[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

STUDIES ON LACTONES RELATED TO THE CARDIAC AGLYCONES. VII. SYNTHESIS OF 3,14-BISDESOXYTHEVETIGENIN AND OF 14-DESOXYTHEVETIGENIN

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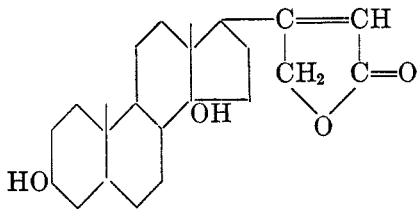
In preceding communications (1, 2), general syntheses for β-substituted $\Delta^{\alpha,\beta}$ -butenolides have been described and applied to the preparation of simple unsaturated lactones analogous to the cardiac aglycones. At the same time a study of the properties of the simple butenolides led to the suggestion that the side chain of the natural cardiac aglycones of the digitalis-strophanthus group is better represented as a $\Delta^{\alpha,\beta}$ -butenolide (3) than as a $\Delta^{\beta,\gamma}$ -butenolide. We have now extended this general study to the preparation of such butenolides containing the sterol ring system as a substituent on the β-carbon atom. The purpose in mind was two-fold: to substantiate the suggestion previously made concerning the position of the side chain double bond by a study of an unsaturated lactone containing a reduced cyclopentanophenanthrene substituent, and to gain information relative to the effect of structure of this group of drugs on physiological activity.

The simplest cardiac aglycones are digitoxigenin, thevetigenin, and uzarigenin. These are structurally identical but stereochemically different, and are represented by formula I.



I

	Stereochemical arrangement relative to C_{10}	
Digitoxigenin	*trans* 3,	*cis* 5
Thevetigenin	*cis* 3,	*cis* 5
Uzarigenin	*cis* 3,	*trans* 5

Of the three aglycones, digitoxigenin and thevetigenin show pronounced cardiotonic activity, whereas uzarigenin is comparatively much weaker in its action (4). It is therefore apparent that one should choose a steroid of the coprostane, or bile acid, series as the starting material for a synthetic lactone, if optimum activity is to be obtained, and that the configuration of the C-3 hydroxyl group is of comparatively minor importance.

The question of whether the presence of one or both of the hydroxyl groups is necessary for cardiotonic activity remains to be answered. There is no information at hand which can be used as a guide in the solution of this problem. Therefore we have prepared a lactone containing neither of the hydroxyl groups in question, and one containing the C-3 hydroxyl group. It is hoped to provide

