and acetylation at C-3 with pyridine and acetic anhydride. Treatment of the diester with excess phenyllithium followed by reacetylation with pyridine and acetic anhydride gave the diol which was not obtained crystalline but was dehydrated directly as described above to give, in 76% yield, the diene XXXIV, m.p. 181–183°, after purification. Oxidation of the diene to the dialdehyde proved to be a difficult problem. Hydroxylation with osmium tetroxide evidently proceeded abnormally since the amorphous product could not be cleaved readily either with sodium periodate or lead tetraacetate. Attention was then directed to the study of the degradation on a model system, namely 3,12 - diacetoxy - bis - norcholanyldiphenylethylene.[37] After a number of unsuccessful experiments with various oxidizing agents, it was discovered that ozonization under controlled conditions gave yields as high as 67% on a 50-mg. scale. The ozonization was carried out in the Rubin ozonizer[38] with standard solutions of ozone in methylene chloride. When applied to the diene XXXIV, this method yielded some of the dialdehyde XXXV which, due to its sensitivity, was not isolated but treated directly with piperidine and acetic acid in benzene[26] to effect cyclization to the more stable unsaturated aldehyde XXXVI. In this way crude material, m.p. 160–165°, with softening at 80°, was obtained in 24% yield, λ_{max} 237 mμ (ε 6,300). Further studies showed that the yield could be improved by carrying out the ozonization in the presence of methanol which serves to trap the intermediary zwitterion as the hydroperoxy methyl ether.[39] The diene XXXIV was thus converted, on ozonization in a solution of methylene chloride and methanol, followed by treatment with zinc dust and acetic acid at 0°, and then cyclization with piperidine and acetic acid in benzene, into the pentacyclic aldehyde XXXVI. The yield of crude material was 45% and on a single crystallization; material, m.p. 202–204°, λ_{max} 238–239 mμ (ε 9,600), was obtained in 34% yield.

Experimental

DL-3α,11β-Dihydroxy-13,14-dehydro-18-nor-D-homo-5β-androstane.[10]—A 190-g. sample of DL-1-methoxy-8α-acetoxy-10aβ-methyl-4bβ,5,6,6aβ,7,8,9,10,10a,10bα-decahydrochrysene[9] (see partial formula D), m.p. 144–145°, was treated with perbenzoic acid, then divided and reduced in two equal portions with lithium and alcohol in ammonia according to procedures already described.[9] In the final chromatography of one of these portions, elution with benzene afforded 26.8 g. of amorphous material. Rechromatography of this fraction on 100 g. of Florisil gave, in addition to some of the dihydroxy ketone, 15.8 g. of amorphous material eluted with benzene. Crystallization of the latter fraction from benzene afforded 2.2 g. (first crop) of material, m.p. 152.5–154°; 2.3 g. (second crop), m.p. 148.5–149.5°; and 1.3 g. (third crop), m.p. 151.5–153.5°. Three recrystallizations of the first-crop material from benzene gave colorless rods, m.p. 156.2–157.3°, $\lambda_{max}^{CH_2Cl_2}$ 2.75 μ (OH).

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.9; H, 10.3.

The ultraviolet spectrum of this substance exhibited only end absorption, and the n.m.r. spectrum showed no absorption corresponding to a vinylic hydrogen. It gave a weakly positive test with tetranitromethane and slowly absorbed a total of 1.04 mole-equivalents of hydrogen over rhodium-on-alumina catalyst in the presence of a trace of perchloric acid.

The diacetate, prepared by the isopropenyl acetate method, crystallized from methanol as colorless needles, m.p. 113.2–113.9°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.8; H, 9.1.

DL-3α,11β-Dihydroxy-17-furfurylidene-18-nor-D-homo-5β-androstane-17a-one (III).—A solution of 27.0 g. of 3α,11β-dihydroxy-18-nor-D-homo-5β-androstane-17a-one,[9] m.p. 169–173°, in 1 l. of methanol was treated with 54 ml. of freshly distilled furfural and 400 ml. of 15% aqueous potassium hydroxide (nitrogen atmosphere) as described previously.[40] The crude product amounted to 28.5 g. (first crop), m.p. 191–194°; and 1.0 g. (second crop), m.p. 193–194°. The total yield in this case was 86%; however, in other runs yields as high as 96% were realized.

(36) G. Roberts, C. W. Shoppee and R. J. Stephenson, *J. Chem. Soc.*, 2705 (1954).

(37) B. Riegel, R. B. Moffett and A. V. McIntosh, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 237.

(38) W. L. Meyer, D. D. Cameron and W. S. Johnson, *J. Org. Chem.*, **27**, 1130 (1962).

(39) R. Criegee, *Ann.*, **583**, 9 (1953).

(40) W. S. Johnson, B. Bannister and R. Pappo, *J. Am. Chem. Soc.*, **78**, 6331 (1956).

Repeated recrystallizations of a specimen from methanol and from acetone–petroleum ether afforded colorless plates, m.p. 193–194°, $\lambda_{max}^{95\%\ EtOH}$ 324 mμ (ε 22,910).

Anal. Calcd. for $C_{24}H_{32}O_4$: C, 74.96; H, 8.39. Found: C, 73.5; H, 8.7.

This analysis is in good agreement with the product containing one-half a molecule of water of crystallization: Calcd. for $C_{24}H_{32}O_4 \cdot 1/2\ H_2O$: C, 73.25; H, 8.45.

DL-1β-(2-Carboxyethyl)-2α-(2-carboxypropyl)-4β,7α-dihydroxy-4bβ-methyl-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone Acetate (V, R = Ac). (a) **Michael Reaction of the Furfurylidene Ketone with Methacrylonitrile.**—To a solution of 1.1 g. of sodium hydride in 58 ml. of anhydrous methanol (nitrogen atmosphere) were added 37 ml. of freshly distilled methacrylonitrile and 48 ml. of tetrahydrofuran (freshly distilled from lithium aluminum hydride), and the mixture was allowed to reflux for 20 min. and then cooled to room temperature. A 37.2-g. sample of the aforementioned furfurylidene ketone, m.p. 196–200°, was added to the mixture with an additional 20 ml. of tetrahydrofuran, and the resulting slurry was allowed to reflux (under nitrogen) for 10 hr. (after 30 min. the solution was homogeneous). The solution was cooled, acidified with 5.5 ml. of glacial acetic acid, diluted with 1:1 ethyl acetate–ether, washed in turn with water, 5% sodium hydroxide solution, again with water, and finally with saturated brine. The organic solution was dried over anhydrous sodium sulfate and the solvent removed by distillation at reduced pressure. The residue amounted to 44.5 g. of amber semicrystalline material, $\lambda_{max}^{95\%\ EtOH}$ 330.2 mμ (ε 18,000).

In preliminary experiments an attempt was made to separate the pure epimers by a combination of chromatography on Florisil and crystallizations from methyl ethyl ketone. In this way it was possible to separate one epimer in an impure state, m.p. 224–234°, and another as a relatively pure substance, m.p. 213–216°. The latter isomer was analyzed.

Anal. Calcd. for $C_{28}H_{37}NO_4$: C, 74.47; H, 8.26; N, 3.10. Found: C, 74.4; H, 8.2; N, 2.9.

(b) **Acetylation.**—The 44.5-g. crude product of the preceding experiment was dissolved in 1.35 l. of freshly distilled isopropenyl acetate by refluxing for 4 hr. The solution was cooled, 4.6 g. of *p*-toluenesulfonic acid monohydrate dissolved in 90 ml. of isopropenyl acetate was added, and the resulting mixture was allowed to stand at room temperature for 48 hr. The resulting cherry-red solution was diluted with an equal volume of ethyl acetate and washed with saturated sodium bicarbonate solution, water, brine, and then dried over anhydrous sodium sulfate. The solvent was distilled under reduced pressure, and the last traces of isopropenyl acetate were removed from the residue by co-distillation with two portions of benzene. The residue amounted to 50.3 g. of tan foam which showed no absorption for the hydroxyl group in the infrared spectrum.

In preliminary experiments an attempt was made to separate the epimeric diacetates IV (R = Ac) by a combination of chromatography on Florisil and crystallization. From the early fractions on the chromatogram which were eluted with 10% ether in benzene, there was obtained by crystallization from methyl ethyl ketone, colorless material, m.p. 228–232°, $\lambda_{max}^{95\%\ EtOH}$ 332.9 mμ. A final crystallization from methanol containing a trace of methyl ethyl ketone afforded colorless material which melted at about 207°, then resolidified and remelted at 234°.

Anal. Calcd. for $C_{32}H_{41}NO_6$: C, 71.75; H, 7.71; N, 2.61. Found: C, 71.6; H, 7.65; N, 2.65.

The fractions eluted with 20% ether in benzene consisted of a mixture of the epimeric diacetates. Further elution with 50% ether in benzene and then with 100% ether afforded material consisting of the second isomer, m.p. 209–212°, $\lambda_{max}^{95\%\ EtOH}$ 332 mμ. Recrystallization from methanol and then from methyl ethyl ketone gave colorless crystals, m.p. 210–213.5°.

Anal. Calcd. for $C_{32}H_{41}NO_6$: C, 71.75; H, 7.71; N, 2.61. Found: C, 71.7; H, 7.9; N, 2.3.

(c) **Ozonolysis and Saponification.**—A solution of 6.5 g. of the crude mixture of diacetates IV (R = Ac) described in the preceding section in 1.1 l. of ethyl acetate was treated with ozone at −78° until a permanent deep blue color developed. This solution was then mixed with a solution of 110 ml. of glacial acetic acid and 67 ml. of water. Then 22 ml. of 30% hydrogen peroxide was added slowly with swirling. The heterogeneous mixture was allowed to stand at room temperature for 48 hr. with occasional swirling. The organic layer was washed thoroughly with 5% aqueous ferrous sulfate (about 1 l.) until the washings were pale green. This solution was then washed with water, and the combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were cooled to −10° and extracted thoroughly with cold 20% sodium hydroxide solution. The combined alkaline extracts were heated on a steam-bath under nitrogen for 40 hr., then cooled, filtered through glass wool, and washed with ether. After cooling to −10°, the solution was acidified with concentrated hydrochloric acid until the *p*H was about 2. The acidic mixture was extracted thoroughly with ethyl acetate, and the combined organic layers were washed with water and dried over anhydrous sodium sulfate. The oily brown residue obtained on evaporation of the solvent at 30° (reduced pressure) amounted to 6.5 g. Crystallization from methyl ethyl ketone gave 1.72 g. (first crop), m.p. 247–248.5°; and 0.72 g. (second crop), m.p. 247–249°; neut. equiv. 209 (calcd. 211). An additional 0.42 g. of product, m.p. 243.5–245°, was obtained by retreating the material from the mother liquors with potassium hydroxide for 40 hr. as described above. The total over-all yield of the epimeric mixture of hydroxy diacids V (R = H) from the furfurylidene ketone was thus 48%. In other experiments, over-all yields of up to 64% were realized, and the melting point of the product ranged from 231–270°; λ_{max}^{mull} 5.7 μ (γ-lactone C=O), 5.82 (acid C=O). The epimeric mixture was separated in the form of the methyl esters as described below.

The methyl esters were prepared from the mixture of acetoxy diacids by treatment with ethereal diazomethane. Chromatography on Florisil gave, in the early fractions eluted with 20% ether in benzene, material which was obtained from methylcyclohexane–ether as colorless crystals, m.p. 120–121°. Two further recrystallizations from ether did not change the melting point.

Anal. Calcd. for $C_{23}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.6; H, 8.3.

The fractions further eluted with 20% ether in benzene up to pure ether consisted of mixtures of the two epimers. The fractions eluted with 1–5% ethanol in ether consisted mainly of the second isomer which, after recrystallization from ether, methanol, benzene and finally methyl ethyl ketone, was obtained as colorless crystals, m.p. 171–172°.

Anal. Calcd. for $C_{23}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.7; H, 8.4.

(d) **Reacetylation.**—A solution of 8.97 g. of the epimeric mixture of hydroxy diacids V (R = H), prepared as described in the preceding section, in 900 ml. of glacial acetic acid (distilled from triacetyl borate) was saturated with anhydrous hydrogen chloride and allowed to stand at room temperature for 48 hr. The solution was then filtered through glass wool, diluted with an equal volume of benzene, and the mixture was distilled under reduced pressure (bath temperature 30–40°). The last traces of acetic acid were removed from the residue by co-distillation with two 100-ml. portions of benzene. The crude acetate V (R = Ac) amounted to 10.5 g. of colorless prisms, m.p. 137–142°; λ_{max}^{mull} 5.65, 5.72, 5.85 μ. In other runs the melting point of the acetoxy diacid epimeric mixture varied from 130–156° to 149–167°. No attempt was made to separate the pure epimers. A single recrystallization of crude material from ethyl acetate gave material, m.p. 251–254°; neut. equiv. 230.5 (calcd. 232.3).

The acetoxy diacid mixture was converted to the dimethyl esters with diazomethane. Chromatography on Florisil gave, in the fractions eluted with 10% ether in benzene, an isomer, m.p. 164–168°, undepressed on admixture with material, m.p. 167–169°, prepared by acetylation of the 121° hydroxy dimethyl ester described above. The 167–169° material was obtained from 95% ethanol as colorless thick hexagonal prisms.

Further elution of the column with higher concentrations of ether afforded low-melting fractions consisting of mixtures of the two epimers. The fractions eluted with pure ether, after crystallization from methanol–acetone followed by absolute ethanol, gave colorless fluffy needles, m.p. 161–162°, undepressed on admixture with the product, m.p. 161–162°, obtained upon acetylation of the 172° hydroxy diester followed by three recrystallizations from 95% ethanol.

Anal. Calcd. for $C_{27}H_{40}O_8$: C, 65.83; H, 8.19. Found: C, 66.1; H, 8.0.

The identity of the acetoxy diesters obtained by the two different procedures showed conclusively that no rearrangement of the lactone occurred under the strongly acidic conditions which were used in the acetylation of the hydroxy diacids.

DL-1β-(2-Acetylethyl)-2α-(2-acetylpropyl)-4β,7α-dihydroxy-4bβ-methyl-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone Acetate (VII). (a) **By the Ketene Acetal Method.**—A solution of 2.30 g. of recrystallized acetoxy diacid mixture V (R = Ac) (a combination of 0.09 g. of first-crop material, m.p. 160–200°, and 2.21 g. of second-crop material, m.p. 130–156°, was used) in 200 ml. of freshly purified[41] thionyl chloride was allowed to stand for 3 days at room temperature. The thionyl chloride was then removed by distillation at reduced pressure (bath temperature below 30°), and the last traces of the reagent were removed from the residue by co-distillation with four portions of benzene. Note that it is extremely important that all traces of acidic or acid-producing materials be eliminated because they catalyze the polymerization of ketene acetal. The partly crystalline diacid chloride residue was cooled

(41) L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1957, p. 345.

in a Dry Ice–acetone-bath, then 200 ml. of cold (0°) freshly distilled dimethyl ketene acetal[14] was added (nitrogen atmosphere). The mixture was then heated rapidly with stirring to the boiling point and was maintained under reflux for 2 hr. The mixture was then distilled at reduced pressure, and the last traces of the reagent were removed by co-distillation with two portions of benzene. The resulting orange pasty residue was dissolved in 150 ml. of 1:3 ether–dioxane, a solution of 3 ml. of concentrated hydrochloric acid in 100 ml. of water was slowly added with stirring, and the resulting mixture was allowed to stir overnight at room temperature. The aqueous layer was separated and extracted thoroughly with ethyl acetate. The combined organic layers were washed with cold 10% potassium bicarbonate solution, water, then saturated brine and dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent under a stream of nitrogen (steam-bath temperature) was dissolved in 40 ml. of methanol, 80 ml. of 5% sodium hydroxide solution was added, and the mixture allowed to stand for 39 hr. at room temperature. The dark brown solution was then acidified to *p*H 2 with concentrated hydrochloric acid, warmed for 40 min. on a steam-bath, cooled and then extracted with 1:1 ethyl acetate–ether. The combined organic layers were washed with cold 5% sodium hydroxide solution, water, saturated brine and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave 1.78 g. (86% yield) of a crude amorphous mixture of the hydroxy diketones VII (H in place of Ac).

The crude hydroxy diketone was dissolved in 24 ml. of dry pyridine containing 12 ml. of acetic anhydride, and the mixture was heated on the steam-bath for 20 min., then cooled, diluted with ice and water, and extracted thoroughly with 1:1 ethyl acetate–ether. The combined organic layers were washed thoroughly with 5% hydrochloric acid followed by water, 10% potassium bicarbonate solution, and saturated brine. The solution was dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure to give 1.90 g. of a tan gummy residue which crystallized on trituration with ether. In this way 1.51 g. of tan crystals, m.p. 141–163°, was obtained. Chromatography of the residue on 20 g. of acid-washed alumina (Merck and Co.) gave, in the fractions eluted with 50% benzene in ether, an additional 0.07 g. of colorless needles, m.p. 136–142°. The over-all yield for the conversion of recrystallized acetoxy dibasic acid mixture to the crystalline mixture of acetoxy dimethyl ketones was thus 69%.

A comparable product obtained from another run was repeatedly recrystallized from ether and finally from isopropyl ether to give **diketone A** as colorless prisms, m.p. 171.5–173°; $\lambda_{max}^{CHCl_3}$ 5.70 μ (γ-lactone C=O), 5.84 (acetate and ketone C=O) and 8.00 (acetate C–O).

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 70.40: H, 8.75. Found: C, 70.4; H, 8.8.

A 1.46-g. portion of the combined crystalline product obtained in the experiment described in detail directly above was dissolved in ethyl acetate–petroleum ether. Seeds of isomer A were added, and the mixture was allowed to stand overnight at room temperature. Isomer A had crystallized on the surface of the flask as clusters of prisms, and the solution contained several large rosettes of fine needles. These two forms were separated mechanically to give 0.89 g. of crude isomer A, m.p. 160–169°, and 0.17 g. of isomer B, m.p. 140–144°. A single recrystallization of the higher-melting fraction gave 0.74 g. of diketone A as colorless prisms, m.p. 168–172°. Repeated recrystallizations of the lower-melting isomer from ether and finally from isopropyl ether afforded the pure **diketone B** as fine colorless needles, m.p. 145–146°. The infrared spectrum was identical with that of isomer A in the region up to 8 μ.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found: C, 70.1; H, 8.7.

