B. Reduction of XII.—A mixture of 260 mg. of trienone XII and 100 mg. of 5% palladium-on-charcoal catalyst in 15 ml. of ethanol was hydrogenated at atmospheric pressure and room temperature. Hydrogen absorption corresponding to three moles was complete in 10 hr. After filtration and evaporation of the solution, a petroleum ether solution of the residue was chromatographed on alumina. Elution with petroleum ether–benzene (3:2) gave 120 mg. of an oil whose infrared spectrum was identical to that of ketone XVII. Its semicarbazone melted at 193–195° and did not depress the melting point of the same derivative of XVII. Further elution of the column yielded oils whose infrared spectra indicated them to be mixtures of XVI and XVII.

C. Reduction of XV.—A mixture of 200 mg. of monoenone XV and 100 mg. of 5% palladium-on-charcoal catalyst in 15 ml. of 95% ethanol was hydrogenated at 740 mm. and 25°. Hydrogen absorption ceased after 1 hr. and an uptake corresponding to the reduction of one double bond. The solution was filtered and evaporated and a petroleum ether solution of the residual oil chromatographed on alumina. Ten fractions were eluted using 2:1 petroleum ether–benzene and 1:1 solvent pair. Their infrared spectra indicated the contents to be mainly XVII mixed with about 10% XVI. A combination of the first two fractions was converted to a semicarbazone, which after two recrystallizations from aqueous ethanol melted at 195–197° dec. No depression was observed in the melting point on admixture with authentic semicarbazone of XVII.

***cis*-4a-Methyl-3,4,4a,10a-tetrahydro-2(1H)-phenanthrone (XX).**—A solution containing 1.50 g. of hydrophenanthrone XV and 3.5 ml. of ethylene glycol in 150 ml. of benzene was distilled until 120 ml. of solution remained. After the addition of 40 mg. of *p*-toluenesulfonic acid hydrate and the attachment of a water separator, the solution was refluxed and stirred for 4 hr. The cooled mixture was washed with sodium bicarbonate solution and water, dried over magnesium sulfate and evaporated, leaving a thick oil. Its infrared spectrum indicated the absence of any ketone. As a consequence the oily ketal was used in the next reaction without further purification. Its ultraviolet spectrum exhibited a maximum at 265 mμ (ε 400).

One gram of the ketal was added to 50 ml. of ethylene glycol containing 2.0 g. of potassium hydroxide and the solution heated under a nitrogen atmosphere at 190° for 5 hr. The cooled mixture then was poured into 250 ml. of water, neutralized with acetic acid, filtered and both residue and filtrate extracted with chloroform. The organic extracts were washed with sodium bicarbonate and water, dried over magnesium sulfate and evaporated. The residue was dissolved in 60 ml. of 95% ethanol containing 10 ml. of 10% sulfuric acid and refluxed on a steam-bath for 1 hr. The cooled reaction mixture was poured into 250 ml. of water and extracted with chloroform. The latter was washed with sodium bicarbonate solution and water, dried over magnesium sulfate and evaporated. A benzene–petroleum ether (3:2) solution of the organic residue was chromatographed on alumina and eluted by the same solvent pair. The infrared spectra of the first fractions showed the presence of a considerable amount of unhydrolyzed ketal, as a consequence of which they were recombined and again put into 10 ml. of 10% sulfuric acid and 40 ml. of 95% ethanol and refluxed on a steam-bath for 1 hr. The resulting hydrolysate, combined with the remaining eluates of the chromatogram, was rechromatographed on alumina and reëluted as a petroleum ether–benzene (1:1) solution. A reaction product of 125 mg. of 4a-methyl-3,4,4a,10a-tetrahydro-2(1H)-phenanthrone, m.p. 65–67°, was obtained, which on two recrystallizations from petroleum ether gave white plates, m.p. 68–69°; spectra: infrared: C=O 1710 cm.$^{-1}$ (s); ultraviolet: $\lambda_{max.}$ 267 mμ (ε 9800); $\lambda_{min.}$ 234 mμ (ε 3000).

Anal. Calcd. for $C_{15}H_{16}O$: C, 84.86; H, 7.60. Found: C, 84.87, 84.36; H, 8.01, 7.61.

Further elution of the column with benzene gave a trace of product whose infrared spectrum was identical with that of starting ketone XV.

Reduction of XX.—A mixture of 55 mg. of ketone XX, 20 mg. of palladium-on-charcoal catalyst in 15 ml. of 95% ethanol was hydrogenated at atmospheric pressure and room temperature. Hydrogen absorption ceased spontaneously at the end of 45 minutes and a one-mole uptake. The solution was filtered and evaporated in a stream of nitrogen. The infrared spectrum of the residue was identical with that of XVII. A benzene solution of the residue was filtered through a short alumina column. The infrared spectrum of the benzene eluate was identical with that of the crude residue. The oil was converted to a semicarbazone, which melted at 191–193° dec. after one recrystallization from ethanol. Its mixed melting point with an authentic semicarbazone of XVII was 192–194° dec. The infrared spectra of the two semicarbazones were identical.

AMES, IOWA

[CONTRIBUTION FROM THE DEPARTMENTS OF MEDICINE AND OF BIOCHEMISTRY, WESTERN RESERVE UNIVERSITY, AND THE LAKESIDE HOSPITAL]

The 20-Epimer of the C_{22}-Lactone from Tigogenin[1]

BY JOHN W. CORCORAN AND H. HIRSCHMANN

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3β,16β-Dihydroxy-20-iso-bisnorallocholanic 22→16-lactone has been prepared. The parent acid lactonized upon acetylation at room temperature. The resulting acetoxy lactone XI opened its lactone ring less readily than the 20-epimer which, however, has the energetically preferred structure. The results are interpreted in terms of steric repulsion of the C-18 and C-21 methyl groups. This concept is useful for assigning configurations at C-20 provided the stability of both isomers can be compared.