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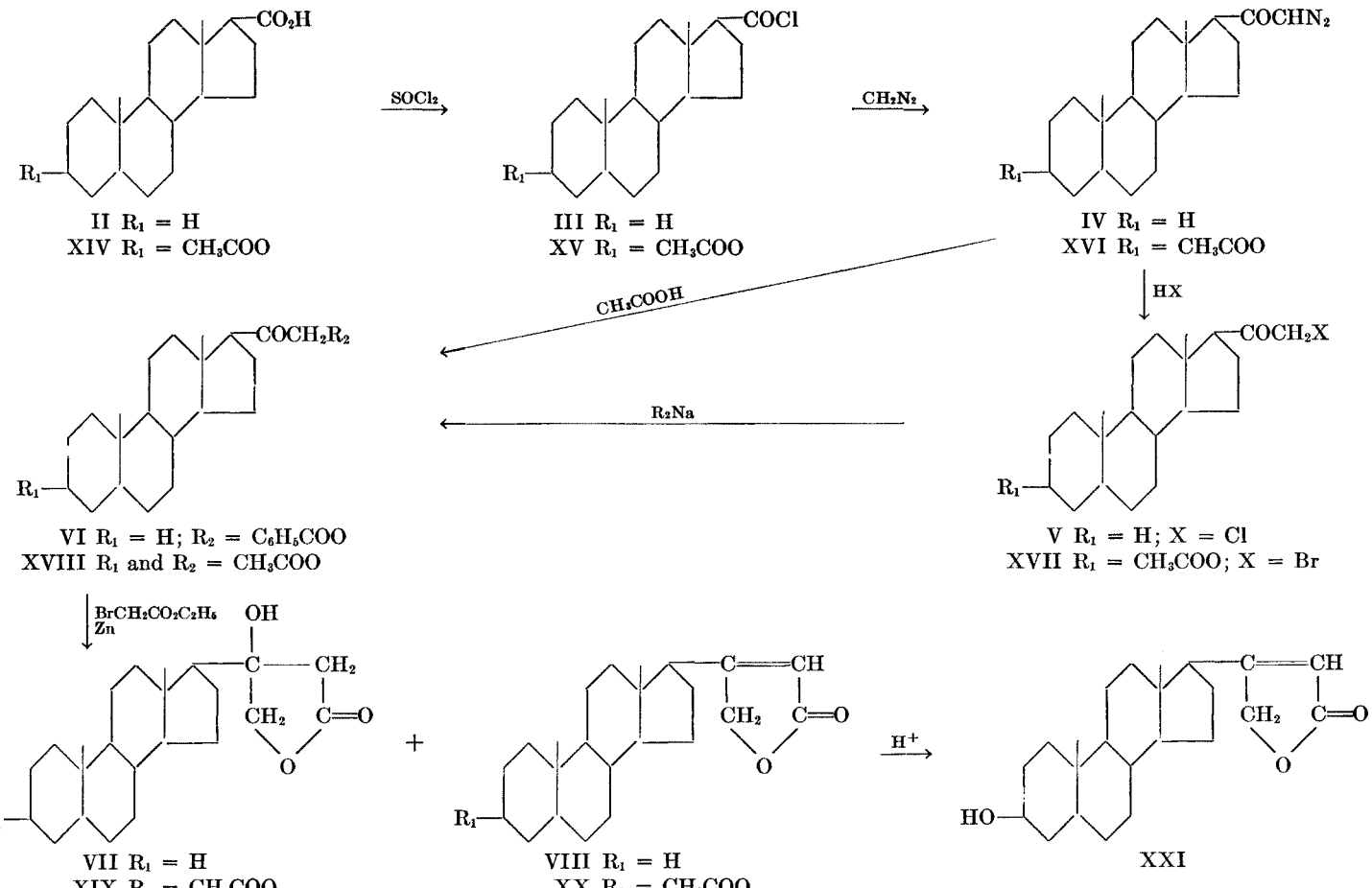

CO2H
SOCl2
COCl
CH2N2
COCHN2
R1
II R1 = H
XIV R1 = CH3COO
III R1 = H
XV R1 = CH3COO
IV R1 = H
XVI R1 = CH3COO
CH3COOH
HX
COCH2R2
R2Na
COCH2X
VI R1 = H; R2 = C6H5COO
XVIII R1 and R2 = CH3COO
V R1 = H; X = Cl
XVII R1 = CH3COO; X = Br
BrCH2CO2C2H5
Zn
OH
C
CH2
CH2
C=O
O
+
CH
H+
HO
VII R1 = H
XIX R1 = CH3COO
VIII R1 = H
XX R1 = CH3COO
XXI

an answer to this question from the results of pharmacological tests of these lactones. A preliminary note dealing with the synthesis of the first of these substances has already appeared (5). During the course of the work here described the preparation of the lactone of 3,21-dihydroxy-$\Delta^{5,6;\ 20,22}$-norcholadienic acid was described by Ruzicka and co-workers (6). In order that the relationship of the synthetic lactones, here described, to the natural aglycones may be apparent, and in order to avoid unduly cumbersome names, we suggest that the lactone of 21-hydroxy-$\Delta^{20,\ 22}$-norcholenic acid be referred to as 3,14-bisdesoxythevetigenin (or digitoxigenin), and that the lactone of 3,21-dihydroxy-$\Delta^{20,22}$-norcholenic acid be referred to as 3-desoxythevetigenin.