When the ketene acetal method was applied to the crude mixture of hydroxy diacids, a 60% yield of crystalline acetoxy diketone mixture was obtained.

(b) By the Acyl Malonic Ester Method.—The method of Bowman,[17] as modified in part by Martin,[16] Valasco and Phillips, was used. All operations through the acylation step were conducted in flame-dried equipment and under an atmosphere of nitrogen.

A solution of 9.8 g. of the crude acetoxy diacid mixture V (R = Ac) in 280 ml. of tetrahydrofuran (distilled from lithium aluminum hydride) was cooled to 0°, then 1.9 g. of sodium hydride was added, and the resulting slurry stirred for 3 hr. at room temperature. The mixture was again cooled with an ice-bath, and 1.0 ml. of pyridine (distilled from barium oxide) followed by 75 ml. of freshly distilled oxalyl chloride was added.[18] After the vigorous foaming had subsided, the cooling bath was removed and stirring continued for 1 hr. The mixture was then concentrated to dryness at reduced pressure and room temperature, and the final traces of excess oxalyl chloride were removed by co-distillation with two portions of benzene.

A solution of sodio dibenzyl malonate was prepared by the cautious addition of 8.4 g. of sodium hydride to an ice-cold solution of 65 g. of dibenzyl malonate[42] in 550 ml. of anhydrous benzene. After the vigorous foaming had subsided, the mixture was heated under reflux for 5.5 hr. The hot solution was then added in one portion to a solution of the diacid chloride, prepared as described directly above, in 100 ml. of anhydrous benzene, and the mixture was heated under reflux for 2.5 hr., then allowed to stand overnight at room temperature. The resulting red solution was cooled (ice-bath) and an ice-cold solution of 40 ml. of concentrated hydrochloric acid in 500 ml. of water was added. The mixture was stirred for about 20 min., then filtered, and the organic layer washed twice with saturated brine. The combined aqueous layers were extracted with ethyl acetate, and the combined organic solutions were dried over anhydrous sodium sulfate. The yellow oily residue obtained upon evaporation of the solvent under reduced pressure was dissolved in 250 ml. of acetone, 2 teaspoonsful of Raney nickel[43] was added, and the mixture was heated under reflux for 30 min. The catalyst was removed by filtration, washed thoroughly with acetone, and the combined filtrates were evaporated to dryness at reduced pressure. The residue was dissolved in 75 ml. of 95% ethanol and 150 ml. of ethyl acetate, and hydrogenated over 0.7 g. of 10% palladium-on-carbon (American Platinum Works) at an initial pressure of 40 p.s.i. After 3 hr., hydrogen absorption was very slow, and an additional 0.5 g. of catalyst was added. Hydrogen up-take ceased after 3.2 mole-equivalents had been absorbed. The catalyst was removed by filtration, replaced by 0.5 g. of fresh catalyst, and the hydrogenation continued, an additional 0.8 mole-equivalent of hydrogen being absorbed. The mixture was filtered, and the filtrate heated under reflux for 5 hr. in order to complete the decarboxylation. The solution was concentrated under reduced pressure to a small volume, and then diluted with 200 ml. of benzene. The volume of this solution was then reduced to about one-half by distillation, and the precipitate which separated (malonic acid) was removed by filtration. The filtrate and washings were combined, diluted with ethyl acetate, and the solution washed thoroughly with saturated potassium bicarbonate solution, followed by brine, and then dried over anhydrous sodium sulfate. The yellow oily residue (7.4 g.) obtained on removal of the solvent at reduced pressure was dissolved in 80 ml. of pyridine containing 40 ml. of acetic anhydride, and the solution was allowed to stand at room temperature overnight. The product was isolated as described above under part a, giving 7.3 g. of yellow oil which yielded 0.86 g. of colorless needles, m.p. 129–161°, on trituration with ether. Chromatography of the mother liquors on 150 g. of acid-washed alumina (Merck and Co.) gave, in the fractions eluted with benzene through 50% ether in benzene, 3.11 g. of crystalline diketone mixture. The fractions eluted with ethyl acetate and then with methanol were combined, reacetylated as above, and rechromatographed to provide an additional 1.32 g. of crystalline diketone mixture. The total yield was thus 54%.

Equilibration of the Epimeric Diketones VII.—A solution of 20 mg. of diketone B, m.p. 142.5–144°, prepared as described in part a above, and 0.2 g. of freshly fused sodium acetate in 2 ml. of anhydrous methanol (distilled from calcium hydride) was heated under reflux for 2.5 hr. in a system protected from atmospheric moisture. The solution was cooled, diluted with 20 ml. of 1:1 ethyl acetate–ether and washed with water, followed by saturated brine. Since the residue obtained upon evaporation of the solvent could not be induced to crystallize, it was believed to have undergone methanolysis of the acetate group at C-3. Therefore the crude product was warmed with 1.5 ml. of 1:2 acetic anhydride–pyridine for 15 min. on the steam-bath. The product was isolated as described under part a of the preceding section, and then fractionally crystallized from ether. A total of 7.8 mg. of diketone A, m.p. 162–166°, was isolated. Recrystallization gave 5.6 mg. of material, m.p. 169–172°, undepressed on admixture with the analytical specimen described above. Also isolated was 7.3 mg. of diketone B, m.p. 135.5–142°, undepressed on admixture with the starting material.

DL-1β-(2-Hydroxyethyl)-2α-(2-hydroxypropyl)-4β,7α-dihydroxy-4bβ-methyl-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone Triacetate (VIII, $R^1 = R^2 = R^3$ = Ac). **(a) From the Epimeric Mixture of Diketones VII.**—A mixture of 0.232 g. of the solid mixture of diketones, m.p. 131–162°, prepared as described above, 20 ml. of methylene dichloride (distilled from phosphorus pentoxide) and 2.60 g. of powdered disodium hydrogen phosphate (Mallinckrodt Chemical Works "analytical reagent grade," dried for 7 hr. at 100° and 100 mm. just prior to use) was cooled with stirring to 0–5°. To this stirred solution was added in one portion a solution of trifluoroperacetic acid in methylene dichloride which was prepared

(42) B. R. Baker, R. E. Schaub, M. S. Querry and J. H. Williams, *J. Org. Chem.*, **17**, 97 (1952). Note that the dibenzyl malonate is sensitive to heat and is preferably distilled rapidly through a short-path system.

(43) Deactivated by heating in acetone for 2 hr. *Cf.* G. B. Spero, A. V. McIntosh, Jr., and R. H. Levin, *J. Am. Chem. Soc.*, **70**, 1907 (1948).

by dissolving 0.16 ml. of 90% hydrogen peroxide (Becco Chemical Division of the Food Machinery and Chemical Corp.) in an ice-cold solution of 1.02 ml. of freshly distilled trifluoroacetic anhydride (Matheson, Coleman and Bell) in 5 ml. of dried methylene dichloride. The resulting mixture was stirred for 1.5 hr. at room temperature, and then for 3.5 hr. at reflux temperature. Ethyl acetate was then added, and the mixture washed with water, followed by saturated brine and dried over anhydrous sodium sulfate. The colorless residue obtained upon evaporation of the solvent at reduced pressure was chromatographed on 12.5 g. of Florisil. The fractions eluted with 20–50% ether in benzene consisted of the crystalline epimeric mixture of the triacetate VIII ($R^1 = R^2 = R^3 =$ Ac) and amounted to a total of 0.175 g. (71% yield), m.p. 119–127°. Further elution with higher proportions of ether in benzene up to pure ether and including 2% ethyl acetate in ether provided an additional 23 mg. of diketone mixture which crystallized on seeding with either of the pure isomers (see below). Trituration with ether gave 20 mg. of product, m.p. 125–138°, and the total yield of the mixture of triacetates was therefore 79%.

With the aid of seeds which were obtained by mechanical separation, it was possible to obtain, from the major chromatographic fraction, the A- and B-isomers in the pure form by fractional crystallization from a mixture of diethyl and diisopropyl ether. Triacetate A (see below) was finally obtained by repeated crystallization from diisopropyl ether in the form of fine colorless needles, m.p. 140–141°.

Anal. Calcd. for $C_{27}H_{40}O_8$: C, 65.83; H, 8.19. Found: C, 66.2; H, 8.35.

Similarly, triacetate B was obtained as colorless plates, m.p. 142–142.5°, depressed on admixture with triacetate A.

Anal. Calcd. for $C_{27}H_{40}O_8$: C, 65.83; H, 8.19. Found: C, 66.1; H, 8.2.

The infrared spectra of both isomers (chloroform solution) showed bands at 5.68 μ (γ-lactone C=O), 5.79 (acetate C=O), 8.00 (acetate C—O), 8.66, 9.79, 10.49 and 10.74. The only major difference in the spectra of isomer A and B was a band at 8.85 μ for the former which appeared at 8.88 μ in the latter case.

In other experiments on a 1.5-g. scale, yields as high as 81–83% of diketone mixture were realized.

(b) **Triacetate A from Diketone A.**—A 0.461-g. sample of diketone A, m.p. 171–172.5°, prepared as described above, was oxidized just as described for the mixture in the preceding experiment. The reaction mixture was diluted with ethyl acetate and washed with water, 5% sodium bicarbonate solution, saturated brine and then dried over anhydrous sodium sulfate. The residue obtained upon evaporation of the solvent under a stream of dry air (steam-bath temperature) crystallized completely on scratching in the presence of a little ether to give 0.483 g. (98% yield) of colorless needles, m.p. 131–136°, with softening at 128°. Chromatography on Florisil gave an 86% recovery of triacetate A as colorless needles, m.p. 138–140°, undepressed on admixture with the analytical specimen described in the preceding experiment.

(c) **Triacetate B from Diketone B.**—A 0.231-g. sample of diketone B, m.p. 143–145°, prepared as described above, was oxidized just as described for the mixture in the preceding experiment. The crude product was crystallized from diisopropyl ether to give 0.182 g. (first crop) of colorless plates, m.p. 138–141°, and 0.016 g. (second crop) of colorless plates, m.p. 137–141°. The melting point of a mixture of first crop material with the analytical sample of triacetate B (see above) was 138–142°.

DL-1β-(2-Hydroxyethyl)-2α-(2-hydroxypropyl)-4β,7α-dihydroxy-4bβ-methyl-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone (VIII, $R^1 = R^2 = R^3 =$ H). (a) **Basic Hydrolysis of Triacetate A.**—A solution of 46 mg. of the aforementioned triacetate, m.p. 139–141.5°, in 1.1 ml. of 1 *N* absolute methanolic potassium hydroxide solution was allowed to stand at room temperature for 6 hr. The mixture was diluted with water and extracted thoroughly with ethyl acetate. The combined organic layers were washed with saturated brine and dried over anhydrous sodium sulfate. The residue (34 mg.), obtained upon evaporation of the solvent under a stream of nitrogen (steam-bath temperature), was crystallized from ethyl acetate to give 25.1 mg. of colorless prisms, m.p. 173–174°. Repeated recrystallizations from acetone raised the melting point to 174.5–175.5°; λ_{max}^{mull} 3.14 μ (associated OH), 5.68 (γ-lactone C=O), 8.68, 9.52 and 10.78.

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.8; H, 9.5.

Reacetylation of a specimen of triol A, m.p. 172–173.5°, with pyridine and acetic anhydride at room temperature regenerated triacetate A in 97% yield. The product was thus obtained as colorless needles, m.p. 139.5–141°, undepressed on admixture with the analytical specimen.

In another experiment, 48.3 mg. of crude triacetate A, m.p. 131–136°, with softening at 128°, obtained directly from diketone A, afforded, after crystallization from acetone, 26 mg. of colorless prisms of triol A, m.p. 170.5–174°. The over-all yield of triol A from diketone A was thus 72%.

(b) **Acid Hydrolysis of Triacetate A.**—A solution of 0.247 g. of the aforementioned triacetate A, m.p. 138–140°, in 75 ml. of absolute methanol containing 3.7 ml. of concentrated hydrochloric acid was heated at reflux for 1.5 hr. (nitrogen atmosphere). The solution was then concentrated at reduced pressure (bath temperature below 30°) to a volume of about 20 ml. Water was added and the mixture extracted thoroughly with ethyl acetate. The crude product, isolated as in the preceding section, amounted to only 0.147 g. (86% yield). Therefore the combined aqueous layers were concentrated to about 40 ml. as above and extracted again with ethyl acetate. This treatment yielded an additional 0.046 g. of product. The total crude material was crystallized from ethyl acetate to yield 0.152 g. (first crop) of colorless prisms, m.p. 172–174°; and 0.006 g. (second crop), m.p. 169.5–172°. The residue from the mother liquors was retreated with 0.3 ml. of concentrated hydrochloric acid in 6 ml. of methanol at reflux for 1.5 hr. under nitrogen. Isolation of the product as described above afforded an additional 0.011 g. of recrystallized material, m.p. 168–172°. The total yield of triol A was thus 92%. The melting point of a mixture of material prepared by this procedure with the analytical specimen (see above) was undepressed.

(c) **Acid Hydrolysis of Triacetate B.**—A 45.7-mg. specimen of triacetate B, m.p. 138.5–140°, prepared as described above, was treated with 0.7 ml. of concentrated hydrochloric acid in 15 ml. of absolute methanol just as described in the preceding experiment. The crude product (31 mg.) on crystallization from ethyl acetate gave 23 mg. (68% yield) of colorless irregular blades of triol B, m.p. 177.5–180°, depressed to 162–166° on admixture with triol A. Repeated recrystallizations of a specimen from ethyl acetate and from acetone–petroleum ether gave colorless blades, m.p. 184–185° (with a small residue finally melting at 189°); λ_{max}^{mull} 2.91 μ, 2.98, 3.09 (OH), 5.75 (γ-lactone C=O), and a weak shoulder at 5.82 (suggesting a trace of acetate or ketone impurity; *cf.* the basic hydrolysis described below).

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 69.2; H, 9.2.

Reacetylation of a specimen of triol B, m.p. 182–184°, with pyridine and acetic anhydride gave, after crystallization from diisopropyl ether, 8.2 mg. of triacetate B, m.p. 140.5–142°, undepressed on admixture with the analytical specimen.

In another experiment, 22 mg. of triacetate B, m.p. 139.5–141.5°, was treated with 0.5 ml. of concentrated hydrochloric acid in 10 ml. of absolute methanol for 19 hr. at room temperature. The product, isolated as described above, was crystallized from acetone to give 13.1 mg. (80% yield) of triol B, m.p. 175–181°.

(d) **Basic Hydrolysis of Triacetate B.**—A solution of 54.0 mg. of triacetate B, m.p. 142–143.5°, in 1.3 ml. of 1 *N* methanolic potassium hydroxide was allowed to stand for 6 hr. at room temperature. The neutral product isolated as described above (part a) amounted to only 31 mg. (77% yield). The alkaline aqueous layers were therefore acidified to *p*H 2 with 5 *N* hydrochloric acid and extracted with ethyl acetate. In this way 7.0 mg. (17% yield) of additional (perhaps relactonized) material was obtained. Crystallization of the 31-mg. fraction from ethyl acetate followed by further trituration with the same solvent afforded 25 mg. (62% yield) of triol B, m.p. 173–174°. Repeated recrystallization from acetone gave a specimen (dimorphic form), m.p. 192–194°, with softening at 186°, the infrared spectrum of which was identical with that of the analytical specimen described above (part c).

The 7-mg. fraction (see above), on crystallization from acetone, yielded 3 mg. of material, m.p. 182.5–192°, undepressed on admixture with the 192–194° sample described above. The infrared spectra of the two specimens were identical.

11β-Hydroxy-Δ^{16}-pregnene-3,20-dione-18-carboxylic Acid (11→18)-Lactone (X). (a) **From Triol A by Direct Oxidation with Sarett Reagent.**—The oxidizing reagent was prepared by dissolving 56.0 mg. of chromium trioxide (Merck and Co. "analytical reagent grade," stored in a desiccator over calcium hydride) in 0.6 ml. of cold anhydrous pyridine (Merck and Co. "analytical reagent grade," distilled from calcium hydride). The solution was stirred for 5 min. with cooling (ice-bath), and to the resulting orange slurry was added a solution of 30.0 mg. of the aforementioned triol A, m.p. 172–174°, in 0.3 ml. of anhydrous pyridine. Two 0.2-ml. portions of pyridine were used to aid in the transfer, and the resulting mixture was allowed to stir for 15 min. and then to stand for 5 hr. at room temperature. The brown mixture was diluted with ethyl acetate, washed with water and the aqueous phase extracted thoroughly with ethyl acetate. The combined organic extracts were washed with water, saturated sodium bicarbonate solution, again with water followed by saturated brine, and finally dried over anhydrous

sodium sulfate. The residue obtained on evaporation of solvent at reduced pressure (bath temperature below 30°) amounted to 35 mg. of colorless oil. This product was dissolved in 4.0 ml. of glacial acetic acid, and 0.8 g. of freshly fused sodium acetate was added. The mixture was then heated under nitrogen for 13 hr. at steam-bath temperature. Initially the flask was swirled occasionally until the solution became homogeneous. The resulting light brown solution was cooled, diluted with water and extracted with ethyl acetate. The combined organic layers were washed with water, followed by saturated brine, and dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent amounted to 25.0 mg. of pale yellow semicrystalline oil, $\lambda_{max}^{95\%\ EtOH}$ 235 mμ (ε 3,312), which was chromatographed on 1.25 g. of acid-washed alumina. Elution with 1% ether in benzene through 2% ether in ethyl acetate gave only 3 mg. of oily material which was discarded. Elution with 5% ether in ethyl acetate through pure ethyl acetate afforded a total of 14 mg. (50% yield) of a solid which, after two recrystallizations from ethyl acetate, afforded 8.0 mg. (29% over-all yield from triol A) of colorless leaflets, m.p. 247–251°; $\lambda_{max}^{95\%\ EtOH}$ 236.6 mμ (ε 7,586); $\lambda_{max}^{CHCl_3}$ 5.68 μ (γ-lactone C=O), 5.85 (saturated ketone C=O), 6.01 (unsaturated ketone C=O) and 6.25 (conjugated C=C). Further purification of comparable material by chromatography on Florisil followed by repeated recrystallizations from ethyl acetate gave colorless hexagonal plates, m.p. 256–258.5°, $\lambda_{max}^{95\%\ EtOH}$ 236.6 mμ.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.95; H, 8.0.