Several years ago[2] in a study of the rates of methanolysis of some steroids carrying acetoxy groups in the 16- and 20-positions an anomaly was encountered which seemed explicable on the following assumption: the space adjacent to the angular methyl group at C-13 is too restricted to readily accommodate bulky substituents of C-20 and, therefore, those conformations are preferred which place the hydrogen rather than the methyl group at C-20 next to the methyl at C-13. One would expect to observe the influence of such methyl–methyl interference on chemical behavior most readily if the rotation around the C-17 to C-20 bond is prevented by ring closure, as the two stereoisomers at C-20 might show differential stability. A good case for such a study would result if C-20 and C-16 were joined in a 5-membered ring on the β-side of the molecule since this situation produces in the models of one of the stereoisomers (Ia) a very close approach of the two methyl groups, C-18 and C-21.

Several investigators have applied this concept of

(1) The data in this paper have been taken from a thesis presented by John W. Corcoran to the Graduate School of Western Reserve University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The investigation was supported by a grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

(2) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **184**, 259 (1950).

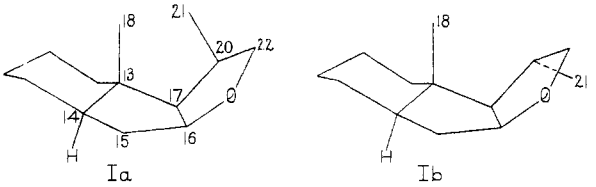
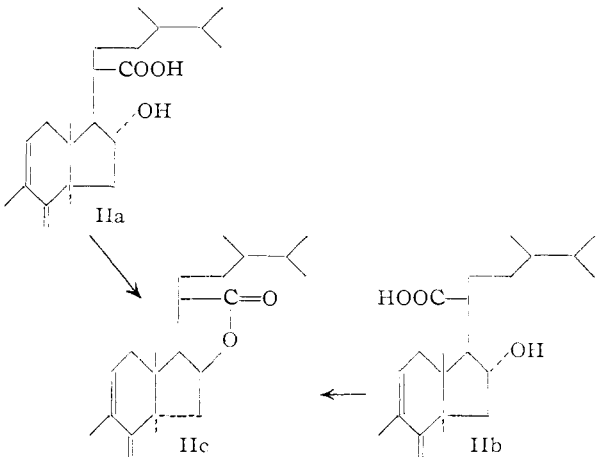
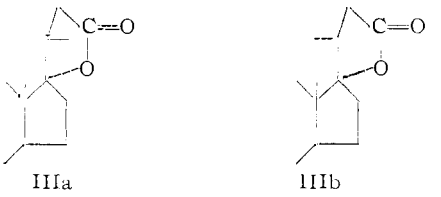
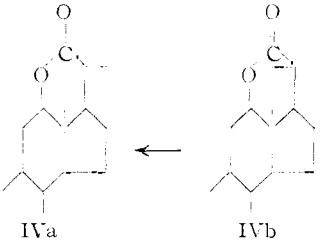


Fig. 1.—Projections of molecular models of rings C, D and E of compound XI (Ia) and of VI (Ib). In the schematic formulas (II to XIII) configurations at C-20 are indicated by formalized projections of models in which the bond leading from C-20 to the substituent written above it, points away from the observer.

methyl–methyl interference. It has been suggested[3] that it might account for the differential reactivity of dihydrosapogenins and dihydropseudosapogenins toward oxidants, and similar views have been expressed more recently by others.[4] Callow and James[5] considered the steric repulsions in Ia too large for such a compound to exist and hence assigned an epimeric structure (as in Ib) to the primary cyclization products of the pseudosapogenins, the so-called cyclopseudosapogenins. Some measure of support for such an extreme position can be derived from the studies of Bowers, *et al.*,[7] in the field of the polyporenic acids. Two hydroxy acids, epimeric at C-20 (IIa and IIb), on treatment with thionyl chloride gave the same lactone (IIc). The reaction is undoubtedly complex as the lactonization also involved inversion at C-16. The structure assigned to the product IIc places the C-22 methylene distal to the C-18 methyl and hence corresponds to the presumably more stable form Ib. The same concept also has been applied to a lactone III in which a 5-membered ring is joined to C-17. This ring system differs from the ones considered thus far in the lesser rigidity of a spirane structure as compared to the fusion of two 5-membered rings. This is expected to facilitate the repulsion of compressing groups by the easier deformation of bond angles. The lactone III was obtained by Ryer and Gebert[8] from sitosterol by oxidation and was assigned structure IIIa since the authors concluded that a compound with the epimeric configuration IIIb could not exist. This assignment, however, is at variance with the views which most investigators hold about the configuration of the sterols and bile acids at C-20.[9] A wider separation of methyl groups than in Ia is to be expected for a third structural type (IVb) which involves a 22→12β-lactone. In this case both isomers (IVa and IVb) have been prepared.[10] The one with the wider separation of methyl groups (IVa) proved to be more stable than its epimer.[11]







Since the use of the concept of methyl–methyl interaction has led in one instance[8] to a result which is contradicted by a large body of evidence, it seemed desirable to appraise the validity of these ideas by a study of a pair of isomers of type Ia and Ib. As the configuration of neither the dihydrosapogenins nor of the cyclopseudosapogenins has been proven beyond a doubt, a simpler example was sought. To this end we attempted the preparation of the 20-epimer of the lactone VI that results from the oxidation of tigogenin acetate (V).[12]

(3) H. Hirschmann, *Ciba Foundation Colloquia on Endocrinology*, **7**, 140 (1953).