The preparation of both of these lactones was accomplished using the method previously described (2). Etiocholanic acid, which was prepared by degradation of cholanic acid according to Wieland, Schlichting, and Jacobi (7) served as the starting material for the synthesis of 3,14-bisdesoxythevetigenin. It has been found that, if the reduction of dehydrocholic acid to cholanic acid be carried out in acetic acid solution, rather than in alcohol (8), an improved yield of a more easily purified product results. The reactions involved in passing from etiocholanic acid to the lactone are shown in formulas II–VIII. In the preparation of etiocholanyl chloride, it is vital that the reaction mixture be kept cold if a satisfactory yield is to be obtained. The reaction of etiocholanyl chloride with diazomethane to yield the diazomethyl ketone (IV) proceeded without difficulty. However, in attempts to prepare 21-acetoxypregnanone-20 in crystalline form, unexpected difficulties were encountered. When the diazomethyl ketone (IV) was warmed with acetic acid in the usual manner, no crystalline product could be isolated from the reaction mixture either before or after chromatographic purification. The reaction of IV with hydrogen chloride proceeded normally, leading to the well defined chloromethyl ketone (V), which after reaction with sodium or potassium acetate, likewise failed to yield a crystalline acetoxymethyl ketone. However, reaction of IV with sodium benzoate resulted in the formation of crystalline 21-benzoxypregnanone-20 (VI), which was used successfully for the subsequent Reformatzky reaction. The crude product of the reaction of VI with ethyl bromoacetate and zinc consisted of a mixture of the hydroxy lactone (VII) and the desired unsaturated lactone (VIII) from which VIII crystallized. The hydroxy lactone (VII) could be converted to VIII by treatment with a solution of hydrogen bromide in acetic acid, as was the case with the simpler lactones described earlier (2).

3,14-Bisdesoxythevetigenin (VIII) as thus prepared gives a strong, positive nitroprusside (Legal) color test, indistinguishable from that displayed by the natural cardiac aglycones. Its ultraviolet absorption curve is shown in Figure 1, together with the curves for strophanthidin, periplogenin, and β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide. The close similarity of the curve for the synthetic lactone with those for strophanthidin, provided allowance is made for the aldehyde group of the latter, and periplogenin, furnishes strong confirmation for assigning the side chain double bond of the natural aglycones to the $\Delta^{\alpha,\beta}$-position. In the earlier paper (3) a slight discrepancy existed between the curves for the

natural aglycones and that for β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide. This discrepancy has now been cleared up by the nature of the curve for the butenolide containing a substituent which is identical with that of the natural lactones, with the exception of the nuclear hydroxyl groups.

Catalytic reduction of the double bond of 3,14-bisdesoxythevetigenin resulted in the formation of the dihydrolactone, which was identical with that previously prepared by a number of workers from digitoxigenin (9), sarmento-