The aqueous sodium bicarbonate extracts from the original work-up were combined, acidified to *p*H 2 with concentrated hydrochloric acid and extracted with ethyl acetate. The combined organic extracts were washed with water followed by saturated brine and dried over anhydrous sodium sulfate. The residue obtained upon evaporation of the solvent amounted to 8.5 mg. (28% yield) of what is probably the diketo acid IX (R = OH). Crystallization of comparable material from another experiment from methyl ethyl ketone afforded a product, m.p. 239–244°.

In several other experiments which were conducted with 15-mg. samples of triol A under slightly varied conditions, the yields of unsaturated ketone based on the extinction coefficient of the 236 mμ band in the ultraviolet were: 37% when the oxidation period was 5 hr. and the cyclization period was 13 hr.; 27%, oxidation period 7 hr., cyclization period 13 hr.; 23%, oxidation period 8 hr., cyclization period 8 hr.; 20%, oxidation period 7 hr., cyclization period 10 hr. in refluxing methanol saturated with sodium acetate; 41% when the oxidation was conducted for 5 hr. with twice the amount of chromium trioxide (68 mg.) in 0.64 ml. of pyridine and the cyclization period was 12 hr.

(b) **From Triol A by Initial Oxidation with N-Bromoacetamide.**—A modification of a previously described procedure[44] for N-bromoacetamide oxidation was employed. To an ice-cold solution of 18.3 mg. of triol A, m.p. 171–172.5°, in 0.6 ml. of acetone, 0.2 ml. of *t*-butyl alcohol and 0.1 ml. of water was added with swirling 27.0 mg. of N-bromoacetamide. The resulting solution was allowed to stand for 6 hr. at 5–10°, then a solution of 0.1 g. of sodium sulfite in 2 ml. of water was added with stirring. Ethyl acetate was added and the solution washed with water, saturated sodium bicarbonate solution, again with water, followed by saturated brine, and finally dried over anhydrous sodium sulfate. The colorless glassy residue (19.6 mg.) obtained upon evaporation of the solvent at reduced pressure was chromatographed on 0.9 g. of Florisil. A major fraction eluted with 2% absolute ethanol in ether amounted to 5.9 mg., m.p. 103–114°; $\lambda_{max}^{CHCl_3}$ 2.94 μ (OH), 5.70 (γ-lactone C=O) and 5.85 (saturated ketone C=O). Recrystallization from ethyl acetate provided 1.5 mg. of colorless cubes, m.p. 143–144°. This substance was not obtained in sufficient quantity for analysis but was undoubtedly the dihydroxy ketone (see Discussion section).

In another experiment with 18.3 mg. of triol A, m.p. 171–173.5°, the reaction was allowed to proceed for 12 hr. at 5–10°. The infrared spectrum of the crude neutral product (20.1 mg.) was identical with that of the 103–114° material described above. This crude product was treated with 20 mg. of chromium trioxide in 0.4 mg. of pyridine for 5 hr. at room temperature as described in the preceding section. The resulting neutral product was treated with 0.8 g. of sodium acetate in 4.0 ml. of glacial acetic acid for 13 hr. at 95–100° as described in the preceding section to give 15.2 mg. of a tan glass, $\lambda_{max}^{95\%\ EtOH}$ 232 mμ (ε 4,000). Crystallization from ethyl acetate afforded 5.3 mg. (38% over-all yield) of pentacyclic material, m.p. 240–251°, undepressed on admixture with the 247–251° material described above.

(c) **From Triol B by Direct Oxidation with Sarett Reagent.**—A 15.0-mg. sample of the aforementioned triol B, m.p. 184–187°, was treated as described above under part a with 28.0 mg. of chromium trioxide and 0.64 mg. of pyridine for 5 hr. at room temperature. Crystallization of the neutral product from ethyl acetate gave 4.0 mg. of material, m.p. 295–304° dec., with some sublimation above 250°. This material, which is tentatively considered to be the dilactone **XI**, was never obtained in a satisfactorily pure state. Several recrystallizations of such material from acetone yielded colorless blades, m.p. 302–305°, with softening and partial resolidification at 286–288°.

Anal. Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found: C, 69.2; H, 7.8.

The residue from the mother liquors of the crystallization of the 295–304° material was treated with 0.4 mg. of sodium acetate in 2 ml. of glacial acetic acid for 12 hr. at the steam-bath temperature. Isolation by the procedure described above (part a) gave 8.5 mg. of semicrystalline material, λ_{max} 233 mμ (ε 2,290). From this extinction coefficient the maximum over-all yield of the unsaturated ketone X is calculated to be 29%. Trituration of this crude product with acetone provided additional dilactone, m.p. 294–302°, undepressed on admixture with the sample described above. Attempts to purify the unsaturated ketone by chromatography and crystallizations were unsuccessful because of difficulty in removing traces of the dilactone.

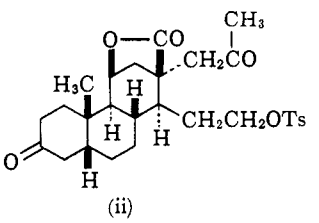
(d) **From Triol B by Initial Oxidation with N-Bromoacetamide.**— An 18.3-mg. sample of triol B, m.p. 183–184.5°, was treated with N-bromoacetamide exactly as described in detail above for the oxidation of triol A (part b). The neutral fraction (17.5 mg.) was chromatographed on 1 g. of Florisil. Elution with benzene through 10% ether in ethyl acetate gave 1.5 mg. of amorphous solid, and further elution with 20% through 50% ethyl acetate in ether afforded 16.8 mg. of solid material which, on crystallization from ethyl acetate–petroleum ether, gave 12.0 mg. (65% yield) of colorless crystals, m.p. 143–145°; $\lambda_{max}^{CHCl_3}$ 2.90 μ (OH), 5.70 (γ-lactone C=O) and 5.85 (saturated ketone C=O). Repeated recrystallizations of such material from ethyl acetate–petroleum ether and from absolute ethanol–petroleum ether gave colorless triangular blades, m.p. 151.5–153°.

Anal. Calcd. for $C_{21}H_{32}O_5$ (the dihydroxy ketone): C, 69.20: H, 8.85; and for $C_{21}H_{30}O_5$ (hydroxy diketone): C, 69.58; H, 8.34. Found: C, 69.4; H, 8.8.

In another run in which the reaction period was reduced to 3 hr., the yield of the above material was considerably diminished. A third experiment with 18.3 mg. of triol B, m.p. 180–182.5° (reaction period 12 hr.), yielded 17.9 mg. of crystalline product, m.p. 140–153°.

For the chromium trioxide oxidation a 55-mg. sample of triol, m.p. 180–182.5°, was oxidized with N-bromoacetamide as described above (6-hr. treatment). Crystallization of the crude product from ethyl acetate gave 23 mg. of crystals, m.p. 144.5–146°. This material and the amorphous residue (32 mg.) from the mother liquor were oxidized separately with 23 mg. of chromium trioxide in 0.43 ml. of pyridine for 5 hr. at room temperature as described in part b above. The resulting neutral products were each treated with 0.8 g. of sodium acetate in 4.0 ml. of glacial acetic acid for 12 hr. (steam-bath temperature) and the product isolated as described above. From the crystalline fraction there was thus obtained 23.8 mg., λ_{max} 239 mμ (ε 4,366), and from the amorphous residue 33.2 mg., λ_{max} 232 mμ (ε 3,467). From the intensity of the extinction coefficient in both products, it may be calculated that the maximum over-all yield of the unsaturated ketone was 49%. Fractional crystallization of the 23.8-mg. product from ethyl acetate gave, in the first fractions, high-melting material which, after recrystallization, melted at 303–307°, undepressed on admixture with the analytical sample of the dilactone. In the later fractions, 1.8 mg. of the unsaturated ketone was obtained, m.p. 244–251°, undepressed on admixture with the analytical specimen.

DL-1β-(2-*p*-Toluenesulfonoxyethyl)-2α-acetonyl-4β-hydroxy-4bβ-methyl-7-keto-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone (ii).—Preliminary to the main investigation (see below), we explored



(ii)

the closure of ring D by the Sarett method[24] prior to introduction of the double bond in ring A. This study, which involved the preparation of the ketone tosylate ii and its cyclization to the substance iii, is reported in the present section.

A 73.2-mg. sample of triol A, m.p. 171.5–173°, was treated with 108 mg. of N-bromoacetamide in 2.4 ml. of acetone, 0.8 ml. of *t*-butyl alcohol and 0.4 ml. of water for 5 hr. at 0–5° just as described above. The total crude neutral product (70.5 mg.) was dissolved in 0.28 ml. of anhydrous pyridine, 4.00 mg. of *p*-

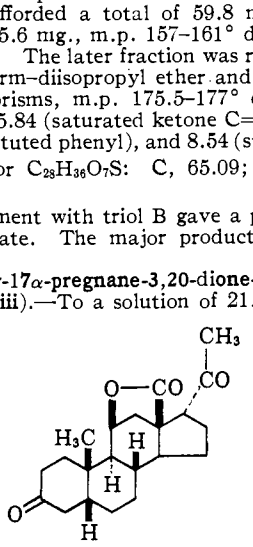
(44) H. L. Herzog, M. A. Jevnik, P. L. Perlman, A. Nobile and E. B. Hershberg, *J. Am. Chem. Soc.*, **75**, 266 (1953).

toluenesulfonyl chloride was added, the mixture stirred until the acid chloride dissolved, and then allowed to stand for 15 hr. at 5–10°. The resulting mixture was neutralized with 1.5 ml. of 5% sodium bicarbonate solution, then ethyl acetate was added, and the organic layer was washed with sodium bicarbonate solution, water, and finally with saturated brine. The solution was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure (bath temperature below 30°). The residue was dissolved in 0.7 ml. of pyridine and transferred with the aid of two 0.3-ml. portions of pyridine to the oxidation reagent prepared (see above) from 160 mg. of chromium trioxide and 1.6 ml. of pyridine. The resulting mixture was stirred for 30 min., allowed to stand for 8.5 hr. at room temperature and then diluted with ethyl acetate and water. The organic layer was washed with saturated sodium bicarbonate solution, water, saturated brine, and then dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent under reduced pressure was chromatographed on 5 g. of Florisil. Elution with 1% ether in benzene through 5% acetone in ether gave 6.1 mg. of amorphous solid. Further elution with 10% acetone in ether afforded a total of 59.8 mg. (58% yield) of colorless prisms; 25.6 mg., m.p. 157–161° dec.; and 32.2 mg., m.p. 153–160° dec. The later fraction was repeatedly recrystallized from chloroform–diisopropyl ether and from ethyl acetate to give colorless prisms, m.p. 175.5–177° dec.; $\lambda_{max}^{CHCl_3}$ 5.68 μ (γ-lactone C=O), 5.84 (saturated ketone C=O), 6.25 (aromatic C=C), 6.66 (substituted phenyl), and 8.54 (sulfonate C=O).

Anal. Calcd. for $C_{28}H_{36}O_7S$: C, 65.09; H, 7.02. Found: C, 65.4; H, 7.1.

A similar experiment with triol B gave a poor yield (13%) of the diketone tosylate. The major product was the dilactone XI.

11β-Hydroxy-17α-pregnane-3,20-dione-18-carboxylic Acid (11→18)-Lactone (iii).—To a solution of 21.0 mg. of the aforementioned diketo tosylate ii, m.p. 157–161°, in 0.8 ml. each of anhydrous benzene and *t*-butyl alcohol was added 0.13 ml. of a 0.48 *N* solution of potassium *t*-butoxide in *t*-butyl alcohol (atmosphere of nitrogen). A precipitate began to form immediately, and the suspension was stirred for 25 min. at room temperature. Water and ethyl acetate were then added, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with saturated sodium bicarbonate solution, water, saturated brine and then dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent under reduced pressure was combined with 4.4 mg. of material isolated from the acidified aqueous solutions by extraction, washing and drying as described above, and the total material was chromatographed on 0.8 g. of Florisil. Elution with 20% ether in benzene through 1% absolute ethanol in ether yielded a total of 7.8 mg. (56% yield) of stout colorless needles, m.p. 181–186°, $\lambda_{max}^{CHCl_3}$ 5.71 and 5.86 μ with only weak absorption in the 6.28 (phenyl) region. Repeated recrystallization of such material from ethyl acetate–diisopropyl ether gave fine colorless needles, m.p. 186–188°.



(iii)

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 72.9; H, 8.1.

Isomerization of this substance to the 17β-epimer was effected by treatment with 5% aqueous methanolic sodium hydroxide solution for 40 hr. at room temperature. The product isolated after recrystallization from ethyl acetate melted at 212–217.5°, undepressed on admixture with a sample, m.p. 216–218.5°, obtained by catalytic hydrogenation over palladium-on-carbon of the unsaturated ketone X. The 17β-isomer was not produced in sufficient quantity for carbon–hydrogen analysis.

DL-1β-(2-Acetoxyethyl)-2α-(2-acetoxypropyl)-4β-hydroxy-4bβ-methyl-7-keto-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone (XII, R^1 = R^2 = H). (a) **Isomer A.**—A solution of 2.0 g. of anhydrous potassium carbonate in 20 ml. of cold water was added to a cooled solution of 0.452 g. of the aforementioned triacetate A, m.p. 137.5–139.5°, in 50 ml. of absolute methanol. The solution was allowed to stand for 2.25 hr. at room temperature and then concentrated to a volume of about 20 ml. The resulting solution was cooled, acidified to *p*H 2 with concentrated hydrochloric acid, diluted with saturated brine and extracted with ethyl acetate. The combined organic extracts were washed with saturated brine and dried over anhydrous sodium sulfate. The colorless glassy residue obtained on evaporation of the solvent at reduced pressure was dissolved in 11 ml. of acetone and 3.7 ml. of *t*-butyl alcohol by warming at the steam-bath temperature. The solution was cooled to 10°, 1.8 ml. of water was added, followed by 0.5 g. of N-bromoacetamide. After stirring for about 1 min., the N-bromoacetamide had dissolved, and fine colorless needles began to separate from the solution. Addition of 1.8 ml. of methylene dichloride effected dissolution of the crystals, and the resulting solution was allowed to stand for 4 hr. at 5–10°. A cold solution of 1.8 g. of sodium sulfite in 20 ml. of water was then added, and the mixture stirred for 5 min. in the cold, and then extracted thoroughly with ethyl acetate. The combined organic extracts were washed with saturated brine and dried over anhydrous sodium sulfate. The residue (containing some acetamide) obtained on removal of the solvent at reduced pressure was dissolved in 10 ml. of pyridine and 5 ml. of acetic anhydride, and the solution allowed to stand for 14 hr. at room temperature. Crushed ice was then added, and the mixture allowed to stand for 10 min. with occasional swirling. Ether and ethyl acetate were added, and the organic layer was washed thoroughly with 5% hydrochloric acid, saturated sodium bicarbonate solution, and finally with water. The aqueous extracts were washed with ethyl acetate, and the combined organic solutions were washed with saturated brine and dried over anhydrous sodium sulfate. The colorless glassy residue obtained on evaporation of the solvent at reduced pressure was chromatographed on 20 g. of acid-washed alumina (Merck and Co.). Elution with 1–10% ether in benzene gave 4.9 mg. of amorphous material. Further elution with 10–20% ether in benzene gave, after crystallization from diisopropyl ether, 7.8 mg. of starting material, m.p. 135–139°, undepressed on admixture with triacetate A. Elution with 50% ether in benzene through 50% ethyl acetate in ether gave 0.279 g. of ether-insoluble material. Recrystallization from acetone–diisopropyl ether afforded a total of 0.224 g. (55% yield) of colorless prisms: 0.148 g. (first crop), m.p. 134–136°; 0.065 g. (second crop), m.p. 133–135°; and 0.011 g. (third crop), m.p. 129–133°, with slight softening at 120°. Repeated recrystallizations of a sample of the first-crop material from ethyl acetate–diisopropyl ether and finally from acetone–diisopropyl ether provided colorless prisms, m.p. 135–136°; $\lambda_{max}^{CHCl_3}$ 5.70 μ (γ-lactone C=O), 5.83 (acetate and ketone C=O) and 8.08 (acetate C—O).

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.9; H, 8.05.

Further elution of the column yielded material which showed absorption in the hydroxyl region in the infrared spectrum. On the assumption that some hydrolysis of acetate had occurred during chromatography, this fraction was reacetylated, rechromatographed and recrystallized to yield a further 0.020 g. of keto diacetate A, m.p. 134–136°. The total over-all yield of recrystallized keto diacetate A was thus 0.244 g. or 54%.

In further experiments, comparable results were realized when 2 instead of 4 mole-equivalents of N-bromoacetamide was used. For larger-scale experiments the lower proportion of oxidizing agent was preferred because the isolation of the product was simplified with the reduced quantity of acetamide. Using this procedure, the yield of keto diacetate A was 50% on a 2.2-g. scale.

(b) **Isomer B.**—The conversion of triacetate B to keto diacetate B was accomplished by the same procedure as described in the preceding experiment except that the hydrolysis period was 1 instead of 2.25 hr. From 0.478 g. of triacetate B, m.p. 141–142.5°, there was thus obtained 0.415 g. of the crude glassy oxidation product. This material was chromatographed on 21 g. of acid-washed alumina. The fraction eluted with 10% ether in benzene amounted to 0.041 g. of crude starting material which, on recrystallization from diisopropyl ether, afforded 0.028 g. (6% recovery) of colorless plates, m.p. 141–142.5°, undepressed on admixture with triacetate B. Continued elution with 10% ether in benzene gave 6 mg. of non-crystalline material. The fractions eluted with 20% ether in benzene through ethyl acetate amounted to 0.346 g. of ether-insoluble crystalline material. Repeated recrystallization of a center fraction from diisopropyl ether–acetone gave colorless blades, m.p. 127–128.5°.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.9; H, 8.2.

Except for a weak band at 8.90 μ in the infrared spectrum (chloroform solution) of keto diacetate B, the spectra of the A- and B-isomers were essentially identical.

On further crystallization experiments with keto diacetate B, a polymorphic form was encountered as colorless prisms, m.p. 147–148.5°.

(c) **Mixture of Epimers.**—The application of the procedure described in detail under part a (2 mole-equivalents of N-bromoacetamide) afforded crystalline keto diacetate mixture in yields up to 62% from the epimeric mixture of triacetates A and B.