(4) M. E. Wall, C. R. Eddy and S. Serota, THIS JOURNAL, **76**, 2849 (1954).

(5) R. K. Callow and V. H. T. James, *Chemistry & Industry*, 691 (1954). In a later paper published jointly with another group[6] the problem of configuration of the sapogenins at C-20 was left open.

(6) R. K. Callow, D. H. W. Dickson, J. Elks, R. M. Evans, V. H. T. James, A. G. Long, J. F. Oughton and J. E. Page, *J. Chem. Soc.*, 1956 (1955).

(7) A. Bowers, T. G. Halsall and G. C. Sayer, *ibid.*, 3070 (1954).

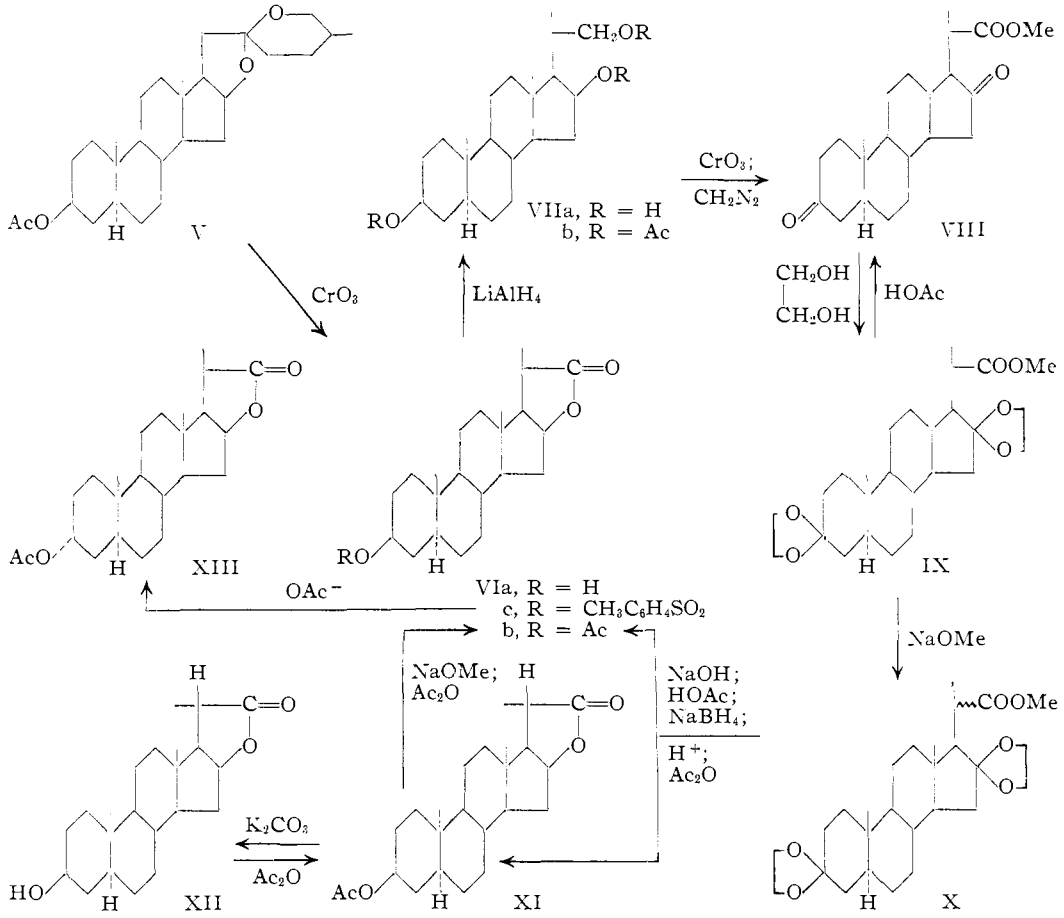
(8) A. I. Ryer and W. H. Gebert, THIS JOURNAL, **74**, 41 (1952).

(9) The evidence recently has been summarized (H. Hirschmann in G. Pincus and K. V. Thimann, "The Hormones," Vol. III, Academic Press, Inc., New York, N. Y., 1955, pp. 528–536). Additional support can be derived from the demonstration that the absolute configurations of C-20 and C-3 are consistent with each other if their relative configurations are formulated in the generally accepted manner (M. Viscontini and P. Miglioretto, *Helv. Chim. Acta*, **38**, 930 (1955)). An opposing assignment follows from the contention that the more stable form of a $\Delta^{12(20)}$-bisnorcholenate has the carboxyl group *trans* to the C-13 to C-17 linkage (D. H. Hey, J. Honeyman and W. J. Peal, *J. Chem. Soc.*, 185, 2648 (1954)). The assumed difference in space requirements of the methyl and carboxyl groups, however, receives no support from the relative blocking effects of these groups on the rotation of biphenyls (R. L. Shriner, R. Adams and C. S. Marvel in H. Gilman, "Organic Chemistry," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 362). The discrepancy between the conclusions that must be drawn from the contentions of Hey, *et al.*, and the results of others is not immediately apparent since the projection formulas of the former are consistent with their arguments only if the projections at C-20 are not made in the conventional manner. (The conventional way is given in the legend to Fig. 1.)