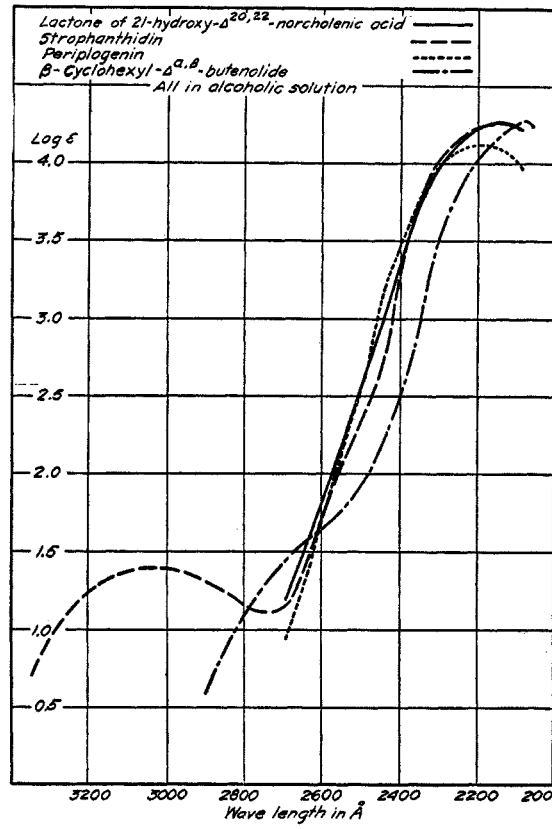
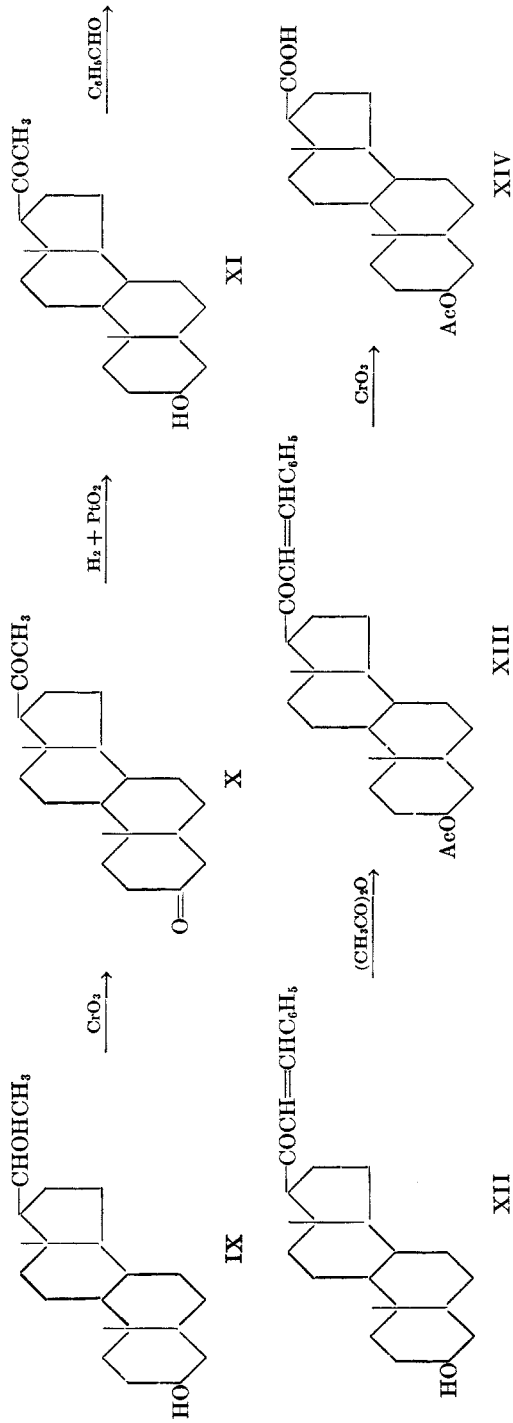


FIGURE 1

genin (10), or digoxigenin (11). A direct correlation between the bile acids and the cardiac aglycones with the carbon skeleton of the side chain of the latter intact, has therefore been accomplished.

The synthesis of 14-desoxythevetigenin was carried out in a similar fashion, proceeding from 3(β)-acetoxyetiocholanic acid. The latter has been described by Reichstein and Fuchs (12), who prepared it by catalytic reduction of methyl 3-keto-$\Delta^{4,5}$-etiocholenate. We have repeated this reduction, and according to our experience, methyl 3(β)-hydroxyalloetiocholanate is the predominating constituent of the fraction of the reduction product precipitated by digitonin.


CHOHCH3
HO
IX
CrO3
COCH3
O
X
H2 + PtO2
COCH3
HO
XI
C6H5CHO
COCH=CHC6H5
HO
XII
(CH3CO)2O
COCH=CHC6H5
AcO
XIII
CrO3
COOH
AcO
XIV

However, too much significance cannot be attached to our failure to duplicate Reichstein and Fuchs' experience, in view of the pronounced effect on such reductions often caused by subjective variations in experimental conditions. In view of this difficulty we have prepared 3(β)-acetoxyetiocholanic acid by degradation of pregnanediol, using substantially the method of Marker and Wittle (13) for the degradation of pregnanediol to etiolithocholic acid.