DL-1β-(2-Acetoxyethyl)-2α-(2-acetoxypropyl)-4β-hydroxy-4bβ-methyl-7-keto-1,2,3,4,4aα,4b,5,6,7,9,10,10aβ-dodecahydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone (XIII). (a) Isomer A.—The following procedure is based upon previously described methods for bromination[44] and dehydrobromination.[22] A 0.194-g. sample of the aforementioned keto diacetate A, m.p. 134–136°, was dissolved in 1.5 ml. of glacial acetic acid (distilled from potassium permanganate) containing 0.05 ml. of a 0.14 *N* solution of hydrobromic acid (prepared by diluting 0.2 ml. of 48% hydrobromic acid to 10 ml. with glacial acetic acid); then a solution of 0.0720 g. of bromine in 1.0 ml. of glacial acetic acid was added with stirring over a period of 10 min. at room temperature. The pale orange solution was stirred for an additional 5 min., 0.2 g. of anhydrous sodium acetate was added, and the resulting mixture stirred for 2 min. and diluted with ethyl acetate. The mixture was washed with water, 10% potassium carbonate solution, then with saturated brine and dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent at reduced pressure was dried for 15 min. at 100° (0.5 mm.) and then for 10 hr. at room temperature in a vacuum desiccator.

The crude bromo ketone was dissolved in 2.2 ml. of dimethylformamide (distilled from phosphorus pentoxide), 0.057 g. of anhydrous lithium chloride was added, and the mixture was warmed to 100° with stirring for 2 hr. (atmosphere of nitrogen). The resulting solution was cooled, diluted with ethyl acetate, and washed with water, then with saturated brine, and dried over anhydrous sodium sulfate. The colorless glassy residue obtained on evaporation of the solvent at reduced pressure amounted to 0.209 g., λ_{max} 237.2 mμ (ε 10,000). After two recrystallizations from ethyl acetate–petroleum ether a total of 0.124 g. (64% over-all yield) of crude unsaturated keto diacetate A was obtained: 0.110 g. (first crop), m.p. 176–180°; and 0.14 g. (second crop), m.p. 170–176°. A sample of the first-crop material was recrystallized twice from ethyl acetate and twice from acetone to yield colorless plates, m.p. 182.5–183.5°; $\lambda_{max}^{95\%\ EtOH}$ 237.7 mμ (ε 17,020); $\lambda_{max}^{CHCl_3}$ 5.69 μ (γ-lactone C=O), 5.81 (acetate C=O), 6.19 (conjugated C=C) and 8.10 (acetate C—O).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 66.9; H, 7.7.

The residue (79.3 mg.) from the combined mother liquors was chromatographed on 2.4 g. of Florisil. Elution with 2% acetone in benzene gave 39.3 mg. of crystalline fractions which, on recrystallization from ethyl acetate–petroleum ether, gave 29 mg. (14% yield) of the chloro ketone XII (R^1 = H, R^2 = Cl) as colorless rods, m.p. 187–193°; $\lambda_{max}^{CHCl_3}$ 5.69 μ (γ-lactone C=O), 5.82 (acetate and ketone C=O) and 8.10 (acetate C—O). Repeated recrystallization from diisopropyl ether–acetone and from ethyl acetate gave colorless irregular needles, m.p. 200–207.5°, which gave a positive Beilstein test.

Anal. Calcd. for $C_{25}H_{35}O_7Cl$: C, 62.19; H, 7.32. Found: C, 62.3; H, 7.3.

Further elution of the chromatographic column described above with 20% acetone in benzene gave, after a glassy fraction, 16.3 mg. of material which, after two recrystallizations from ethyl acetate–petroleum ether, afforded 9.4 mg. of prisms, m.p. 171–176°, undepressed on admixture with the analytical specimen of the unsaturated ketone A. The over-all yield of this product from the keto diacetate A was thus 0.124 g. or 58%.

(b) Isomer B.—The procedure for the bromination and dehydrobromination of the keto diacetate B was essentially the same as that described in the preceding experiment for isomer A except that lithium bromide was used in place of the chloride for the dehydrohalogenation step. A solution of 0.770 g. of keto diacetate B, m.p. 146–148°, in 8 ml. of acetic acid containing 6 drops of 0.14 *N* hydrobromic acid in acetic acid was treated with a solution of 0.268 g. of bromine in 9.7 ml. of acetic acid just as described above. The crude bromo ketone (0.940 g.) was heated in 10 ml. of dimethylformamide with 0.870 g. of anhydrous lithium bromide for 8 hr. at 100°. The crude dehydrohalogenation product isolated as described in the preceding experiment was chromatographed on 50 g. of Florisil. Elution with 2% acetone in benzene gave a total of 0.212 g. (28% yield) of DL-1β-(2-acetoxyethyl)-2α-(2-acetoxypropyl)-4β-hydroxy-4bβ-methyl-7-keto-1,2,3,4,4aα,4b,7,8,8aβ,9,10,10aβ-dodecahydrophenanthrene-2β-carboxylic acid (2→4)-lactone (XIV), m.p 163–167°, $\lambda_{max}^{95\%\ EtOH}$ 227 mμ. A sample purified by repeated recrystallizations from acetone–diisopropyl ether gave colorless prisms, m.p. 167–168°; $\lambda_{max}^{95\%\ EtOH}$ 228 mμ (ε 11,700); $\lambda_{max}^{CH_2Cl_2}$ 5.66 μ (γ-lactone C=O), 5.78 (acetate C=O) and 5.96 (conjugated C=O).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 67.3; H, 7.5.

Further elution with 5% acetone in benzene afforded a total of 0.392 g. (52% yield) of the title compound, m.p. 150–153°. Comparable material produced from preliminary experiments (lithium chloride procedure) was purified by repeated recrystallizations from ethyl acetate to give colorless prisms, m.p. 154–155°, $\lambda_{max}^{95\%\ EtOH}$ 237.9 mμ (ε 17,220).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 66.9; H, 7.6.

In the experiments utilizing lithium chloride for the dehydrohalogenation step, the chloro ketone B (XII, R^1 = H, R^2 = Cl) was isolated, as in the A-series, by a combination of chromatography and recrystallization. The analytical specimen was obtained by repeated recrystallizations from diisopropyl ether in acetone and from methanol; m.p. 210–212°, with slight previous softening; $\lambda_{max}^{CHCl_3}$ 5.69 μ (γ-lactone C=O), 5.82 (acetate and saturated ketone C=O) and 8.10 (acetate C=O). The spectrum also exhibited a shoulder at 5.92 μ (unsaturated ketone C=O) and a band at 6.22 (conjugated C=C), suggesting that the product was contaminated with unsaturated ketone.

Anal. Calcd. for $C_{25}H_{35}O_7Cl$: C, 62.19; H, 7.32. Found: C, 63.3; H, 7.25.

Treatment of 2.52 g. of the mixture of keto diacetates A and B by the procedure described in the preceding section (part b) gave a 34% yield of Δ¹-unsaturated keto diacetate mixture, 53% of crystalline Δ⁴-unsaturated ketone diacetate mixture and a 2.5% yield of intermediate fractions containing both the Δ¹- and Δ⁴-unsaturated products.

Hydrogenation of the Δ¹-Unsaturated Keto Diacetate Mixture (XIV).—A solution of 0.730 g. of the Δ¹-unsaturated keto diacetate mixture described in the preceding section in 30 ml. of 95% ethanol was hydrogenated over 50 mg. of 10% palladium-on-carbon (Engelhard Industries, Inc.) at room temperature and atmospheric pressure. After 30 min. the absorption of gas ceased with the uptake of about 0.7 mole-equivalent. The mixture was filtered, and the residue obtained on evaporation of the filtrate amounted to 0.739 g. This product was combined with 0.164 g. from a similar run and chromatographed on 40 g. of Florisil. The fraction eluted with 2–5% acetone in benzene consisted of crystalline keto diacetate mixture XII (R^1 = R^2 = H) and amounted to 0.779 g. (87% yield). Bromination and dehydrobromination of this material as described above gave the Δ¹- and Δ⁴-keto diacetates in 32 and 50% yields, respectively.

DL-1β-(2-Acetoxyethyl)-2α-(2-acetoxypropyl)-4β-hydroxy-4bβ-methyl-7-ethylenedioxy-1,2,3,4,4aα,4b,5,6,7,8,10,10aβ-dodecahydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone (XV, R^1 = R^2 = Ac). (a) Isomer A.—A modification of a previously described procedure[23] was employed. A solution of 100 mg. of the aforementioned unsaturated keto diacetate A (XIII), m.p. 179–181°, and 30 mg. of *p*-toluenesulfonic acid monohydrate in 20 ml. of 2-methyl-2-ethyl-1,3-dioxolane[23] was distilled slowly through a 4-in. Vigreux column (nitrogen atmosphere) so that 8 ml. of distillate was collected in 2 hr. The resulting pale yellow solution was cooled, 5 ml. of 10% potassium bicarbonate solution was added with swirling, and then benzene was added. The aqueous layer was extracted with benzene, and the combined organic layers were washed with saturated brine and dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent at reduced pressure was chromatographed on 5.5 g. of Florisil. Elution with benzene through 10% ether in benzene afforded 17 mg. of non-crystalline oily material. Further elution with 20% ether in benzene through 1% absolute ethanol in ether afforded 90.2 mg. of colorless needles, m.p. 127–143°, which showed only end absorption in the ultraviolet spectrum. Recrystallization from diisopropyl ether–ethyl acetate provided a total of 61.8 mg. (62% yield) of the ketal: 52.0 mg. (first crop), m.p. 152–153°; and 15.8 mg. (second crop), m.p. 150–152.5°. Two recrystallizations of the first-crop material from diisopropyl ether–acetone gave fine colorless needles, m.p. 154.5–156°; $\lambda_{max}^{CHCl_3}$ 5.68 μ (γ-lactone C=O), 5.80 (acetate C=O) and 8.08 (acetate C—O).

Anal. Calcd. for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 65.9; H, 7.9.

Further elution of the chromatographic column with 2% absolute ethanol in ether through acetone gave 34 mg. (26% recovery) of a crystalline product. Recrystallization from ethyl acetate–petroleum ether gave 20.4 mg. of prisms, m.p. 166–176°, undepressed on admixture with the starting material.

(b) Isomer B.—A solution of 112 mg. of the aforementioned unsaturated keto diacetate B, m.p. 148–151°, and 30 mg. of *p*-toluenesulfonic acid monohydrate in 20 ml. of the dioxolane (see above) was distilled so that 9 ml. of distillate was collected in 4 hr. The product was processed just as described for isomer A in the preceding section. The crude yellow crystalline product, on trituration with ether, yielded 117 mg. of needles, m.p. 130–159°. The extinction coefficient in the ultraviolet spectrum at 237 mμ was 3631, indicating that 30–40% of starting material was still present. Recrystallization from diisopropyl ether–ethyl acetate afforded 69 mg. (56% yield) of almost colorless blades, m.p. 160–168°. Repeated recrystallization of such material from diisopropyl ether–acetone gave colorless blades, m.p. 174.5–175°. The infrared spectrum of this material was essentially identical with that of isomer A (see above) except for an additional band at 8.71 μ in the latter.

Anal. Calcd. for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 66.1; H, 7.9.

From the residue of the combined mother liquors, there was obtained, after chromatography on Florisil followed by chromatography on acid-washed alumina, additional ketal B which, after crystallization, amounted to 6.3 mg. of colorless blades, m.p. 170–172.5°. The total ketal product (75.3 mg.)—less 7.0 mg. which constituted the analytical specimen—was recrystallized from diisopropyl ether–methanol to give 64.3 mg. (58% yield): 54.5 mg. (first crop), m.p. 172.5–174°; and 9.8 mg. (second crop), m.p. 169–171°.

The chromatographies mentioned above also yielded in the later eluates a total of 23.5 mg. (21% recovery) of starting material as colorless prisms, m.p. 131–145°. Recrystallization from diisopropyl ether–ethyl acetate gave 16.6 mg. of pure unsaturated keto diacetate B, m.p. 149–151.5°, undepressed on admixture with the analytical specimen.

(c) **Mixture of Epimers.**—A solution of 1.32 g. of the aforementioned crystalline mixture of Δ^4-unsaturated keto diacetates A and B and 120 mg. of *p*-toluenesulfonic acid monohydrate in 100 ml. of the dioxolane (see above) was distilled so that 50 ml. of distillate was collected after 6 hr. The resulting solution was processed just as described above under part a, and the crude product was chromatographed on 75 g. of Florisil. The total crystalline crude ketal diacetate mixture amounted to 1.05 g. Crystallization from ether gave 0.354 g. of colorless needles, m.p. 142–158°. In addition, a total of 0.444 g. of non-crystalline material, $\lambda_{max}^{95\%\ EtOH}$ 236 mμ (ε 11,000), was obtained and consisted largely of recovered starting material (about 20% recovery estimated from the extinction coefficient of the ultraviolet spectrum).

DL-1β-(2-Hydroxyethyl)-2α-(2-hydroxypropyl)-4β-hydroxy-4bβ-methyl-7-ethylenedioxy-1,2,3,4,4aα,4b,5,6,7,8,10,10aβ-dodecahydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone (XV, $R^1 = R^2 = H$). (a) **Isomer A.**—A 62.0-mg. sample of the aforementioned ketal diacetate A, m.p. 151–153°, was dissolved in 2.0 ml. of anhydrous methanol (distilled from potassium hydroxide) by warming at steam-bath temperature. The resulting solution was cooled to room temperature, 2.0 ml. of 10% methanolic potassium hydroxide was added with swirling, and the resulting solution allowed to stand for 6 hr. at 26°. Crystallization could not be induced by the addition of water so chloroform was added, and the aqueous phase extracted with chloroform. The combined organic layers were washed with saturated brine and dried over anhydrous sodium sulfate. The glassy residue obtained on evaporation of the solvent at reduced pressure was crystallized from ethyl acetate to give 40.1 mg. (78% yield) of colorless rods, m.p. 185–187°. Comparable material from another experiment was repeatedly recrystallized from ethyl acetate and finally from diisopropyl ether–ethyl acetate to give colorless needles, m.p. 185–185.5°.

Anal. Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 67.8; H, 8.2.

The aqueous alkaline solution from the above experiment was acidified with hydrochloric acid to liberate and effect relactonization of hydroxy acid. Isolation of the product as described above gave 1.3 mg. of relactonized material which only partly crystallized on seeding with the pure material.

(b) **Isomer B.**—The 54.5-mg. sample of the aforementioned ketal diacetate B, m.p. 172.5–174°, was hydrolyzed exactly as described in the preceding section for isomer A. The neutral fraction yielded, after crystallization from ethyl acetate, 34.1 mg. (75% yield) of material, m.p. 196–199.5°, and the basic fraction yielded 11.7 mg. of relactonized material, m.p. 205–208°. Recrystallization of the former product from ethyl acetate gave 18 mg., m.p. 209–213°, undepressed on admixture with the 205–208° material. Repeated recrystallizations from aqueous acetone containing a trace of pyridine (necessary to prevent hydrolysis of ketal residue) gave colorless blades, m.p. 206–208.5°.

Anal. Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 68.3; H, 8.65.

(c) **Mixture of Epimers.**—A 0.326-g. portion of the aforementioned ketal diacetate mixture, m.p. 142–158°, was dissolved in 35 ml. of methanol with warming, then cooled to slightly below room temperature. A solution of 1.4 g. of potassium carbonate in 14 ml. of water was added with stirring, and the resulting pale yellow mixture allowed to stand for 8 hr. at room temperature. Saturated brine was then added with stirring, and the mixture extracted with benzene–ethyl acetate followed by ethyl acetate, and the combined organic layers were dried over anhydrous sodium sulfate. Evaporation of the solvent at reduced pressure gave a colorless glassy product which on trituration with ethyl acetate provided 0.255 g. (94% yield) of colorless prisms, m.p. 127–134°. Acidification of the alkaline aqueous phase followed by isolation as described above afforded 15 mg. (5.6% yield) of relactonized material as a yellow glass.

DL-1β-(2-*p*-Toluenesulfonoxyethyl)-2α-(2-ketopropyl)-4β-hydroxy-4bβ-methyl-7-ethylenedioxy-1,2,3,4,4aα,4b,5,6,7,8,10,-10aβ-dodecahydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone (XVI).—To an ice-cold solution of 138 mg. of the aforementioned ketal diol mixture, m.p. 127–134°, in 4.9 ml. of pyridine (stored over and distilled from barium oxide) was added with stirring 72 mg. of *p*-toluenesulfonyl chloride, m.p. 69.4–69.6°. The flask containing the solution was chilled and allowed to stand for 16 hr. at 8°; then 1 ml. of cold ethyl acetate was added, followed by 1 ml. of 5% sodium bicarbonate solution. The mixture was stirred for 5 min., then diluted with 5% sodium bicarbonate solution and ethyl acetate. The aqueous layer was extracted with chloroform, and the combined organic layers were dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure (bath temperature 20–25°), and the last traces of pyridine were removed from the residue by co-distillation with benzene. The colorless foamy residue amounted to 227 mg., λ_{max} 224 mμ (ε 10,500) (note that the pure material has an extinction of 11,600). To an ice-cold slurry of 220 mg. of anhydrous chromium trioxide in 2.2 ml. of anhydrous pyridine was added a solution of the crude *p*-toluenesulfonate described directly above in 0.5 ml. of pyridine. An additional 0.9 ml. of pyridine was used to assist in the transfer. The flask containing the solution was sealed and allowed to stand at room temperature for 9 hr. The product was isolated as described directly above for the precursor to give, after the co-distillation with benzene, 222 mg. of tan glassy residue which was chromatographed on 12 g. of Florisil. Elution with benzene and 5% ethyl acetate in benzene gave 32 mg. of unidentified amorphous material. Further elution with 10% ethyl acetate in benzene gave a total of 162 mg. (86% yield) of crystalline keto *p*-toluenesulfonate, m.p. 116–119°. Recrystallization of comparable material from another experiment from methylene dichloride–ether gave small colorless needles, m.p. 113–114° dec., $\lambda_{max}^{95\%\ EtOH}$ 224 mμ (ε 11,600); $\lambda_{max}^{CH_2Cl_2}$ 5.67 μ (γ-lactone C=O), 5.84 (saturated ketone C=O), 6.25 (aromatic C=C), and 8.48 (S=O). The melting point varied with the rate of heating.

Anal. Calcd. for $C_{30}H_{38}O_8S$: C, 64.50; H, 6.86. Found: C, 64.25; H, 6.9.