(10) M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **27**, 1631 (1944).

(11) D. Arigoni, B. Riniker and O. Jeger, *ibid.*, **37**, 878 (1954).

(12) R. Tschesche and A. Hagedorn, *Ber.*, **68**, 1412 (1935).



Esters, but not salts, of bisnorcholanic acids isomerize readily at C-20 in reaction with base.[10,13,14] However, when the lactone VIb was subjected to this treatment no product of isomerization could be detected. This suggested that the interaction of methyl groups may render the 20-iso compound so unstable as to displace the equilibrium completely in favor of the 20-normal compound.[15] We have, therefore, carried out the epimerization at C-20 with an ester of bisnorallocholanic acid so substituted at C-16 that a 16β-hydroxy group could be regenerated but that no cyclization would occur on treatment with base.

A bisnorallocholanate with a ketal group at C-16 seemed suitable for this purpose. It was obtained in the following manner. The lactone acetate VIb was reduced with lithium aluminum hydride to the triol VIIa which was reoxidized with chromic acid. The main product was the desired 3,16-dioxobisnorallocholanic acid which was esterified (VIII) with diazomethane and ketalized at C-3 and C-16 (IX) with ethylene glycol. Treatment with sodium methoxide induced spectral changes suggestive of partial epimerization (X). However, separation of the isomers was deferred until the hydrolyses of the ester and of the ketals and the reduction of the resulting carbonyl groups had been carried out, since it was expected that differential ease of lactonization would facilitate the fractionation of the 20-epimers. Moreover the separation of the two 20-epimeric ketals X would have been quite futile since isomerization at C-20 was expected to occur again during the alkaline ester hydrolysis which is a slow process in the presence of a 16-ketal grouping.

When the mixture of 3,16-dihydroxybisnorallocholanates was acidified, an acidic and a neutral fraction were obtained. The latter upon acetylation showed a spectrum similar to that of the starting lactone VIb and gave a pure specimen of this compound upon recrystallization. The acidic fraction, which could not be purified readily by recrystallization, was treated with acetic anhydride and pyridine at room temperature. This converted the bulk into neutral material which furnished a new compound XI. Its analysis and infrared spectrum showed that it was not an acid anhydride[16] but again an acetoxy lactone isomeric with the starting compound VIb. Since the method of preparation permitted inversion not only at C-20 but also at C-3, C-16 and C-17 it was necessary to establish the structure. When the new lactone was treated with sodium methoxide, it was quantitatively isomerized to yield, after acidification and reacetylation, the starting lactone VIb. The only labile asymmetric center in the lactone is believed to be at C-20 and hence the structural differences between the two

(13) H. Wieland, O. Schlichting and R. Jacobi, *Z. physiol. Chem.*, **161**, 80 (1926).

(14) M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **28**, 875 (1945).

(15) H. Hirschmann, F. B. Hirschmann and J. W. Corcoran, *J. Org. Chem.*, **20**, 572 (1955).

(16) See, *e.g.*, G. Roberts, C. W. Shoppee and R. J. Stephenson, *J. Chem. Soc.*, 3178 (1954).

lactones were ascribed to epimerism at this site. At higher temperatures than were used in this experiment, 3-hydroxysteroids also can be epimerized with alkoxide.[17] Thus there seemed to be a very remote possibility that isomerism at C-3 might be involved.

Although this interpretation would be consistent with the molecular rotation difference[18] between the two acetoxy lactones, it was contradicted by the infrared spectra of compound XI and of its parent lactone XII which were clearly indicative of an equatorial rather than an axial oxygen function at C-3.[20] To demonstrate beyond any doubt that compound XI was not the 3-epimer of the starting compound VIb, we have prepared this 3α-acetoxy lactone XIII by acetolysis of the 3β-tosylate VIc.[21] The resulting product XIII had the expected infrared characteristics[20] of an axial 3-acetoxy compound and differed in all its properties from either of the acetoxy lactones VIb and XI. It is concluded from these observations that the compounds VIb and XI must differ in their configurations at C-20 and that their structural differences are confined to this asymmetric center.

The two acetoxy lactones VIb and XI show striking differences in their infrared spectra which readily permit their differentiation. Of particular diagnostic value is an intense, narrow band which appears at 8.61 μ in the 20-iso compound and near 8.51 μ in VIb and other 22→16-lactones of the 20-normal series when measurements are made in carbon disulfide (Table I). The intensity and frequency of this absorption indicates that it results from the acyl–oxygen stretching of the lactone group. In contrast to this shift, two other bands associated with the lactone ring are not affected by the epimerism at C-20. These are the carbonyl stretching band and an intense peak near 9.83 μ which is ascribed to the alkyl-oxygen stretching at C-16.

The spectrographic differences permitted a study of the two lactones on a small scale. No interconversion was observed when either lactone VIb or XI was heated in toluene to 190° which is in contrast to the isomerization of 3α,12β-dihydroxybisnorcholanic 22→12-lactone under these conditions.[11] When a solution of the iso-lactone XI in alcohol containing hydrochloric acid was heated under a reflux again no isomerization could be detected. Treatment of the normal acetoxy lactone VIb with excess potassium carbonate in aqueous methanol at 20° gave predominantly the acid, 3β,16β-dihydroxybisnorallocholanic acid. Under identical conditions the iso-lactone XI yielded mainly a neutral fraction containing the parent lactone XII which could be reacetylated to the acetoxy 20-iso-lactone XI. This rather surprising difference in rates of lactone hydrolysis affords a very effective procedure for the fractionation of mixtures of the two 20-epimeric lactones.