Pregnanedione, prepared by oxidation of pregnanediol (14), was selectively reduced at carbon atom 3 to yield a mixture of isomeric pregnanolones (15). The pregnanol-3(β)-one-20 (XI) was isolated from this mixture by precipitation with digitonin and then condensed with benzaldehyde to yield XII (13). Oxidation of XII after protection of the hydroxyl group by acetylation, yielded 3(β)-acetoxyetiocholanic acid (XIV).

In the subsequent reactions (XIV–XXI) no unexpected difficulties were encountered. The diacetoxy ketone (XVIII) was prepared directly from the diazomethyl ketone (XVI) rather than by way of the intermediate halomethyl ketone (XVII) which was necessary in the above case. However, a discrepancy between the properties of 3(β),21-diacetoxypregnanone-20 prepared by us, and those of the same substance reported by Marker, Crooks, and Wagner (16) should be noted. These workers prepared their compound by a less direct and unambiguous method. Unfortunately we have been unable to secure a sample of the material described by these workers for direct comparison. However, apparently 3(β)-acetoxy-21-bromopregnanone-20 (XVII), as prepared by us from the diazomethyl ketone (XVI), agrees in properties with the same substance described by Marker *et al.* (16). It should be emphasized that the subsequent reactions carried out with our 3(β),21-diacetoxypregnanone-20 (XVIII) furnish convincing proof of the correctness of the structure assigned to it. The possibility of a rearrangement about carbon atom 17 is excluded by the results of the hydrogenation of 3,14-bisdesoxythevetigenin (VIII). If inversion had taken place, the reduction product should have been isomeric with the reduction product of the natural aglycones at carbon atom 17. Since the natural aglycones are known to possess the configuration of etiocholanic acid at this point (17), it follows that no inversion has occurred. The exact cause for this discrepancy remains to be worked out.

The Reformatzky reaction of XVIII with ethyl bromoacetate led to the usual mixture of the unsaturated lactone (XX) and the 20-hydroxy lactone (XIX). In this case separation of the two was easily accomplished by chromatographic adsorption of XIX on aluminum oxide. Under the conditions used, XX was not adsorbed. Finally, deacetylation of XX with dilute hydrochloric acid gave 14-desoxythevetigenin (XXI).

The pharmacological examination of these substances will be reported elsewhere.

We wish to express our appreciation to the Schering Corporation for the gift of the methyl 3(β)-$\Delta^{5,6}$-etiocholenate used in this work. Our thanks are likewise due to Parke, Davis and Company for the gift of the pregnanediol used.

EXPERIMENTAL

All melting points are corrected for stem exposure, except as noted.

Cholanic acid. Dehydrocholic acid was reduced in 40-g. runs by dissolving it in 450 cc. of glacial acetic acid and adding 300 g. of amalgamated zinc (20 mesh) and 450 cc. of concentrated hydrochloric acid. Dry hydrogen chloride gas was passed through the solution while refluxing for 30 hrs. The solution was then cooled and the cholanic acid solidified to a white crystalline mass on top of the liquid. The acid solution was decanted from the solid acid and the latter was thoroughly washed with water. The residual zinc was carefully washed with chloroform and the cholanic acid thus obtained was combined with the main crop and recrystallized three times from acetic acid. It melted at 163–164° (uncorr.). Wieland and Boersch (8) report the melting point 164° (uncorr.). From 300 g. of cholic acid, 113 g. of cholanic acid was obtained.

Etiocholanyl chloride (III). A mixture of 4 cc. of absolutely pure thionyl chloride and 500 mg. of etiocholanic acid was kept in the refrigerator at 0° with occasional shaking until all the acid had gone into solution. This required about 60 hrs., after which the solution was allowed to stand at room temperature for several hours. The thionyl chloride was then removed under reduced pressure with the aid of several concentrations with dry benzene. The acid chloride crystallized readily, was slightly yellow, and melted at 80–86°. It was used without further purification.