DL-1β-(2-Mesitylenesulfonoxyethyl)-2α-(2-ketopropyl)-4β-hydroxy-4bβ-methyl-7-ethylenedioxy-1,2,3,4aα,4b,5,6,7,8,10,10aβ-dodecahydrophenanthrene-2β-carboxylic Acid (2→4)-Lactone.—The procedure was essentially the same as that described in the preceding section. A cold solution of 46 mg. of the ketal–diol mixture in 0.2 ml. of pyridine was treated with 50 mg. of mesitylenesulfonyl chloride, m.p. 54.5–55.4°, and allowed to stand at 8° for 48 hr. The crude product, isolated as described above, was oxidized with 87 mg. of chromium trioxide in a total of 1.7 ml. of pyridine. After 24 hr. the product was isolated as described above and chromatographed on 3.5 g. of Florisil to yield, on elution with 5–10% ethyl acetate in benzene, 51.4 mg. of crystalline ester. Recrystallization from chloroform–ether provided colorless prisms, m.p. 161–162°; $\lambda_{max}^{95\%\ EtOH}$ 232 mμ (ε 10,900), 276 (1,800), 285 (1,800); $\lambda_{max}^{CH_2Cl_2}$ 5.69 μ (γ-lactone C=O), 5.84 (saturated ketone C=O), 7.34, 8.44 and 8.48 (S=O).

Anal. Calcd. for $C_{32}H_{42}O_8S$: C, 65.51; H, 7.22. Found: C, 65.2; H, 7.4.

DL-3-Ethylenedioxy-11β-hydroxy-17α-Δ^5-pregnene-20-one-18-carboxylic Acid (11→18)-Lactone (XVII).—To a solution of 110 mg. of the aforementioned crude crystalline keto *p*-toluenesulfonate in 3.5 ml. each of anhydrous benzene and anhydrous *t*-butyl alcohol was added 1.3 ml. of a 0.22 *N* solution of potassium *t*-butoxide in *t*-butyl alcohol, and the solution was stirred for 30 min. at room temperature (atmosphere of nitrogen). The mixture was then cooled (ice-bath) and diluted with ice-cold ethyl acetate and 5 ml. of cold 0.5% hydrochloric acid. The layers were immediately separated, and the organic phase, while still cold, was washed with 10% potassium bicarbonate solution followed by brine. The combined aqueous layers were extracted with ethyl acetate, and all of the combined organic layers were dried over sodium sulfate. Evaporation of the solvent at reduced pressure (bath temperature 35–40°) gave a yellow oil containing crystalline material. Filtration and washing with cold 1:1 ethyl acetate–ether provided 16 mg. of colorless needles, m.p. 160–162°. The residue from the mother liquor was chromatographed on 3.5 g. of Florisil. The fraction eluted with 5–10% ether in benzene amounted to 18 mg. of colorless needles, m.p. 142–146°. The fractions eluted with ethyl acetate were shown by the infrared spectrum to contain *p*-toluenesulfonate, so that they were combined (31 mg.) and retreated in 1 ml. each of benzene and *t*-butyl alcohol with 0.44 ml. of 0.22 *N* potassium *t*-butoxide in *t*-butyl alcohol as described above. The product was processed just as described above to give an additional 7 mg. of colorless needles, m.p 138–150°. The total yield of crude crystalline cyclization product was 41 mg. (54%).

A comparable product from a preliminary experiment starting with crude non-crystalline keto *p*-toluenesulfonate was purified by rechromatography to give colorless needles, m.p. 149–156°. Two recrystallizations from ethyl acetate–petroleum ether gave colorless needles, m.p. 134–139°, with resolidification and remelting at 195–199°; $\lambda_{max}^{CHCl_3}$ 5.68 μ (γ-lactone C=O), 5.85 (saturated ketone C=O) and 9.07 (ketal C—O). Wettstein and

co-workers[26] reported m.p. 190–193° with loss of water of crystallization between 135–150°.

In this same preliminary experiment a second product, m.p. 199–207°, was separated by chromatography and recrystallization from ethyl acetate–petroleum ether. On admixture of this material with authentic DL-3-ethylenedioxy-11β-hydroxy-Δ⁵-pregnene-20-one-18-carboxylic acid (11→18)-lactone (XVII),[27] the melting point was 198–214°. The infrared spectra of the two samples were essentially identical.

Cyclization of the aforementioned keto mesitylenesulfonate by the procedure described above gave comparable yields of the 17α-acetyl ketal.

Conversion of the 17α-Isomer XVII into the 17β-Form XVIII.—A solution of 10.0 mg. of the 17α-acetyl ketal XVII, m.p. 184–190°, and 40 mg. of anhydrous potassium carbonate in 2.0 ml. of methanol and 0.3 ml. of water was heated under reflux for 1 hr. (atmosphere of nitrogen). The resulting solution was diluted with 5 ml. of water and concentrated to a volume of 4–5 ml. under reduced pressure. Chloroform was added, and the mixture was acidified to *p*H 2 with 5% hydrochloric acid with cooling. The solution was then washed with 10% potassium bicarbonate solution, water, followed by saturated brine and dried over anhydrous sodium sulfate. The solid residue obtained on evaporation of the solvent was chromatographed on 0.5 g. of Florisil. Elution with 5–20% ether in benzene gave a total of 3.7 mg. of ether-soluble 17α-isomer, m.p. 125–131° and 153–157.5°. Further elution with 50% ether in benzene through ethyl acetate gave 6.0 mg. of ether-insoluble crystals, m.p. 192–198°. Recrystallization from diisopropyl ether–methylene dichloride gave 4.0 mg. of colorless blades, m.p. 209.5–213.5°, undepressed on admixture with the authentic material (see above).

DL-11β-Hydroxy-Δ⁴-pregnene-3,20-dione-18-carboxylic Acid (11→18)-Lactone.—A solution of 2.5 mg. of the 17β-acetyl ketal prepared as described in the preceding section in 0.5 ml. of acetone containing 0.9 mg. of *p*-toluenesulfonic acid monohydrate and 0.015 ml. of water was heated at reflux for 1 hr. (atmosphere of nitrogen). About 1 ml. of water was added, and then the solution was evaporated under reduced pressure in order to remove the acetone. The resulting suspension was diluted with 3 ml. of 5% potassium bicarbonate solution and extracted with methylene dichloride. The combined organic layers were washed with saturated brine and dried over anhydrous sodium sulfate. The residue obtained upon evaporation of the solvent was crystallized from ethyl acetate–petroleum ether to give 1.1 mg. of colorless blades, m.p. 215–218.5°, undepressed on admixture with authentic material, m.p. 211.5–217°.[27] The infrared spectra of the two materials were identical.

DL-11β-Hydroxy-17α-Δ⁴-pregnene-3,20-dione-18-carboxylic Acid (11→18)-Lactone.—A 3.0-mg. sample of the aforementioned 17α-acetyl ketal XVII was treated just as described in the preceding section for the hydrolysis of XVIII. After evaporation of the acetone, there was obtained 2.2 mg. of colorless material, m.p. 209–214°. Recrystallization from ethyl acetate–petroleum ether afforded 1.5 mg. of colorless rods, m.p. 224.5–228° (reported[26] m.p. 224–227°); $\lambda_{max}^{CHCl_3}$ 5.68 μ (γ-lactone C=O), 5.86 (ketone C=O), 6.02 (unsaturated ketone C=O) and 6.19 (conjugated C=C).

DL-3-Ethylenedioxy-11β,20-dihydroxy-17α-Δ⁵-pregnene-18-al (11→18)-Lactol (XIX).—The following procedure is a modification of that already described for the partial reduction of an 11→18-steroid lactone.[45] A 50.0-mg. sample of the 17α-acetyl ketal XVII (a mixture of two fractions, m.p. 190.5–192° and 193.5–195.5°) in a 6-ml. round-bottomed flask equipped with a side arm and a magnetic stirring bar was dried in a desiccator for 3 hr. at 26° (0.1 mm.) over calcium hydride; and, with a slow stream of dry nitrogen passing through the system *via* the side arm, there was added with stirring a solution of 10.4 mg. of lithium aluminum hydride in 4.1 ml. of anhydrous tetrahydrofuran. The solution, which immediately became homogeneous, was allowed to stand for 2 hr. at 26°. A solution of 2 drops of water in 1 ml. of tetrahydrofuran was then added dropwise with stirring, and the resulting suspension was filtered and the residue washed with tetrahydrofuran and then with methylene dichloride. Evaporation of the combined filtrates and washings under a stream of nitrogen gave 50.2 mg. of solid, m.p. 180–192°, with some previous softening. Recrystallization from ethyl acetate–petroleum ether gave 41.4 mg. (82% yield) of colorless prisms, m.p. 190–193.5°, $\lambda_{max}^{CHCl_3}$ 2.88 μ (OH) and 9.1 (ketal C–O). The residue from the mother liquors was chromatographed on 0.45 g. of Florisil. Elution with 2–4% acetone in benzene gave 3.7 mg. of additional lactol, m.p. 185–191°, undepressed on admixture with the above material. The total yield was thus 44.8 mg. or 89%.

Material from a comparable run on repeated recrystallization from ethyl acetate–petroleum ether was obtained as fine colorless prisms, m.p. 193–194°.

(45) J. v. Euw, R. Neher and T. Reichstein, *Helv. Chim. Acta*, **38**, 1423 (1955).

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.7; H, 8.55.

DL-11β,20-Dihydroxy-17α-Δ⁴-pregnene-3-one-18-al (11→18)-Lactol (XX, R = H).—A solution of 40.0 mg. of the lactol ketal XIX, m.p. 190–193.5°, described in the preceding experiment, in 4.0 ml. of acetone containing 7.2 mg. of *p*-toluenesulfonic acid monohydrate and 0.12 ml. of water was heated under reflux for 1 hr. (atmosphere of nitrogen). The resulting solution was diluted with 4.0 ml. of water, concentrated until cloudy, and seeded with material from a preliminary run. After 1.5 hr., 32.1 mg. of colorless blades, m.p. 208–231°, crystallized. Comparable material from another run was repeatedly recrystallized from methanol–ethyl acetate to give colorless microcrystals, m.p. 249–251 dec.; λ_{max}^{KB} 2.96 μ (OH), 6.60 (unsaturated ketone C=O) and 6.19 (conjugated C=O). Another specimen, similarly prepared, melted at 247–248.5° dec., $\lambda_{max}^{95\%\ EtOH}$ 242 mμ (ε 15,500).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73; and for $C_{21}H_{30}O_4 \cdot 2/3H_2O$: C, 70.40; H, 8.81. Found: C, 70.5; H, 8.6.

DL-21-Desoxy-17α-aldosterone Methyl Ether (XXI).—To a solution of 33.6 mg. of crude lactol unsaturated ketone XX (R = H), prepared as described in the preceding section, in 6.0 ml. of anhydrous methanol was added a solution of 4.5 mg. of *p*-toluenesulfonic acid monohydrate in 0.5 ml. of anhydrous methanol. The resulting solution was stirred for 5 min. and then allowed to stand for 26 hr. at room temperature (atmosphere of nitrogen). Saturated potassium bicarbonate solution (1.0 ml.) was then added with rapid stirring, followed by 2.0 ml. of water and then the solution was concentrated almost to dryness under reduced pressure. Chloroform was added to the residue, and the solution was washed with water, then with saturated brine. The combined aqueous layers were washed with chloroform, and the combined organic solutions were dried over anhydrous sodium sulfate. Evaporation of the solvent gave 35.2 mg. of colorless glassy residue which crystallized from diisopropyl ether to give 28.1 mg. (79% yield) of the **lactol methyl ether XX** (R = CH_3) as colorless prisms, m.p. 168–169.5°; $\lambda_{max}^{CHCl_3}$ 2.89 μ (OH), 6.02 (unsaturated ketone C=O) and 6.19 (conjugated C=C).

A solution of 35.0 mg. of crude unrecrystallized lactol methyl ether, prepared as described in the preceding experiment, in 0.20 ml. of anhydrous pyridine was transferred with the aid of 0.12 ml. of pyridine to Sarett reagent prepared from 34.0 mg. of chromium trioxide and 0.34 ml. of anhydrous pyridine. The mixture was stirred for 15 min. and then allowed to stand for 16 hr. at room temperature. The crude product was isolated as in the oxidation experiments described above, and amounted to 34.5 mg. of a colorless glass which crystallized from diisopropyl ether to yield 26.3 mg. of colorless needles, m.p. 165.5–167.5°, with slight previous softening. Comparable material from another experiment was obtained after recrystallization from ethyl acetate–petroleum ether as large colorless plates, m.p. 166.5–168°, with slight previous softening; $\lambda_{max}^{95\%\ EtOH}$ 240 mμ (ε 18,400); $\lambda_{max}^{CH_2Cl_2}$ 5.86 μ (γ-lactone C=O), 6.00 (unsaturated ketone C=O), 6.19 (conjugated C=C) and 8.94 (ether C—O).

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.5; H, 8.2.

Epimerization of the 17α-Acetyl Lactol Methyl Ether (XXI).—A 3.0-mg. sample of the aforementioned 17α-acetyl lactol methyl ether, m.p. 165.5–167.5°, was dissolved in a 1.0-ml. aliquot of a solution which was prepared from 0.2 g. of anhydrous potassium carbonate, 1.5 ml. of water and 10 ml. of methanol. The resulting solution was heated under reflux for 3 hr. (atmosphere of nitrogen), then 3 ml. of water was added, and the mixture distilled at reduced pressure to eliminate most of the methanol. Then saturated brine was added, the mixture extracted with chloroform, and the combined organic layers dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent at reduced pressure was chromatographed on 1.5 g. of Florisil. Elution with 2–50% ether in benzene provided a total of 1.6 mg. (53% yield) of crude starting material, m.p. 163–167°. On recrystallization there were obtained colorless needles, m.p. 167–168.5°, undepressed on admixture with the starting material. Continued elution with 2% ethyl acetate in ether gave 0.2 mg. of an intermediate fraction, and further elution with 5–25% ethyl acetate in ether afforded 0.7 mg. (20% yield) of crystalline material. Recrystallization from methylene dichloride–petroleum ether gave 0.3 mg. of material, m.p. 159–166.5°, which was undoubtedly the 17β-isomer; $\lambda_{max}^{CHCl_3}$ 5.85 μ (saturated ketone C=O), 6.01 (unsaturated ketone C=O) and 6.19 (conjugated C=C). A mixture of this material with the 17α-isomer melted at 136–154°.

When the above experiment was repeated, but the heating period was extended to 6 hr., the ratio of products isolated was essentially unaltered.

DL-17α-Aldosterone Acetate Methyl Ether (XXII, R = CH_3).—The following procedure is a scaled-down version of the previously reported[30] method. Our exploratory experiments were carried

out with progesterone which was thus converted on a 21-mg. scale into desoxycorticosterone acetate in 35% yield.

A mixture of a 23.3-mg. sample of the 17α-acetyl lactol methyl ether XXI, m.p. 165–168°, 39.0 mg. of diethyl oxalate and 0.30 ml. of anhydrous *t*-butyl alcohol was warmed to 70° with stirring until the solution became homogeneous (atmosphere of nitrogen). The solution was then cooled to 55°, and 0.040 ml. of 4.6 *N* methanolic sodium methoxide was added with stirring. The greenish yellow pasty mixture was allowed to stir for 15 min. at room temperature, and then a solution of 11.0 mg. of glacial acetic acid in 0.5 ml. of anhydrous methanol was added, followed, after 3 min., by a solution of 16.5 mg. of iodine in 0.30 ml. of anhydrous methanol. The color of the solution faded to pale brown within 20 min., and, after a total of 2.5 hr., 130 mg. of freshly fused potassium acetate was added with stirring, and the solution was allowed to stand at 26° for 24 hr. The mixture was poured into 7 ml. of ice-water contained in a centrifuge tube, two 1.0-ml. portions of 95% ethanol being used to aid in the transfer. The finely divided yellow precipitate was separated by centrifugation, washed with three 1-ml. portions of water and then dried to give 24.0 mg. (72% yield) of crude 3-glyoxolate of XXII (R = CH_3) which gave a purple color with 2% alcoholic ferric chloride.

The aqueous solution and washings were combined, diluted with saturated brine and extracted with chloroform. The combined organic layers were washed with saturated brine, and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave 7.5 mg. of a yellow-green glassy material which gave a purple color with alcoholic ferric chloride solution. This product was treated separately as described below. The crystalline 24.0-mg. fraction was dissolved in 0.43 ml. of anhydrous methanol containing 20.0 mg. of freshly fused sodium acetate (atmosphere of nitrogen), the solution was cooled (ice-bath) and a solution of 7.37 mg. of bromine in 0.10 ml. of anhydrous methanol was added slowly with stirring over a 5-min. period. After the addition was complete, the mixture containing the colorless precipitate was stirred for an additional 3 min.; then 0.050 ml. of 0.92 *N* methanolic sodium methoxide was added. The resulting slurry was allowed to warm to room temperature for 1 hr. with stirring; then 0.035 ml. of glacial acetic acid and 14.0 mg. of zinc powder were added, and the mixture was stirred vigorously for 1 hr. at room temperature (atmosphere of nitrogen). The mixture was diluted with 10 ml. of chloroform and the solution washed with 5 ml. of saturated brine, followed by 3 ml. of 10% potassium bicarbonate. The aqueous solutions were extracted with chloroform, and the total combined organic layers were washed with saturated brine and dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent was chromatographed on 1 g. of Florisil. The early fractions eluted with benzene through 5% ether in benzene amounted to a total of 2.5 mg. and were not further studied. From the later fractions, eluted with 10–20% ether in benzene, it was possible to obtain, after recrystallization from diisopropyl ether–ether, a total of 3.3 mg. of crystalline starting material XXI: 2.7 mg. (first crop), m.p. 158–165°, undepressed on admixture with starting material; and 0.6 mg. (second crop), m.p. 152–161°. The infrared spectrum of the first-crop material was identical with that of the starting material. The spectrum of the residue from the recrystallizations, however, displayed bands characteristic of the 21-acetoxy compound (see below). Since further elution of the column with solvents up through 10% ethyl acetate in ether afforded material which could not be purified on crystallization, all of these fractions, along with the residue from the mother liquors of the recrystallization, were combined and rechromatographed on Florisil. Elution with benzene followed by 1% acetone in benzene afforded a total of 2.9 mg. which was not further studied. Elution with 2% acetone in benzene gave a total of 6.7 mg. of material which, after two crystallizations from diisopropyl ether–diethyl ether gave an additional 0.8 mg. of starting material, m.p. 158–165°. Further elution with 3–25% acetone in benzene gave 1.5 mg. of crystalline material which was combined with the residue from the mother liquor of the 0.8-mg. product, and recrystallized twice from diisopropyl ether to give 1.6 mg. of prisms, m.p. 129–134°; $\lambda_{max}^{CHCl_3}$ 5.74 μ (α-keto acetate C=O), 5.79 (α-acetoxy ketone C=O), 6.02 (unsaturated ketone C=O), 6.19 (conjugated C=C), 8.12 (acetate C—O) and 8.94 (lactol C—O).