Our initial small-scale experiment which effected the inversion of the 20-iso-lactone with sodium methoxide did not conclusively demonstrate the greater stability of the normal lactone, since it was found that the reaction, although conducted with carefully dried reagents, had proceeded with complete hydrolysis and had furnished the final product by relactonization in the subsequent work-up. In such a situation two explanations other than greater stability of the normal lactone had to be considered: (1) the driving force of the isomerization is the faster rate of hydrolysis of the normal lactone which disturbs the equilibrium of the two lactones to yield irreversibly the normal carboxylate ion; (2) the main product of the isomerization is not the normal lactone but methyl 3β,16β-dihydroxybisnorallocholanate[22] which then undergoes hydrolysis. Although neither of these explanations accounts very plausibly for the completeness of the inversion we attempted to exclude them by more direct evidence. When the reaction time was shortened to 10 minutes, enough neutral material was retained to permit its spectrographic examination. The curve exhibited a lactone but no ester peak in the 6μ range. The fingerprint region of the spectrum of this material and of its acetate showed that the chief constituent was the normal lactone. We conclude, therefore, that it is more stable than its 20-epimer.

TABLE I

ABSORPTION MAXIMA ASCRIBED TO STRETCHING VIBRATIONS OF LACTONE RINGS OF 16β-HYDROXYBISNORALLOCHOLANIC 22→16-LACTONES

Solvent	Config. at C-20	Substituent at C-3	Cpd.	Assignment, μ Carbonyl	Acyl-oxygen	Alkyl-oxygen
CS_2	Normal	β-Acetoxy	VIb	5.64	8.51	9.84
CS_2	Normal	β-Tosyloxy	VIc	5.64	8.51[a]	9.83
CS_2	Normal	α-Acetoxy	XIII	5.64	8.50	9.82[b]
CS_2	Iso	β-Acetoxy	XI	5.64	8.61	9.83
CH_2Cl_2	Normal	β-Hydroxy	VIa	5.67	8.44	9.84
CH_2Cl_2	Iso	β-Hydroxy	XII	5.67	8.54	9.84

[a] A tosylate band is expected to contribute to this maximum. [b] An acetate band is expected to contribute to this maximum.

(17) A. Windaus, *Ber.*, **49**, 1724 (1917); W. Hueckel, "Theoretical Principles of Organic Chemistry," Vol. I, Elsevier Publishing Co., Houston, Texas, 1955, p. 512.

(18) $\Delta[M]_D^{3\alpha-3\beta}$ +53° for 3-acetoxysteroids with 5α-configuration (D. H. R. Barton and W. Klyne, *Chemistry & Industry*, 755 (1948)); $\Delta[M]_D^{XI-VIb}$ +54°. This value agrees also with $\Delta[M]_D^{(20\text{-iso})-(20\text{-}n)}$ for 22 → 12β-lactones[10] (+62°). This accord, however, is considered to be fortuitous since the orientation of the substituents of C-20 relative to the remainder of the molecule are greatly altered by the different point of attachment of the lactone ring. The uncertainties which beset the use of optical rotational differences for assigning configurations of methyl bisnorcholanates are well brought out by the effect of substitution at C-12 on the size and *sign* of $\Delta[M]_D$ reported for 20-epimeric pairs[10,14,19] ($\Delta[M]_D^{(20\text{-iso})-(20\text{-}n)}$ +60° for 12α-acetoxy, and −180° for 12β-acetoxy substituted methyl 3α-acetoxybisnorcholanates[14]).

(19) M. E. Herr and F. W. Heyl, THIS JOURNAL, **74**, 3627 (1952).

(20) (a) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954); (b) H. Rosenkrantz and L. Zablow, THIS JOURNAL, **75**, 903 (1953).

(21) This method of epimerizing 3β-hydroxy-5-allosteroids was described by P. A. Plattner and A. Fuerst (*Helv. Chim. Acta*, **26**, 2266 (1943)) and is indicated particularly if the reduction of the 3-ketone in an acid medium fails to give the axial hydroxyl (H. Hirschmann, F. B. Hirschmann and M. A. Daus, THIS JOURNAL, **74**, 539 (1952)). It was used here since hydrogenation of methyl 3,16-dioxobisnorallocholanate (VIII) with platinum in acetic acid gave mainly the 3β-hydroxy lactone VIa and no detectable product with the absorption characteristics of a 3α-hydroxysteroid.

(22) Facile methanolysis of strained[23] δ-lactones has been observed with both 20-epimers of 3α-acetoxy-12β-hydroxybisnorcholanic 22 → 12-lactone.[10] In contrast, the equilibrium between the methyl ester and the lactone of 3α,12α-dihydroxy-17-isoetianic acid appears to be in favor of the γ-lactone even in the presence of large amounts of methoxide ion.[23]

(23) M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **29**, 1218 (1946).