21-Diazopregnanone-20 (IV). Etiocholanyl chloride prepared from 500 mg. of etiocholanic acid was dissolved in 7 cc. of dry benzene and the solution was slowly added to a twice distilled solution of diazomethane, prepared from 5 g. of nitrosomethylurea, in 60 cc. of ether. The temperature during the addition was kept at −14° and the solution was kept in a freezing mixture for 2 hrs. longer, after which it was allowed to stand at room temperature for 12 hrs. The solution was filtered, and after removal of the solvent, the diazo ketone remained as a yellow crystalline solid which melted at 80–106°. It was used without further purification.

Attempted preparation of 21-acetoxypregnanone-20. One cubic centimeter of pure glacial acetic acid was added to the crystalline diazo ketone from 100 mg. of etiocholanic acid. The solution was then warmed on the steam-bath, during which it turned brown and evolved nitrogen. After heating for 30 min. a brown oil separated. Acetic acid was removed under reduced pressure and the non-crystalline residue was chromatographed over aluminum oxide. The acetoxy ketone could not be obtained crystalline.

In another experiment, 50 mg. of 21-chloropregnanone-20 (see below) was refluxed with 150 mg. of fused sodium acetate and 1 cc. of glacial acetic acid. Nothing crystalline could be obtained after chromatographing. In another experiment, 100 mg. of 21-chloropregnanone-20 was refluxed with 170 mg. of potassium acetate in 2 cc. of 90% alcohol for 4.5 hrs. The chloride ion in the reaction mixture was determined gravimetrically and 45 mg. of silver chloride was obtained. The calculated amount of silver chloride is 44 mg. Hence the reaction apparently took the desired course. However, the acetoxy ketone again could not be obtained crystalline.

21-Chloropregnanone-20 (V). A stream of dry hydrogen chloride was passed into an ice-cold solution of 21-diazopregnanone-20, from 100 mg. of etiocholanic acid, in 30 cc. of dry ether for 10 min. After evaporation of the ether, first on the steam-bath and then *in vacuo*, a yellow oil remained which crystallized on addition of a few drops of pentane. The chloro ketone was recrystallized from alcohol and formed colorless prisms which melted at 103–105°. The yield was 75 mg. $[\alpha]_D^{25}$ 125° ± 2° [c = 0.850 in chloroform].

Anal. Calc'd for $C_{21}H_{33}ClO$: C, 74.8; H, 9.9; Cl, 10.5.
Found: C, 75.0; H, 10.2; Cl, 10.7.

21-Benzoxypregnanone-20 (VI) A mixture of 68 mg. of pure 21-chloropregnanone-20, 62 mg. of sodium benzoate, 0.12 cc. of water and 1.2 cc. of absolute alcohol was refluxed for 5.5 hrs. Upon cooling, the benzoxy ketone crystallized as fine white needles. The mixture

was diluted and extracted with ether; the ether extract was washed with water and dried over sodium sulfate. On evaporation of the solvent the ketone remained as a colorless oil which quickly solidified. After two recrystallizations from absolute alcohol, it melted at 158–159°. The yield was 45 mg. $[\alpha]_D^{25}$ 113° ± 2° [c = 0.406 in chloroform].

Anal. Calc'd for $C_{28}H_{38}O_3$: C, 79.6; H, 9.1.
Found: C, 79.6; H, 9.1.

A small amount of unreacted chloro ketone was recovered from the mother liquors.