The 7.5 mg. of non-crystalline 2-glyoxolate adduct (see above) was treated just as described above for the crystalline glyoxolate with bromine, sodium methoxide and zinc and acetic acid. Chromatography of the crude product on 0.4 g. of Florisil gave, after elution of 1.7 mg. of gummy material with 5% ether in benzene, a total of 3.5 mg. (elution with 10–25% ether in benzene) of material which on crystallization from ether–petroleum ether gave 2.9 mg. of colorless prisms, m.p. 130–134.5°. The infrared spectrum of this material was identical with that of the 129–134° material described above. The over-all yield of the 21-acetoxy compound XXII, therefore, was 20%. Sufficient material was not available for obtaining a carbon–hydrogen analysis.

In another experiment it was found that the products could be separated effectively by paper chromatography. Thus the crude product from 25.8 mg. of the 17α-acetyl lactol methyl ether XXI, treated essentially as described above, was chromatographed on four 6-in. sheets of Whatman No. 1 paper with a formamide–cyclohexane–benzene system (4:1). The fraction with R_f 0.7 was eluted with 80% aqueous tetrahydrofuran and after trituration with ether gave 8.2 mg. of starting material, m.p. 155–167°. The fractions with R_f about 0.5 were eluted with 80% aqueous tetrahydrofuran to give on trituration with ether 6.8 mg. of colorless rods, m.p. 128–139°.

DL-17α-Aldosterone Acetate (XXII, R = H).—A 2.8-mg. sample of the acetoxy lactol methyl ether XXII (R = CH_3), described in the preceding section, was dissolved in 0.4 ml. of 70% acetic acid in water and the solution was warmed for 1 hr. at 70° (atmosphere of nitrogen). Chloroform was added and the solution washed with saturated sodium bicarbonate solution, water, then with saturated brine and finally over anhydrous sodium sulfate. Since the infrared spectrum of the residue obtained upon evaporation of the solvent was essentially identical with that of the starting material, the total product was retreated again exactly as described above. The crude product of the second hydrolytic treatment was chromatographed on 0.15 g. of Florisil. Early eluates afforded 0.8 mg. which, on crystallization from diisopropyl ether–ether, gave 0.3 mg. of starting material, m.p. 130.5–133°. Further elution gave 0.6 mg. of non-crystalline material, and finally elution with 5–10% acetone in benzene provided 1.5 mg. of colorless microcrystals, m.p. 164–167°. Recrystallization from diisopropyl ether–methylene dichloride gave 1.3 mg., m.p. 166–169°; $\lambda_{max}^{CHCl_3}$ 2.89 μ (OH), 5.74 (α-keto acetate C=O), 5.79 (α-acetoxy ketone C=O), 6.00 (unsaturated ketone C=O), 6.19 (conjugated C=C), and 8.10 (acetate C—O). Wettstein and co-workers[26] reported m.p. 158.5–162°. An infrared spectrum of the latter product[27] was essentially identical with the spectrum of our 169° material.

DL-Aldosterone.—The procedure for the epimerization of aldosterone[32] was used. A mixture of 1.1 mg. of DL-17α-aldosterone-21-acetate (XXII, R = H) (combined fractions, m.p. between 165–168° and 167–170°) and 0.2 ml. of 0.1 *N* potassium carbonate in 80% methanol in water was stirred at room temperature in an atmosphere of nitrogen. After 10 min. a small piece of Dry Ice and 0.2 ml. of water were added to the clear solution, and the mixture was concentrated at reduced pressure in order to remove most of the methanol. The cloudy solution was diluted with saturated brine and extracted thoroughly with methylene dichloride. The combined organic layers were washed with water, then with saturated brine and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave a partly crystalline residue. This material was streaked on Whatman No. 1 paper and developed in a propylene glycol–toluene system for 20 hr. Two streaks were observed, one with R_s (cortisone) 1.25 and the other 0.9. Since the R_s value for aldosterone on the same sheet was 1.24, the 1.25 fraction was eluted with 80% aqueous tetrahydrofuran. Trituration of the residue with acetone and then with ether gave about 0.2 mg. of off-white prisms, m.p. 142–147° (with a small portion melting at 160°). Note that the reported melting point of DL-aldosterone is not characteristic; reported values are 154°, 183–185°, 197–204°.[26] The solution ($CHCl_3$) infrared spectrum of our sample was determined in the laboratories of Dr. R. N. Jones[33] and was found to be identical within experimental error with that of an authentic sample of DL-aldosterone.

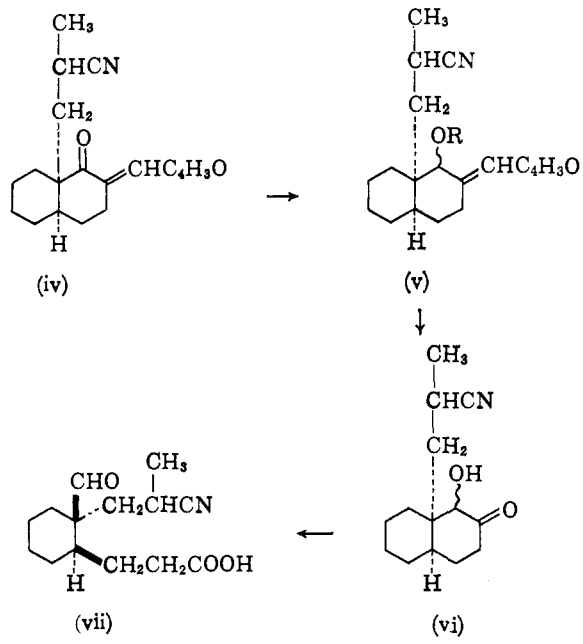
The streak with R_s at 0.9 was considerably more intense than the aldosterone fraction. Since it was undoubtedly mainly 17α-aldosterone, the fraction was eluted and treated again with 0.1 *N* potassium carbonate exactly as described above. Rechromatography gave two streaks, one with R_s (cortisone) 1.3, which was identical with the R_s value of authentic DL-aldosterone placed on the same sheet. This fraction was eluted and submitted for biological testing in the laboratories of Dr. Edgar Gordon.[34] The activity was indistinguishable from that of authentic DL-aldosterone.

The experiment described directly above was carried out after a series of runs on a 100-γ scale were completed. In one such experiment, 100 γ of DL-17α-aldosterone-21-acetate was treated with 0.2 ml. of 0.1 *N* potassium carbonate in 80% methanol and water. The total crude product isolated as described above was spotted on Whatman No. 1 paper in a propylene glycol–toluene system. Two major spots were noted with R_s (cortisone) 1.21 and 0.83. Aldosterone spotted on the same sheet had R_s 1.23. The R_s at 0.83 was the more intense of the two. There was also a suggestion of a faint spot at 0.37. The spot corresponding to aldosterone was eluted with 80% aqueous tetrahydrofuran and rechromatographed in the system formamide–chloroform, giving a single spot R_f 0.65 (aldosterone R_f 0.66).

Model Experiments

With Stephen D. Darling and Robert A. Clement

2-Furfurylidene-9-(2-cyanopropyl)-*cis*-1-decalone (iv).—To a solution of 1.2 g. of sodium hydride in 12 ml. of anhydrous



methanol was added a solution of 50 g. of 2-furfurylidene-1-decalone[40] in 180 ml. of anhydrous dioxane followed by 35 g. of methacrylonitrile. After 4.5 hr. at room temperature, 10 ml. of acetic acid was added, most of the solvent was evaporated at reduced pressure (steam-bath temperature) and the residue was dissolved in ether. The solution was washed with 10% potassium carbonate solution followed by water and dried over anhydrous magnesium sulfate. The residue obtained on removal of the solvent amounted to 66 g. of a red oil which was used directly in the subsequent reactions.

In a preliminary experiment the comparable crude product was chromatographed on Florex in an unsuccessful attempt to separate the epimeric mixture. The main fraction which was eluted with 30% benzene in petroleum ether was evaporatively distilled at reduced pressure to give a colorless oil, $\lambda_{max}^{95\%\ EtOH}$ 328.8 mμ.

Anal. Calcd. for $C_{19}H_{24}O_2N$: C, 76.24; H, 7.80. Found: C, 76.4; H, 7.7.

Reduction of the furfurylidene ketone iv to the alcohol v (R = H) was effected by sodium borohydride. To a cooled (ice-bath) solution of 66 g. of the aforementioned furfurylidene ketone iv in 1200 ml. of methanol and 200 ml. of water was added, with stirring, 50 g. of sodium borohydride. After 8 hr. at about 1°, the ultraviolet absorption at 329 mμ due to the unsaturated ketone had vanished. Water was added, and the mixture was extracted thoroughly with benzene. The combined benzene extracts were washed with water, ice-cold 10% hydrochloric acid, ice-cold 10% potassium carbonate solution and then dried over anhydrous potassium carbonate. The gummy residue obtained on evaporation of the solvent was triturated with ether–petroleum ether, and the solid which was formed amounted to 47 g., m.p. 105–132°; λ_{max} 2.87 μ (OH), 4.44 (CN). The tetrahydropyranyl ether v (R = 2-tetrahydropyranyl) was prepared in the conventional manner from 40 g. of the crude hydroxy compound v (R = H) and 160 ml. of dihydropyran in petroleum ether–benzene containing 0.5 g. of *p*-toluenesulfonic acid monohydrate. Sufficient solvent was used to dissolve about one-half of the substrate. The mixture was then stirred at room temperature. After 35 min. all of the solid had dissolved and after 2 hr. the mixture was washed with cold 5% potassium carbonate solution and dried over anhydrous potassium carbonate. The red oily residue obtained upon evaporation of the solvent amounted to 56 g. and was contaminated with polymeric material from the dihydropyran. There was no absorption in the infrared corresponding to OH. The ultraviolet spectrum exhibited bands at 263, 270 and 282 mμ, typical of a double bond conjugated with a furan nucleus. The hydroxy ketone vi was prepared by ozonization and hydrolysis of the crude tetrahydropyranyl ether. A solution of 3.6 g. in 150 ml. of ethyl acetate containing 2.2 g. of pyridine was cooled (Dry Ice-bath) and treated with ozone until the solution developed a pale blue color. The mixture which now contained a white precipitate was immediately hydrogenated over 1 g. of 6% palladium-on-strontium carbonate at an initial pressure of 40 p.s.i. After 1 hr. the test (starch–iodide) for oxidants was negative, and the solution was filtered, diluted with ether, washed with saturated sodium bicarbonate solution, followed by cold 10% potassium hydroxide solution, and dried over anhydrous potassium carbonate. The residue obtained on evaporation of the solvent amounted to 1.9 g. of a partially crystalline orange oil, λ_{max} 4.48 and 5.83 μ (CN) (C=O).

A solution of 2.5 g. of the ozonization product, prepared as described in the preceding experiment, in 70 ml. of methanol and 25 ml. of water containing 0.5 g. of *p*-toluenesulfonic acid monohydrate was allowed to stand at room temperature for 14 hr. Benzene was added, and the mixture was washed thoroughly with saturated sodium bicarbonate solution and dried over anhydrous magnesium sulfate. The residue obtained on evaporation of the solvent amounted to 1.48 g. of partially crystalline yellow oil (crude vi); λ_{max} 2.92 (OH), 4.48 (CN) and 5.85 μ (C=O).

2-(2-Cyanopropyl)-2-formyl-β-cyclohexylpropionic Acid (vii).—To a solution of 10.2 g. of the aforementioned hydroxy ketone vi in 360 ml. of methanol was added a solution of 10.7 g. of sodium metaperiodate in 240 ml. of water. The mixture was allowed to stand for 19 hr.; then 10 ml. of concentrated hydrochloric acid was added. After dilution with water, the mixture was extracted with benzene, and the combined benzene layers were washed thoroughly with saturated sodium bicarbonate solution and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave 1.4 g. of neutral material which was not further investigated. The combined bicarbonate solutions were acidified with hydrochloric acid and extracted thoroughly with benzene. The combined benzene layers were dried over anhydrous magnesium sulfate, and then the solvent was evaporated to give 6.7 g. of the aldehydo acid vii as a colorless viscous oil. This material was converted directly to the **semicarbazone** by treatment with semicarbazide hydrochloride and sodium acetate in dilute alcohol. The crude crystalline product amounted to 2.1 g., m.p. 181–185° dec. Two recrystallizations from dilute ethanol gave colorless prisms, m.p. 186–191° dec.

Anal. Calcd. for $C_{15}H_{24}N_4O_3$: C, 58.42; H, 7.84. Found: C, 58.6; H, 8.05.

2-(2-Carboxypropyl)-2-methyl-β-cyclohexylpropionic Acid (VI, **R = OH**).—A solution of 9.1 g. of the aforementioned semicarbazone, 25 g. of potassium hydroxide, 6 ml. of hydrazine hydrate and 180 ml. of diethylene glycol was heated under reflux for 1 hr. in a bath maintained at 170°. The reflux condenser was removed and the volatile components were permitted to distil until the vapor temperature reached 220°. The condenser was then replaced and the mixture allowed to reflux for 4 hr. It was then cooled, 50 ml. of water was added, and the mixture heated on the steam-bath for 11.5 hr. to complete the hydrolysis of the nitrile group. The mixture was diluted with water and washed with benzene. Evaporation of the benzene layer gave only 29 mg. of neutral material which was not further investigated. The aqueous layer was acidified with concentrated hydrochloric acid and extracted thoroughly with benzene. The combined benzene extracts were dried over anhydrous magnesium sulfate. Evaporation of the solvent afforded 8.0 g. of acidic material which was evaporatively distilled at reduced pressure (<0.1 mm.). At a temperature of about 100°, 0.9 g. of a mobile yellow oily distillate was obtained which was insoluble in aqueous potassium carbonate and was not further investigated. The main portion of the material (6.1 g.) distilled at 170–200° as a pale yellow viscous oil which gradually solidified: neutral equivalent 128 (calcd. 128.2).

After six recrystallizations from benzene, a small amount of one of the epimers was isolated as colorless plates, m.p. 137–140°.

Anal. Calcd. for $C_{14}H_{24}O_4$: C, 65.60; H, 9.44. Found: C, 65.7; H, 9.4.

The residue from the first crystallization mentioned above was triturated with methylcyclohexane. This treatment gave 4.0 g. of crystalline material, m.p. 97–103°, which was probably a mixture of the two epimers.

2-(2-Formylpropyl)-2-methyl-β-cyclohexylpropionaldehyde (VI, **R = H**).—A solution of 1.0 g. of the aforementioned diacid, m.p. 97–103°, in 20 ml. of thionyl chloride was allowed to stand at room temperature for 13 hr. The solution was evaporated to dryness under reduced pressure, and then 50 ml. of benzene was added, and the evaporation process repeated in order to remove traces of thionyl chloride. The diacid chloride thus obtained was subjected to the modified Rosenmund reduction.[46] A mixture of the acid chloride, 400 ml. of benzene and 1.0 g. of dried 10% palladium-on-carbon was placed in a three-necked 500-ml. round-bottomed flask equipped with a thermometer, hydrogen inlet tube and a condenser. The top of the condenser was fitted with an exit tube which was arranged so that the effluent gases bubbled through an aqueous solution of phenolphthalein indicator contained in a second flask which was fitted with a buret charged with standard base so that the liberated hydrogen chloride could be titrated as the reaction proceeded. This second flask contained an outlet tube by which the pressure of the whole system could be reduced (water separator). The reaction

(46) W. S. Johnson, D. G. Martin, R. Pappo, S. D. Darling and R. A. Clement, *Proc. Chem. Soc.*, 58, (1957), footnote p. 7.

flask was heated with an oil-bath at 56°, and the pressure in the system, which was regulated by a needle valve, was adjusted to approximately 115 mm.; under these conditions the benzene solution refluxed smoothly with an inside temperature of approximately 31°. When hydrogen was admitted *via* the inlet tube, reduction proceeded smoothly and was 94% complete, as indicated by titration, after 5 hr. The solution was filtered and utilized directly in attempts to prepare the enamine. No attempt was made to isolate the dialdehyde because of its expected instability.

An aliquot of the benzene solution containing 8.6% of the total reaction product was added to a solution of 0.4 g. of 2,4-dinitrophenylhydrazine in 2 ml. of concentrated sulfuric acid, 3 ml. of water and 10 ml. of 95% ethanol. The two-phase mixture was allowed to boil at steam-bath temperature, and the volume was maintained constant by occasional addition of ethanol. After most of the benzene had thus been replaced by ethanol, the solution was cooled and 0.18 g. (92% yield) of the **bis-2,4-dinitrophenylhydrazone** of the dialdehyde V (R = H) separated. After two recrystallizations from ethanol–ethyl acetate, the derivative was obtained as a yellow microcrystalline powder, m.p. 192–200°.

Anal. Calcd. for $C_{26}H_{32}N_8O_8$: C, 53.42; H, 5.71. Found: C, 53.8; H, 5.7.

Attempts to form the piperidine enamine of the dialdehyde resulted in the formation, after acid hydrolysis, of material having a maximum in the ultraviolet spectrum at 233 mμ, suggesting the formation of an unsaturated aldehyde by intramolecular aldol condensation. In the hope that the crude material contained some of the desired bis-enamine, it was ozonolyzed[13] and then treated under conditions for cyclization (see above). The product thus obtained showed no indication of absorption in the ultraviolet for the expected α,β-unsaturated carbonyl system.