The demonstration that 16β-hydroxybisnorallocholanic acids of either configuration at C-20 can undergo ring closure without inversion under rather mild conditions and that the resulting lactones are not unusually susceptible to hydrolysis clearly contradicts the view that compounds of type Ia cannot occur. Arguments based on this assumption, therefore, do not permit one to exclude a 20-iso structure for the very reactive cyclopseudosapogenins. Our findings, we believe, make also untenable the position that compounds of the type IIIb cannot exist. As Ryer and Gebert did not compare their products with the 20-epimeric structures, we see little persuasiveness in the deduction that the long alkyl chain of the sterols is attached to the β-facet of C-20.[24]

The conversions of the natural sapogenins to compounds with known configurations at C-20 indicate an arrangement as in Ib.[15,25] The configuration at C-20 can be expected to be retained upon oxidation to the lactone by means of chromic acid since neither the starting compound[26] V nor the 20-epimer of the isolated lactone is isomerized at C-20 by treatment with mineral acid. Accordingly, the most likely formulations for the lactone from tigogenin and for its 20-epimer are those given in formulas VI and XII, respectively. If these assignments are accepted we have observed that the lactone with the α-orientation[24] of the carboxylate VI is more stable than its epimer and hence have found an order of stability which is the reverse of that shown by esters[13,14] of bisnorcholanic acid and their lactones[11] attached to C-12. The reason for this reversal should lie in some characteristic of the attachment to C-16. It seems that interference of the methyl groups is by far the most likely explanation and in this sense it may be said that the concept has been verified by our experiments. Conversely, if we accept *a priori* the repulsion of methyl groups as a factor which allows one to predict the lesser stability of structure XI as compared to structure VIb, our experiments support the configuration at C-20 which was assigned to the stable lactone and to the sapogenin from which it was derived.

Interference of methyl groups seems to provide also a plausible explanation for the observation that the more stable lactone is hydrolyzed more readily than the epimer with the higher energy content. A molecular model of the 20-iso-lactone (Ia) indicates a distance between the methyl groups which is only about one-half of the sum of their van der Waals radii.[27] The strain resulting from such a compression is expected to be relieved in part by deformation of bond angles leading to a closer approach of the C-21 methyl to the carbonyl group. This in turn should increase the steric hindrance which an α-methyl group normally exerts on the addition of hydroxyl ion to the ester carbonyl.

(24) The terms α and β are applied to C-20 as proposed by L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948).

(25) R. E. Marker and D. L. Turner, THIS JOURNAL, **63**, 767 (1941); I. Scheer and E. Mosettig, *ibid.*, **77**, 1820 (1955); H. Hirschmann, F. B. Hirschmann and M. A. Daus, *J. Biol. Chem.*, **178**, 751 (1949). These reactions take place in a strongly acid medium and could proceed with inversion at C-20, if a 22-ketone is an intermediate.[6] The equilibration of simpler 22-ketones (P. Wieland and K. Miescher, *Helv. Chim. Acta*, **32**, 1922 (1949)) suggests that if isomerization at C-20 took place during the conversions it would result not in complete inversion but yield a mixture of both 20-epimers. As yet, however, no evidence against the stereospecificity of these conversions has been presented.

(26) R. K. Callow and V. H. T. James, *J. Chem. Soc.*, 1671 (1955).

(27) J. M. Robertson, "Organic Crystals and Molecules," Cornell University Press, Ithaca, N. Y., 1953, p. 227.

Experimental[28]

Oxidation of Tigogenin Acetate (V).—The procedure followed was essentially that of Tschesche and Hagedorn[12] except that the oxidant (chromium trioxide) was added in one portion and that the reaction time was shortened to 20 minutes. The neutral fraction was purified by chromatography. These changes raised the yield of 3β-acetoxy-16β-hydroxybisnorallocholanic 22→16-lactone (VIb) to 22% (m.p. 220–222°, $[\alpha]^{27}D$ −48° (*c* 1.2); λ_{max} 5.77, 8.06 and 9.73 μ (acetate[20]) and lactone peaks as given in Table I).

Bisnorallocholane-3β,16β,22-triol (VIIa) and Triacetate (VIIb).—A mixture of 2.6 g. of 3β-acetoxy-16β-hydroxybisnorallocholanic 22→16-lactone (VIb), 6 g. of lithium aluminum hydride, 555 ml. of tetrahydrofuran, and 150 ml. of dry ether was stirred for 90 minutes. The resulting triol VIIa (2.33 g.) was isolated in the usual manner and used for the next step without purification. For purposes of characterization an aliquot was acetylated with acetic anhydride and pyridine at room temperature. The triacetate VIIb after recrystallization from ligroin (b.p. 90–96°) had m.p. 117–119° and $[\alpha]^{28}D$ +52° (*c* 0.6, 95% ethanol).

Anal. Calcd. for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31. Found: C, 70.63; H, 9.40.

Recently the compound was prepared also by Klass, *et al.*,[29] who reported m.p. 117–119°, $[\alpha]D$ +53° (chloroform).

Methyl 3,16-Dioxobisnorallocholanate (VIII).—A solution of 2.27 g. of bisnorallocholane-3β,16β,22-triol (VIIa) in 190 ml. of acetic acid was stirred and kept in a bath (10–15°) while a solution of 2 g. of chromium trioxide in 11.5 ml. of 87% acetic acid was added during 5 minutes. The temperature of the bath was allowed to rise to 20° (1 hr.) and kept there for another hour. The excess of oxidant was reduced with methanol. The neutral reaction product (762 mg.) was again reduced with lithium aluminum hydride and re-used for another oxidation. The acidic fraction (1.59 g.) was dissolved in methanol (20 ml.) and treated with an ethereal solution (30 ml.) of diazomethane for 30 minutes. The product (1.65 g.) was chromatographed on silica gel–Celite (40 g.) and eluted with 10% ether in benzene. The crystalline fractions (1.47 g.) were recrystallized from butanone; yield 1.03 g., m.p. 220–223°, $[\alpha]^{27}D$ −109° (*c* 0.65), $\lambda_{max}^{dioxane}$ 294 mμ (ε 53), $\lambda_{max}^{CH_2Cl_2}$ 5.85 (3-ketone) and 5.78 μ (ester and 16-ketone).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.67; H, 9.27.