Investigation of an Alternative Approach

DL-3α,11β-Dihydroxy-13α-(2-cyanoethyl)-17-furfurylidene-18-nor-D-homo-5β-androstane-17a-one (XXIII, R¹ = R² = H). (a) Using β-Methoxypropionitrile.—To a cold (5°) solution of 48 mg. of sodium hydride in 6 ml. of anhydrous methanol was added slowly 1.20 ml. of acrylonitrile. The mixture was allowed to stand at room temperature for 1 hr., then cooled again to 5°, 1.15 g. of the aforementioned furfurylidene ketone III, m.p. 191–194°, was added, and the solution was stirred at room temperature for 2 hr., then heated at reflux for 2 hr. Since considerable starting furfurylidene ketone remained undissolved after this treatment, 4 ml. of anhydrous tetrahydrofuran was added, whereupon the solution became homogeneous. The mixture was heated for an additional 8 hr. at reflux, then cooled to 5° and acidified with acetic acid. Water was added, and the mixture extracted thoroughly with ethyl acetate. The organic layers were washed with saturated sodium bicarbonate solution, then with water and dried over anhydrous magnesium sulfate. The solvent was evaporated by distillation at reduced pressure, and excess β-methoxypropionitrile was removed from the residue by distillation at 100° (1 mm.). The product was crystallized from a small volume of ethyl acetate, and the crystals were washed with hot petroleum ether and then with ether to give 0.750 g., m.p. 183–187°. A second crop amounting to 0.170 g., m.p. 170–180°, was obtained from the mother liquors, and on recrystallization gave an additional 0.070 g. of satisfactory material, m.p. 185–188°. Repeated recrystallizations of such material from methanol gave colorless plates, m.p. 193–194°, $\lambda_{max}^{95\%\ EtOH}$ 329.8 mμ (ε 20,900): λ_{max}^{mull} 2.85 μ (OH), 4.47 (CN), 6.07 (unsaturated ketone C=O) and 6.35 (aromatic C=C).

Anal. Calcd. for $C_{27}H_{32}O_4N$: C, 74.11; H, 8.06; N, 3.20. Found: C, 74.2; H, 8.0; N, 3.1.

(b) Using Acrylonitrile Directly.—To a solution of 0.120 g. of sodium hydride in 4.0 ml. of anhydrous methanol was added 3.84 g. of the furfurylidene ketone III, m.p. 191–194°, and 5.0 ml. of anhydrous tetrahydrofuran. The mixture was cooled to 5°, and 4.0 ml. of acrylonitrile was added dropwise with stirring. After 15 min. at 0°, there was no evidence of reaction so the solution was heated (for 2 min.) until it became homogeneous and was then allowed to stand at room temperature for 1 hr. The product was isolated as described in the preceding section and amounted to 1.75 g., m.p. 186–189° (40% yield). The second crop amounted to 0.155 g., m.p. 176–183°. Additional material could be obtained from the residue only by chromatography. In this way it was possible to obtain another 0.730 g. of product, m.p. 187–190°, raising the total yield to 63%. The procedure described above (part a), however, is preferred. The experiment described directly below in which the residues were acetylated before chromatography showed that dicyanoethylation product was produced when acrylonitrile was used directly.

The combined residues (3.3 g.) from three runs, in which acrylonitrile was used directly as described above, were dissolved in 200 ml. of isopropenyl acetate, 1.5 g. of *p*-toluenesulfonic acid monohydrate was added, and the mixture was allowed to stand at room temperature for 20 hr. The solution was poured into excess saturated sodium bicarbonate and the mixture extracted with ethyl acetate. The organic layers were dried over anhydrous magnesium sulfate, and the residue obtained on evaporation of the solvent at reduced pressure was chromatographed on 30 g. of Florisil. Elution with benzene gave a total of 1.17 g. of material which was crystallized from methanol to yield 0.53 g. (first crop), m.p. 229–233°; and 0.06 g. (second crop), m.p. 220–227°. This material was shown by recrystallization and mixed melting point determinations to be identical with the diacetate XXIII (R¹ = R² = Ac) described directly below. Elution with 5% ethyl acetate in benzene gave a total of 1.62 g. which on crystallization from methanol yielded 0.60 g. of **DL-11β-acetoxy-3-(2-cyanoethoxy)-13α-(2-cyanoethyl)-17-furfurylidene-18-nor-D-homo-5β-androstane-17a-one** (XXIII, R¹ = CH_2CH_2CN, R² = Ac) as colorless rectangular prisms, m.p. 212–214°. Recrystallization from methanol raised the melting point to 218–219°, $\lambda_{max}^{95\%\ EtOH}$ 332 mμ (ε 18,650).

Anal. Calcd. for $C_{32}H_{40}O_5N_2$: C, 72.15; H, 7.57. Found: C, 72.3; H, 7.6.

Treatment of 40 mg. of this material in 10 ml. of methanol with 10 ml. of 2% sodium bicarbonate solution by heating at reflux for 40 min. gave a product which was recognized by the infrared spectrum as starting material. The failure of the substance to undergo hydrolysis under these conditions served to demonstrate that the acetate residue was located at C-11 rather than C-3.

DL-3α,11β-Dihydroxy-13α-(2-cyanoethyl)-17-furfurylidene-18-nor-D-homo-5β-androstane-17a-one Diacetate (XXIII, R¹ = R² = Ac).—A solution of 0.290 g. of the aforementioned Michael adduct XXIII (R¹ = R² = H), m.p. 187–190°, in 20 ml. of isopropenyl acetate containing 0.13 g. of *p*-toluenesulfonic acid monohydrate was allowed to stand overnight at room temperature. Excess saturated sodium bicarbonate solution was added, the mixture extracted with ethyl acetate–ether, and the organic layers were dried over anhydrous magnesium sulfate. The residue obtained on evaporation of the solvent at reduced pressure was crystallized from methanol to give 0.295 g. (first crop), m.p. 226–230°; and 0.02 g. (second crop), m.p. 207–219°. Repeated recrystallizations of first-crop material from acetone and from ethyl acetate give fine colorless needles, m.p. 236–236.5°, $\lambda_{max}^{95\%\ EtOH}$ 333.0 mμ (ε 26,400).

Anal. Calcd. for $C_{31}H_{39}O_6N$: C, 71.37; H, 7.54. Found: C, 71.1; H, 7.4.

Proof of Configuration of the Michael Adduct.—A cooled (−70°) solution of 45 mg. of the aforementioned diacetate XXIII (R¹ = R² = Ac), m.p. 228–231°, in 40 ml. of ethyl acetate was treated with ozone until the solution became a pale blue color; then 20 ml. of 3% sodium bicarbonate solution, 0.1 ml. of 30% aqueous hydrogen peroxide and 5 ml. of methanol were added with stirring. The mixture was allowed to warm to room temperature and was stirred at this temperature for 4 hr. Most of the organic solvent was removed by distillation at room temperature and the residue was extracted with benzene. Enough solid potassium hydroxide was added to the aqueous layer to bring the concentration to about 10% hydroxide, and the solution was heated at 100° for 35 hr. It was then cooled, washed with benzene and the aqueous layer was acidified and extracted thoroughly with ethyl acetate. The organic layers were washed with water and dried over anhydrous magnesium sulfate. The residue obtained on evaporation of the solvent at reduced pressure was crystallized from acetone–petroleum ether to give 20 mg. of **DL-1β,2α-di-(2-carboxyethyl)-2α-(2-carboxypropyl)-4β,7α-dihydroxy-4bβ-methyl-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic acid (2→4)-lactone** (XXV), m.p. 267–269°. Two recrystallizations from methyl ethyl ketone gave colorless prisms, m.p. 268–269°; λ_{max}^{mull} 2.90 μ (OH), 5.70 (γ-lactone C=O) and 5.90 (carboxyl C=O).

Anal. Calcd. for $C_{22}H_{32}O_7$: C, 64.68; H, 7.90. Found: C, 64.5; H, 7.9.

In another experiment, 90 mg. of the diacetate XXIII (R₁ = R² = Ac), m.p. 228–232°, was treated with ozone and hydrogen peroxide just as described directly above. Proportional amounts of reagents and solvents were employed, and the product was isolated by acidification of the aqueous layer prior to treatment with potassium hydroxide. The product was isolated by extraction with ethyl acetate as described above, and the crude residue obtained upon evaporation of the solvent was crystallized from ethyl acetate to give 65 mg. (77% yield) of **DL-1β-(2-carboxyethyl)-2α-(2-cyanoethyl)-4β,7α-diacetoxy-4bβ-methyl-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-carboxylic acid (XXIV)**, m.p. 201–204°. Repeated recrystallizations from ethyl acetate afforded colorless prisms, m.p. 209–210°.

Anal. Calcd. for $C_{26}H_{37}O_8N$: C, 63.52; H, 7.48. Found: C, 63.45; H, 7.6.

3α,11β,17a-Trihydroxy-13α-(2-cyanoethyl)-17-furfurylidene-18-nor-D-homo-5β-androstane-17a-one.—To a cooled (0°) solution of 1.75 g. of the aforementioned furfurylidene ketone XXIII

($R^1 = R^2 = H$), m.p. 186–189°, in 230 ml. of methanol containing 16 ml. of water was added 1.5 g. of sodium borohydride. The mixture was allowed to stir at −10° for 60 hr. by which time the maximum at 330 mμ in the ultraviolet spectrum had completely disappeared. Excess acetic acid was added slowly and the mixture distilled at reduced pressure to remove most of the methanol. The mixture was extracted with ethyl acetate, and the combined organic layers were washed with water, saturated sodium bicarbonate solution and dried over magnesium sulfate. A 0.525-g. portion of the 1.85 g. of residue obtained upon removal of the solvent at reduced pressure was chromatographed on 20 g. of Florisil. The fractions eluted with 10–50% ethyl acetate in benzene amounted to 0.445 g. of crystalline material which probably represented a mixture of the C-17a epimeric alcohols, m.p. 150–180°. Recrystallization of the combined fractions from acetone gave 0.230 g. (first crop), m.p. 192–194°; and 0.065 g. (second crop), m.p. 189–193°. Repeated recrystallization of this material from acetone gave fine colorless rods, m.p. 200–200.5°; $\lambda_{max}^{95\%\ EtOH}$ 262 mμ (ε 19,800), 269 (21,900), 281 (15,200); $\lambda_{max}^{95\%\ EtOH}$ 264 mμ (ε 19,400), 278 (13,900).

Anal. Calcd. for $C_{27}H_{37}O_4N$: C, 73.77; H, 8.48. Found: C, 73.4; H, 8.3.

1β-(3,3-Dimethoxypropyl)-2α-(2-cyanoethyl)-4β-hydroxy-4bβ-methyl-7α-acetoxy-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXVII).—A solution of 1.10 g. of the aforementioned Michael adduct XXIII ($R^1 = R^2 = H$), m.p. 192–195°, in 5 ml. each of methylene dichloride and methanol was added with swirling to a saturated solution of ozone in 250 ml. of methylene dichloride at −70°. The pale blue solution was diluted with 200 ml. of methanol (cooled to −70°), then 1.5 g. of sodium borohydride in 5 ml. of water was added. The mixture was allowed to warm up to 0°, then two additional 1.5-g. portions of sodium borohydride were added after 1.5 hr. and after 7.5 hr. After a total of 19 hr. at 0°, the borohydride was completely consumed. Therefore the solution was acidified to *p*H 5 with acetic acid, and an additional 2.5 g. of sodium borohydride was added, followed by additional 1.5-g. portions after 22 and again after 27 hr. After a total reaction period of 42 hr., the solution was acidified to *p*H 5 with acetic acid, and the solvents were largely removed by concentration at reduced pressure (room temperature). Final traces of methanol were removed by co-distillation with portions of ethyl acetate. The residue was diluted with water and extracted thoroughly with ethyl acetate. The combined organic layers were washed with cold 5% hydrochloric acid, cold water, cold saturated potassium bicarbonate solution, again with cold water, and finally dried over anhydrous sodium sulfate. The colorless amorphous residue (0.85 g.) obtained upon removal of the solvent at reduced pressure was used directly for the periodate oxidation described below.

In another run, incomplete extraction of the aqueous solution gave only about 70% of the crude product. Further extraction of the aqueous solution after concentration gave an additional 26% of material which crystallized from aqueous methanol. This product was one of the stereoisomeric forms of **3α,11β,17,17a-tetrahydro-13α-(2-cyanoethyl)-18-nor-D-homo-5β-androstane (XXVI).** Repeated recrystallizations from aqueous methanol gave colorless rods, m.p. 137–140°, which, as shown by the analysis, was a hemihydrate. This material could not be induced to crystallize from non-aqueous solvents.

Anal. Calcd. for $C_{22}H_{37}O_2N \cdot 1/2\ H_2O$: C, 68.36; H, 9.39. Found: C, 68.4; H, 9.5.

A solution of 0.138 g. of sodium metaperiodate in 3 ml. of water was added to a cold (ice-bath) solution of 0.103 g. of the crude tetrahydroxy compound XXVI, described directly above, in 5 ml. of methanol. The mixture was allowed to stand in the dark for 19 hr. at 23°. The colorless solution which had deposited crystals of sodium iodate was diluted with 20 ml. of saturated brine and extracted thoroughly with ethyl acetate. The combined organic layers were washed with saturated brine, saturated potassium bicarbonate solution, again with saturated brine and finally dried over anhydrous sodium sulfate. The colorless amorphous residue (0.117 g.) obtained on evaporation of the solvent at reduced pressure was dissolved in 10 ml. of anhydrous methanol, 11 mg. of *p*-toluenesulfonic acid monohydrate was added, and the solution was allowed to stand in the dark for 2 days at room temperature. The mixture was neutralized with excess saturated potassium bicarbonate solution and concentrated by evaporation under a stream of nitrogen to remove most of the methanol. The mixture was diluted with water, extracted thoroughly with ether, and the combined organic layers were washed with water, followed by saturated brine and then dried over anhydrous sodium sulfate. The gummy residue (0.126 g.) obtained on removal of the solvent at reduced pressure was chromatographed on 4.5 g. of Florisil. The fraction (98 mg.) eluted with 10% ether in benzene was acetylated with 2 ml. of acetic anhydride and 4 ml. of pyridine. After standing at room temperature overnight, the solution was poured into ice-cold saturated potassium bicarbonate solution and extracted with ether. The combined organic layers were washed with water, followed by saturated brine and dried over anhydrous sodium sulfate. The thick oily residue (0.108 g.) obtained on evaporation of the solvent at reduced pressure was crystallized from methanol to give 53 mg. (first crop) of colorless prisms, m.p. 126–129°; and 34 mg. (second crop), m.p. 124–128°.

Comparable material from another experiment was obtained by repeated recrystallization from methanol as colorless prisms, m.p. 126–127°, λ_{max}^{mull} 4.46 μ (CN) and 5.80 (acetate C=O).

Anal. Calcd. for $C_{27}H_{43}O_6N$: C, 67.89; H, 9.08; CH_3O, 19.49. Found: C, 67.9; H, 9.1; CH_3O, 19.8.

1β-(2-Formylethyl)-2α-(2-cyanoethyl)-4β-hydroxy-4bβ-methyl-7α-acetoxy-1,2,3,4,4aα,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXX).—The procedure for the selective hydrolysis of the acetal XXVII is an adaptation of a published method.[47] To a slurry of 0.360 g. of the aforementioned acetal XXVII, m.p. 123–125°, in 3 ml. of ether was added 9 ml. of 70% acetic acid in water, whereupon the mixture became homogeneous. The solution was allowed to stand at room temperature for 16 hr. and was then extracted with ether. The combined organic layers were washed thoroughly with water, followed by saturated sodium bicarbonate solution and finally dried over anhydrous magnesium sulfate. The residue (0.337 g.) obtained on evaporation of the solvent at reduced pressure was triturated with ether to give 0.305 g. of colorless crystals, m.p. 83–85°, with evolution of gas. Recrystallization from ether gave colorless plates, m.p. 84–86°, which appeared to contain ether of solvation. Recrystallization from other solvents gave only poorly defined crystals which melted over a range up to 105° (polymorphic forms?). When the 84–86° material was dried at room temperature (0.2 mm.), it turned to a glass; λ_{max}^{film} 3.70 μ (aldehydic C—H), 4.47 (CN), 5.84 (aldehyde and acetate C=O).

Anal. Calcd. for $C_{25}H_{37}O_5N$: C, 69.57; H, 8.64; CH_3O, 7.19. Found (after drying in the combustion boat at 100° (0.1 mm.) for 4 hr.): C, 70.3; H, 8.6; CH_3O, 6.9. Calcd. for $C_{25}H_{37}O_5N \cdot (C_2H_5)_2O$: C, 68.88; H, 9.37. Found (after drying at room temperature for 2 hr.): C, 69.0; H, 8.6.

1β-(3,3-Dimethoxypropyl)-2α-(3-hydroxy-3,3-diphenylpropyl)-4β-hydroxy-4bβ-methyl-7α-hydroxy-1,2,3,4,4aα,4b,5,6,7,-8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXVIII, R = H).—A solution of 0.440 g. of the aforementioned cyano acetal XXVII, m.p. 124–126°, in 30 ml. of methanol and 10 ml. of 10% aqueous potassium hydroxide was heated under reflux for 10 hr. When the methanol was removed by distillation at reduced pressure, some alkali-insoluble material remained, so additional potassium hydroxide and methanol were added to make the solution 10% with respect to the hydroxide and 40% with respect to methanol. Heating at reflux was then continued for an additional 4 hr. Most of the methanol was removed by distillation at atmospheric pressure. The aqueous residue was washed with benzene, then cooled to 5° and acidified to *p*H 4 with ice-cold 3 *N* hydrochloric acid. The mixture was quickly extracted with ethyl acetate. The combined organic layers (total volume 200 ml.) were diluted with 20 ml. of methanol and then treated with excess diazomethane in ether. After 10 min. at room temperature, the solution was concentrated at reduced pressure. The pale yellow residue (0.390 g.) was chromatographed on 6 g. of Florisil. The fraction eluted with ether amounted to 0.344 g. (80% yield) of crude amorphous ester which was used directly in the reaction with phenyllithium as described below.

A solution of phenyllithium, prepared from 0.210 g. of lithium and 2.4 g. of bromobenzene,[48] in 10 ml. of ether was added to a solution of 0.110 g. of the amorphous ester, described directly above, in 20 ml. of anhydrous ether (atmosphere of nitrogen). The solution was stirred at room temperature for 3 hr., and absolute ethanol was added dropwise to decompose the excess phenyllithium. Water was added, and the mixture was extracted with 1:1 ethyl acetate–ether. The combined organic layers were washed with water and dried over anhydrous magnesium sulfate. The oily residue (0.277 g.) obtained on evaporation of the solvent at reduced pressure was chromatographed on 7 g. of Florisil. The fraction eluted with ether amounted to 0.125 g. of the carbinol XXVIII; λ_{max}^{film} 2.95 μ (OH) and 6.26 (aromatic C=C). Crystallization of this product was successful only from solvents containing acetone, and the analysis showed that the product crystallized with 1 mole-equivalent of acetone of solvation. Thus a single crystallization of the crude product from acetone–petroleum ether gave 0.120 g. of colorless fluffy needles, m.p. 100–102°; λ_{max}^{mull} 2.95 (OH), 5.84 (acetone C=O), 6.26 (aromatic C=C). The intensity of the carbonyl band in the infrared spectrum was not diminished after the crystalline product was dried at 50° (0.2 mm.) for 24 hr.

(47) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, *J. Am. Chem. Soc.*, **77**, 12 (1955).

(48) J. C. W. Evans and C. F. H. Allen, *Org. Syntheses*, **2**, 22 (1943).

Anal. Calcd. for $C_{30}H_{38}O_7$: C, 73.86; H, 8.99; CH_3O, 14.31. Found: C, 74.2; H, 8.95; CH_3O, 14.2.

1β-(3,3-Dimethoxypropyl)-2α-(3,3-diphenyl-2-propenyl)-4β-hydroxy-4bβ-methyl-7α-acetoxy-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,-10aβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXIX).—A mixture of 48 mg. of the aforementioned carbinol XXVIII (R = H), m.p. 100–102°, 1 ml. of pyridine and 0.5 ml. of acetic anhydride was heated at 100° for 8 min. The solution was cooled, poured into ice-cold saturated sodium bicarbonate solution and extracted with ether. The organic layers were washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent at reduced pressure gave 50 mg. of colorless amorphous acetate XXVIII (R = Ac). This material failed to crystallize even after chromatography and was used directly for the dehydration step described directly below.

A solution of 75 mg. of the crude acetate XXVIII (R = Ac) in 10 ml. of anhydrous benzene, 1.0 ml. of pyridine and 0.5 ml. of thionyl chloride was allowed to stand at 0° for 15 min. It was then poured into ice-cold saturated sodium bicarbonate solution and extracted with ether. The combined organic layers were dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. Since this product still contained halogen (Beilstein test), it was dissolved in 20 ml. of pyridine and heated at 100° for 15 min. The solvent was removed by distillation at reduced pressure, and the residue chromatographed on 5 g. of Florisil. The fractions eluted with ether amounted to 65 mg. of the crude olefin. Crystallization from methanol gave 54 mg. (first crop) of colorless rods, m.p. 154–158°; and 10 mg. (second crop), m.p. 148–155°. Recrystallization from ether yielded a solvated form which melted at about 105° with evolution of gas, resolidified and then remelted at 154–156°. Repeated recrystallizations of a specimen from methanol gave colorless rods, m.p. 159–160°; $\lambda_{max}^{95\%\ EtOH}$ 250.5 mμ (ε 15,900), λ_{min} 236 (13,200).

Anal. Calcd. for $C_{39}H_{52}O_6$: C, 75.94; H, 8.40. Found: C, 75.8; H, 8.5.

1β-(2,2-Dimethoxyethyl)-2α-(2-cyanoethyl)-4β-hydroxy-4bβ-methyl-7α-acetoxy-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXXI).—A solution of 0.710 g. of the crude amorphous cyano aldehyde XXX, prepared by the selective hydrolysis of acetal described above, in 10 ml. of benzene containing 5 ml. of piperidine was allowed to stand at room temperature for 10 min., then heated under reflux for 3 hr. (atmosphere of nitrogen). The solvents were removed under reduced pressure, leaving 0.820 g. of a colorless oil, λ_{max}^{ether} 233 mμ (ε 8,300); λ_{max}^{Nujol} 5.78 μ (acetate C=O), 6.09 (C=O).

A cold (−70°) solution of 0.810 g. of the crude enamine described directly above in 60 ml. of methylene dichloride and 1.5 ml. of pyridine was treated with ozone until the solution turned a blue color; then 3 g. of zinc dust and 6 ml. of glacial acetic acid were added. The mixture was stirred and allowed to warm to 0° and to remain at that temperature for 30 min. It was then filtered, washed with saturated sodium bicarbonate solution and dried over anhydrous magnesium sulfate. The residue (0.750 g.) obtained on evaporation of the solvent at reduced pressure was chromatographed on 25 g. of Florisil. The fractions eluted with benzene through ether amounted to 0.310 g. of the cyanoaldehyde: λ_{max}^{film} 3.70 μ (aldehyde C—H), 4.47 (CN) and 5.79 (acetate C=O). This amorphous product could not be induced to crystallize and was used directly for the formation of the acetal.

A solution of the 0.310 g. of amorphous aldehyde in 60 ml. of anhydrous methanol containing 40 mg. of *p*-toluenesulfonic acid monohydrate was allowed to stand at room temperature for 9 hr. The product was isolated as described above for the homologous aldehyde, and the crude oily acetal (0.325 g.) was chromatographed on 12 g. of Florisil. The fractions eluted with ether amounted to 0.285 g. of an oil which crystallized from petroleum ether to give 0.230 g. (first crop) of irregular colorless prisms, m.p. 149–152°; and 0.030 g. (second crop), m.p. 147–151°. Recrystallization from methanol and again from ether gave colorless rods, m.p. 150–152°.

Anal. Calcd. for $C_{36}H_{49}O_6N$: C, 67.36; H, 8.91; CH_3O, 20.08. Found: C, 67.4; H, 9.0; CH_3O, 20.1.

1β-(2,2-Dimethoxyethyl)-2α-(3-hydroxy-3,3-diphenylpropyl)-4β-hydroxy-4aβ-methyl-7α-hydroxy-1,2,3,4,4aα,5,6,7,8,8aβ,9,-10,10bβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXXII).—A solution of 0.220 g. of the aforementioned cyano ketal XXXI, m.p. 149–152°, in 5 ml. of methanol and 10 ml. of 10% aqueous potassium hydroxide was heated under reflux for 17 hr. The acidic material was isolated with special care as described above for the higher homolog and treated immediately with diazomethane. Chromatography of the crude methyl ester (0.235 g.) on 6 g. of Florisil gave, in the fractions eluted with ether, 0.220 g. of oily ester which could not be induced to crystallize and was used directly in the reaction with phenyllithium described below.

Excess phenyllithium (about 50 mole-equivalents prepared as described above) in 20 ml. of ether was added to a solution of 0.215 g. of the crude ester described directly above in 20 ml. of anhydrous ether (atmosphere of nitrogen). The crude oily product (0.310 g.) which was isolated as described above for the higher homolog was chromatographed on 6 g. of Florisil. The fractions eluted with ether amounted to 0.267 g. and crystallized on trituration with a small volume of ether to give 0.252 g. of needles, m.p. 116–119°. Recrystallization from ether gave fine colorless needles, m.p. 116–118°; $\lambda_{max}^{95\%\ EtOH}$ 252 mμ (ε 530), 258 (590), 264 (440); λ_{min} 243 mμ (ε 440) and 255 (500).

Anal. Calcd. for $C_{36}H_{50}O_6$: C, 74.70; H, 8.71. Found: C, 74.7; H, 8.8.

Selective acetylation of the diol XXXII by the procedure described above for the higher homolog gave a colorless amorphous monoacetate which, on dehydration according to the thionyl chloride–pyridine procedure described above for the higher homolog, gave the olefinic compound as a colorless amorphous product; $\lambda_{max}^{95\%\ EtOH}$ 251 mμ (ε 17,300), λ_{min} 235 (14,800). The over-all yield from the crystalline dihydroxy compound was 90%.

1β,2α-Di-(2-carbomethoxyethyl)-4β-hydroxy-4bβ-methyl-7α-acetoxy-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXXIII).—A solution of 0.260 g. of the crude aldehyde XXX, prepared as described above, in 2 ml. of pyridine was added to the Sarett reagent prepared from 1.5 g. of chromium trioxide and 15 ml. of pyridine; then 5 drops of water was added, and the dark mixture allowed to stand at room temperature for 12 hr. The mixture was chilled, acidified to *p*H 5 with ice-cold 5% aqueous hydrochloric acid and then rapidly extracted with 1:1 ether–ethyl acetate. The organic layers were washed thoroughly with water until neutral and dried over anhydrous sodium sulfate. The residue (0.300 g.) obtained on evaporation of the solvent was dissolved in benzene and extracted with four 25-ml. portions of 5% potassium hydroxide solution. The combined alkaline solutions were brought up to 10% in hydroxide by the addition of 6 g. of solid potassium hydroxide; then the mixture was heated under reflux for 23 hr. (atmosphere of nitrogen). The solution was cooled, excess solid sodium dihydrogen phosphate was added, followed by acetic acid until the *p*H was 5. The mixture was immediately extracted with ethyl acetate and the combined organic layers (300 ml.) were washed thoroughly with water until neutral, and dried over anhydrous sodium sulfate. The ethyl acetate solution of the diacetate was diluted with 60 ml. of methanol and then treated with excess ethereal diazomethane. The crude diester obtained upon evaporation of the solvent at reduced pressure was acetylated by treatment with pyridine and acetic anhydride overnight at room temperature. The mixture was poured into ice-cold saturated potassium bicarbonate solution and extracted with ether. The combined organic layers were washed with water and dried over anhydrous sodium sulfate. The oily residue (0.430 g.) obtained upon evaporation of the solvent at reduced pressure was chromatographed on 7 g. of Alcoa alumina. The fractions eluted with benzene and 5% ether in benzene amounted to 0.402 g., and crystallized on trituration with ether. Crystallization from 2 ml. of diisopropyl ether gave 0.151 g. (first crop) of large colorless prisms, m.p. 103–108°; and 0.105 g. (second crop), m.p. 100–105°. Recrystallization of such material from diisopropyl ether followed by two further recrystallizations from methylcyclohexane gave colorless prisms, m.p. 117–118.5°.

Anal. Calcd. for $C_{31}H_{42}O_8$: C, 65.56; H, 8.56. Found: C, 65.9; H, 8.6.

1β,2α-Di-(3,3-diphenyl-2-propenyl)-4β-hydroxy-4bβ-methyl-7α-acetoxy-1,2,3,4,4aα,4b,5,6,7,8,8aβ,9,10,10aβ-perhydrophenanthrene-2β-aldehyde 2β,4β-Lactol Methyl Ether (XXXIV).—A solution of 0.103 g. of the diester XXXIII, m.p. 112–116°, prepared as described directly above, in 5 ml. of anhydrous ether was added to a solution of phenyllithium, prepared from 5 g. of freshly distilled bromobenzene and 0.438 g. of lithium, in 10 ml. of anhydrous ether. The reaction was carried out and also the product was isolated as described in detail above for the preparation of the diol XXVIII (R = H). The crude product was chromatographed on 18 g. of Florisil. The fractions eluted with 5–50% ether in benzene consisted of the crude trihydroxy compound which amounted to 0.149 g. This material was selectively acetylated at C-3 by treatment with 5 ml. of acetic anhydride and 10 ml. of pyridine overnight at room temperature. The crude monoacetate, which was isolated as described in detail above for the preparation of XXVIII (R = Ac), was dissolved in 10 ml. of benzene containing 2 ml. of anhydrous pyridine and treated with 1 ml. of thionyl chloride for 30 min. at 0°. The crude product, which was isolated as described in detail above under the preparation of XXIX, was dissolved in 15 ml. of pyridine and heated at 100° for 30 min. The solvent was then removed under a stream of dry nitrogen (steam-bath temperature), and the last traces of pyridine were eliminated by adding portions of toluene and evaporating to dryness under dry nitrogen. The crude diolefin XXXIV (0.162 g.) was chromatographed

on 10 g. of Alcoa alkaline alumina. The fractions eluted with benzene (0.142 g.) were combined and crystallized from petroleum ether to give 0.113 g. (76% yield), m.p. 173–177°. Successive recrystallizations from acetone–petroleum ether, acetone–methanol, and twice from petroleum ether gave colorless prisms, m.p. 181–183°, $\lambda_{max}^{95\% EtOH}$ 250 mμ (ε 31,700).

Anal. Calcd. for $C_{49}H_{54}O_4$: C, 83.25; H, 7.70. Found: C, 83.2; H, 7.7.

3α-Acetoxy-11β-hydroxy-Δ^{16}-5β-androstene-18,20-dialdehyde 11β,18-Lactol Methyl Ether (XXXVI).—Chamber B of the Rubin ozonolysis apparatus[38] was charged with 8 ml. of methylene dichloride, the apparatus was then cooled in a Dry Ice–acetone bath, and ozone was admitted until the solvent was saturated. Chamber A of the apparatus was then charged with a solution of 100 mg. of the diolefin XXXV, m.p. 173–177°, prepared as described directly above, in 1 ml. of methylene dichloride and 8 ml. of anhydrous methanol; then the solution of ozone was transferred from chamber B into chamber A by the application of a positive pressure of nitrogen. The mixture was stirred magnetically at −70° for 9 min. and was then allowed to warm to 0° (ice-bath) for 2 min. Four-tenths gram of zinc dust and 2 ml. of glacial acetic acid were immediately added, and the solution was stirred at 0° for 1.5 hr., during which period three additional 0.4-g. portions of zinc and 2-ml. portions of acetic acid were added. The mixture was filtered, the filtrate neutralized with sodium bicarbonate solution and the mixture extracted with ether. The combined organic layers were washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue dissolved in methylene dichloride, dried again over anhydrous sodium sulfate and concentrated. This crude oily dialdehyde was immediately dissolved in 35 ml. of benzene, about 15 mg. of anhydrous crystalline piperidine hydroacetate was added, and the mixture was stirred at room temperature for 4 hr. Ether was added, and the solution was washed with water, saturated brine, dilute acetic acid, saturated sodium bicarbonate solution, again with brine and finally dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent under reduced pressure was chromatographed on 15 g. of Florisil. The fractions eluted with 30% ether in benzene through ether amounted to 25 mg. of glassy material which crystallized on trituration with ether to give 18.7 mg. of colorless crystals, m.p. 202–204°, after sintering at 190°; $\lambda_{max}^{95\% EtOH}$ 238–239 mμ (ε 9,600); $\lambda_{max}^{CHCl_3}$ 3.4–3.45 μ (aldehyde C—H), 5.85 (acetate C=O), 5.95 (unsaturated aldehyde C=O). On slow crystallization from ether the substance was obtained as colorless needles having the same melting point.

Anal. Calcd. for $C_{33}H_{32}O_5$: CH_3O, 7.99. Found: CH_3O, 7.9.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES, LOS ANGELES 24, CALIF.]

Mold Metabolites. IX. Contribution to the Elucidation of the Structure of Althiomycin[1]

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The structure of the mold metabolite, althiomycin (probable formula $C_{27}H_{28}O_{10}N_8S_3$), has been investigated. Methanolysis (acid-catalyzed) of the substance gave 4-methoxy-Δ^3-pyrrolin-2-one, whose structure was established by degradation and synthesis. Strong acid hydrolysis of the amorphous products of the methanolysis provided thiazole-4-carboxylic acid, identified through its physical properties and by comparison with an authentic sample. When subjected to a Moore and Stein cation exchange resin column, the acid hydrolysate of althiomycin was demonstrated to contain cysteine, ammonia and an unknown and non-identified amino acid. The presence of a cysteine unit was confirmed through identification of cysteic acid in the hydrolysate of the oxidation product of the original antibiotic. When treated with acetic anhydride and pyridine, althiomycin gave an acetylated degradation product (acetylalthiomycin) whose probable formula is $C_{38}H_{47-49}O_6N_8S_2$. Hydrolysis and spectral studies demonstrated that acetylalthiomycin contained the same structural units identified as hydrolysis products of althiomycin itself. Strong acid hydrolysis of acetylalthiomycin gave carbon dioxide, ammonia, formic acid, acetic acid and hydrogen sulfide as volatile products. A provisional structure for acetylalthiomycin is considered.

A new sulfur-containing antibiotic was isolated at the Upjohn Company in 1957 from culture no. 116a of an unspecified mold.[3] In the same year, a group of Japanese investigators[4] reported the isolation of althiomycin, and comparison of this material with that of the Upjohn group demonstrated the identity of the two materials.[3]

Characterization.[3]—Althiomycin by isothermal distillation exhibited a molecular weight of 708, and the formula $C_{27}H_{28}O_{10}N_8S_3$ is the most consistent with the analytical data. The substance contains two methoxyl groups and no terminal methyl groups. The pure material, m.p. 180–181.6° dec., gave a rotation of $[\alpha]^{25}D$ +37.8° (*c* 2, 1:1 95% ethanol–methylene chloride). The antibiotic gave negative ninhydrin, biuret, ferric chloride, Sakaguchi, Benedict, anthrone, Molisch and Wegand tests, and positive Tollens and Tommila tests. Titration of the material with 2 *N* hydrochloric acid produced a well defined end point at pK_a 11.2, the equivalent weight being 256, but the antibiotic underwent decomposition during the titration, and a degradation product was probably involved.[3]

Methanolysis of Althiomycin.—Methanolysis of althiomycin in 0.25 *N* hydrogen chloride in methanol at 25° gave a solution which when neutralized with a strong anion exchange resin produced a precipitate (64% by weight, labeled fraction A) and a water-soluble fraction. From the latter was obtained 17% by weight of a paper chromatographically pure crystalline material, which was recrystallized first from ethanol–benzene and then from benzene to give 10% by weight of white prisms.

This compound was demonstrated to be 4-methoxy-Δ^3-pyrrolin-2-one (I), by the following experiments. Elemental analysis and molecular weight determination established $C_5H_7O_2N$ as the molecular formula. The substance (m.p. 133–134°) sublimed readily at 90° and 0.2 mm., was optically inactive, and formed a very unstable hydrogen chloride adduct. Methoxyl determination demonstrated the presence of one such group. Its ultraviolet spectrum (95% ethanol) contained two λ_{max}, one at 210 mμ (ε 23,000) and a second at 279 mμ (ε 9). The infrared spectrum (potassium bromide disk) contained a large number of well defined bands: 3150 cm.$^{-1}$ (NH or OH group); 1660, 1635 and 1610 cm.$^{-1}$ (associated with an unsaturated lactam structure).

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(3) T. E. Eble and G. B. Whitfield, private communication.

(4) H. Yamaguchi, Y. Nakayama, K. Takeda and K. Tawara, *J. Antibiotics* (Japan), **10A**, 195 (1957).