Methyl 3,16-Bisethylenedioxybisnorallocholanate (IX).—A mixture of 750 ml. of benzene, 6 ml. of ethylene glycol, 565 mg. of crude 3-dioxolane (see below) and 1.5 g. of *p*-toluenesulfonic acid (added in 2 portions at 0 and 5 hours) was heated for 9 hours while 310 ml. of distillate was removed through a helix-packed column equipped with a total condensation partial take-off still head. The warm mixture was treated with 3 g. of sodium hydroxide in 37 ml. of methanol and then extracted and washed. The product (648 mg.) was promptly chromatographed on Florisil (16 g.). Benzene and benzene +20% ether gave crystalline eluates (622 mg.) which were mainly bisdioxolane (infrared spectrum). Recrystallization from ligroin gave IX (m.p.

(28) All melting points are corrected. Compounds were dried for analysis, rotation and spectroscopy at 80° except VIIb and VIc which were dried at 70° and at room temperature, respectively. Unless when noted otherwise, rotations were measured in chloroform, ultraviolet spectra in 95% ethanol and infrared spectra in carbon disulfide. Reaction products were isolated by extraction with ether or ether–chloroform and then washed with acid or alkali as required and with water. The term acidic fraction applies to products extracted from the organic phase with alkali even if they lactonized upon acidification and isolation. The silica gel–Celite mixture (2:1) which was used in chromatography was prewashed as recommended by K. N. Trueblood and E. W. Malmberg (THIS JOURNAL, **72**, 4112 (1950)).

(29) D. L. Klass, M. Fieser and L. F. Fieser, *ibid.*, **77**, 3829 (1955).

198.5–200°, $[\alpha]^{28}D$ −19° (*c* 0.65, 95% ethanol); $\lambda_{max}^{CS_2}$ 5.76 μ; no carbonyl absorption in ultraviolet).

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 70.23; H, 9.00.

If aqueous sodium carbonate was used for neutralization instead of methanolic sodium hydroxide, the main product still had the carbonyl absorption (ultraviolet and infrared) of a 3-dioxolane. Such material (1.29 g.) was obtained first from compound VIII (1.19 g.) in this manner, before the importance of the removal of tosyl derivatives had been recognized.

Hydrolysis of 3 mg. of the diketal IX with dilute acetic acid as described by Oliveto, *et al.*,[30] gave a product with the infrared spectrum of methyl 3,16-dioxobisnorallocholanate (VIII).

Partial Epimerization of Methyl 3,16-Bisethylenedioxybisnorallocholanate (IX).—A mixture of sodium methoxide (7.8 g. of sodium in 78 ml. of dry methanol) and of 345 mg. of IX in 10 ml. of dry benzene was kept in a sealed tube for 2 hours at 78° and then separated into neutral X (266 mg.) and acidic (80 mg.) fractions. Both were processed as outlined below to yield the 20-iso-lactone XI. Longer heating caused no further changes in the infrared spectrum of the neutral fraction.

3β-Acetoxy-16β-hydroxy-20-isobisnorallocholanic 22→16-Lactone (XI).—A mixture of 222 mg. of partially epimerized methyl 3,16-bisethylenedioxybisnorallocholanate (X) in 35 ml. of methanol and 1 ml. of benzene and of 10 g. of potassium hydroxide in 5.6 ml. of water was heated under a reflux for 2 hours and after removal of methanol separated into neutral (12 mg.) and acidic (234 mg.) products. The latter in 20 ml. of acetic acid and 8 ml. of water was heated on a steam-bath for 30 minutes. The product (204 mg.) in 41 ml. of methanol was treated with 1 g. of sodium borohydride in 8 ml. of water at 29° for 7 hours and then shaken with cold ether–chloroform (3:1) and 100 ml. of cold 1 *N* hydrochloric acid. After 20 minutes at room temperature the organic phase was separated and fractionated into acidic (115 mg.) and neutral (58 mg.) material. The latter was acetylated at room temperature to yield 66 mg. of crude VIb. A pure specimen (14 mg., m.p. 217–219°) was obtained by recrystallization from methanol and identified by mixture m.p. and infrared spectrum.

The acidic fraction (115 mg.) was treated with 4 ml. of pyridine and 2 ml. of acetic anhydride for 13 hours and again separated into neutral (81 mg.) and acidic (38 mg.) material. The latter was not purified readily by recrystallization and was not investigated further. The main neutral fraction (81 mg.) was chromatographed on 4 g. of silica gel–Celite. The middle eluates (benzene + 2% ether) gave 21.4 mg. of compound XI upon recrystallization from methanol. It crystallized either as needles or as broad plates. The analytical specimen gave m.p. 227–229°, $[\alpha]^{27}D$ −34° (*c* 0.7) and infrared peaks characteristic[20] of a 3β-acetoxy group (5.77, 8.06 (single maximum), and 9.73 μ). A mixture with acetoxy lactone VIb (m.p. 220–221°) melted at 204–218°.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.47; H, 9.62.

The mother liquors which contained some acetoxy lactone VIb were not effectively purified by chromatography and recrystallization, but gave compound XI by selective hydrolysis; 44.5 mg. of such material in 8 ml. of methanol and 102 mg. of potassium carbonate in 2 ml. of 50% methanol were mixed and kept at 20° for 41 hours and then separated into acidic and neutral material. The latter (26.4 mg.) was reacetylated and recrystallized to yield 11.4 mg. of pure compound XI.

Hydrolysis of Lactones; 3β,16β-Dihydroxy-20-isobisnorallocholanic 22→16-Lactone (XII).—A mixture of 9.2 mg. of the 20-iso-lactone XI in 4.0 ml. of methanol and of 51.4 mg. of potassium carbonate in 1.0 ml. of 50% methanol was kept at 20° for 22 hours and then separated into acidic (0.6 mg.) and neutral (7.9 mg.) material. The latter was recrystallized from acetone to give the hydroxy lactone XII (m.p. 248–253° on rapid heating, with decomposition; $\lambda_{max.}^{CH_2Cl_2}$ 2.78 and 9.66 μ (3β-hydroxyl); $\lambda_{min.}$ 10.02 μ (a 3α-hydroxy group would have caused strong absorption at this wave length[20])). When the crude neutral fraction of another run was reacetylated, a product with the infrared spectrum of the starting material XI was obtained.

Hydrolysis of 9.9 mg. of the normal lactone VIb run simultaneously with that of XI under exactly the same conditions gave 6.8 mg. of acidic and 2.7 mg. of neutral products.

Isomerization of Lactones.—A mixture of 7.8 mg. of compound XI in 0.5 ml. of dry benzene and of sodium methoxide (200 mg. of sodium and 2 ml. of dry[31] methanol) was kept in a sealed tube at 78° for 10 minutes. The mixture was diluted with dry ether and rapidly washed with cold water until neutral. The ether layer was evaporated in a current of nitrogen without application of heat to yield a crystalline residue (1.2 mg.) which had a single infrared peak in the carbonyl region (5.67 μ in methylene chloride). The infrared spectrum after acetylation (pyridine and acetic anhydride at room temperature) showed an intense peak at 8.51 μ and all other maxima of the 20-normal acetoxy lactone VIb. In addition there was a small amount of extraneous absorption which did not include a peak or shoulder near 8.61 μ. The sample therefore contained no detectable amounts of starting material.

The alkaline washings after acidification, extraction and acetylation gave 6.8 mg. of neutral acetate. Its infrared spectrum was in very close accord with that of the acetoxy lactone VIb. The m.p. after recrystallization from ethanol was 218–220° and was not depressed by admixture of an authentic specimen prepared from tigogenin acetate.

When an identical experiment was performed with the lactone VIb as starting material the products showed infrared curves virtually identical with those of the products obtained above.

The following conditions gave somewhat contaminated starting materials which gave, however, no infrared evidence for isomerization: (a) heating of 2.4 mg. of lactone XI (or VIb) in 1 ml. of dry toluene in a sealed tube at 190° for 6 hours; (b) heating of 3 mg. of XI in 3 ml. of ethanol and 0.3 ml. of concentrated hydrochloric acid under a reflux for 2 hours, followed by acetylation.

3β-*p*-Toluenesulfonyloxy-16β-hydroxybisnorallocholanic 22→16-Lactone (VIc).—A solution of 101 mg. of 3β,16β-dihydroxybisnorallocholanic 22→16-lactone (VIa) (m.p. 237–239°) in 1.5 ml. of pyridine containing 322 mg. of *p*-toluenesulfonyl chloride was kept at 23° for 47 hours. The mixture was chilled, diluted with 0.6 ml. of water and gave by extraction (methylene chloride and ether) 144 mg. of neutral residue. It was recrystallized from acetone to yield 121 mg. of tosylate VIc, m.p. 168.5–173.5° on fast heating (with decomposition). Slower heating resulted in sharper but lower m.p.

Anal. Calcd. for $C_{29}H_{46}SO_5$: C, 69.56; H, 8.05. Found: C, 69.61; H, 8.32; residue, 0.47.

Ultraviolet spectrum in methylene chloride above 240 mμ showed λ_{max} 273 (ε 394) and 262.5 mμ (ε 590). The infrared maxima at 8.42 and 9.10 μ appear to be characteristic of the tosyloxy group.[20a]

Acetolysis of Tosylate VIc; 3α-Acetoxy-16β-hydroxybisnorallocholanic 22→16-Lactone (XIII).—3β-*p*-Toluenesulfonyloxy-16β-hydroxybisnorallocholanic 22→16-lactone (VIc) (110 mg.), 264 mg. of fused sodium acetate and 4 ml. of dry acetic acid were refluxed for one hour under anhydrous conditions. The neutral product (78 mg.) was chromatographed on a Florisil column (3 g., 200 mesh). Elution with benzene containing 1% ether gave 34 mg. of crystalline material. Its infrared spectrum (λ_{max} 5.64, 8.50, 9.82 μ) showed the presence of a γ-lactone but no acetate band. This product is believed to be mainly Δ²-16β-hydroxybisnorallocholanic 22→16-lactone.[21]

Further elution with benzene containing up to 10% ether gave 33 mg. of eluate which was recrystallized from ethanol to yield 26 mg. of compound XIII (m.p. 240–243°, with slight discoloration; $[\alpha]^{29}D$ −31° (*c* 0.7)).

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.56; H, 9.78.

The acetate peaks were at 5.76, 7.96, 8.04 (inflection), 8.09 and 9.82 μ and indicate the α configuration at C-3.[20]

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(30) E. P. Oliveto, T. Clayton and E. B. Hershberg, THIS JOURNAL, **75**, 486 (1953).

(31) N. Bjerrum and L. Zechmeister, *Ber.*, **56**, 894 (1923).