3,14-Bisdesoxythevetigenin (3,14-bisdesoxydigitoxigenin) (VIII). To a solution of 600 mg. of pure 21-benzoxypregnanone-20 and 1.8 g. of ethyl bromoacetate in 13 cc. of dry benzene, was added 900 mg. of 60 mesh granulated zinc. After distilling off some of the benzene in order to secure absolutely anhydrous conditions, the solution was refluxed for 30 min., after which an additional 0.2 cc. of ethyl bromoacetate and 200 mg. of zinc was added and refluxing was continued for 15 min. After the addition of 0.4 cc. of absolute alcohol the solution was boiled for another hour. The addition compound was broken up in the usual manner with dilute hydrochloric acid, and the lactone was extracted with ether. The residue, after evaporation of the ether, deposited crystals on standing several days in the refrigerator. The crystalline material was separated from adhering oil by careful washing with a mixture of pentane and ether. As thus obtained, the crystalline material represents a mixture of the two possible isomeric hydroxy lactones (VII) and the desired unsaturated lactone (VIII). After repeated recrystallization from alcohol, a small amount of the unsaturated lactone was obtained. Complete conversion of the original mixture into the butenolide was effected by dissolving 220 mg. of the crystalline material in a mixture of 2 cc. of glacial acetic acid and 4 cc. of glacial acetic acid saturated with dry hydrogen bromide, and refluxing for 1.5 hrs. with a bath temperature of 135–145°. The mixture was then poured into cold sodium bicarbonate solution and the lactone was extracted with ether. After evaporation of the ether, the unsaturated lactone was recrystallized from alcohol, using a small amount of decolorizing carbon. It crystallized as stout needles which melted at 167–168° and gave a strong positive nitroprusside (Legal) test. $[\alpha]_D^{25}$ 11° ± 1.5° [c = 0.316 in methanol].

Anal. Calc'd for $C_{28}H_{34}O_2$: C, 80.6; H, 10.0.
Found: C, 80.4; H, 10.1.

An additional amount of the lactone was obtained by treating the oil removed from the crystals by the ether-pentane washing with hydrogen bromide in acetic acid as before. After removing some colored amorphous material from the butenolide by solution in alcohol and chilling, the latter crystallized from the alcoholic solution on seeding.

Hydrogenation of 3,14-bisdesoxythevetigenin. An absolute alcoholic solution of 40 mg. of the above unsaturated lactone was hydrogenated in the presence of 20 mg. of platinum oxide. The product crystallized readily and was recrystallized repeatedly from absolute alcohol until the crystalline form no longer changed. There was thus obtained 12 mg. of long rectangular plates which melted at 187–189°. When mixed with hexahydrodianhydrothevetigenin [prepared from digitoxigenin (9)], which melted at 187–189°, the melting point was not depressed.

Anal. Calc'd for $C_{23}H_{36}O_2$: C, 80.3; H, 10.5.
Found: C, 80.4; H, 10.6.

The physical constants for this substance, which has been obtained from several natural aglycones, are shown in Table I, from which the identity of the lactone prepared from etiocholanic acid with those of plant origin, is obvious.

Pregnanol-3(β)-one-20 (XI). A solution of 2.6 g. of pregnanedione (X) in 40 cc. of 90% acetic acid was added to a suspension of 130 mg. of Adams platinum oxide catalyst, which had been previously reduced, in 40 cc. of 90% acetic acid. This mixture was then shaken in

an atmosphere of hydrogen until 185 cc. of wet hydrogen at 760 mm. and 24° had been absorbed. The solution of the hydrogenated product in 118 cc. of absolute alcohol was added to a solution of 5.2 g. of digitonin in 470 cc. of alcohol. After adding 200 cc. of water, the mixture was left at room temperature for 12 hrs. The crystalline digitonide was filtered, washed with ether, and decomposed by dissolving the dried material in 10 cc. of dry pyridine and precipitating the digitonin by addition of 120 cc. of dry ether. After removal of the pyridine by repeated washing with dilute hydrochloric acid, the ether solution was dried and the solvent was removed, leaving the crystalline pregnanolone, which was used for the next reaction without further purification. The yield was 30 to 35%.

An additional quantity of pregnanolone was obtained by isolating the other products of the reduction, reoxidizing them to pregnanedione and repeating the above procedure. For this purpose, the mother liquors from the digitonide precipitate were completely freed from alcohol *in vacuo*. The residue was then thoroughly extracted with ether, leaving digitonin undissolved. The material extracted by the ether was then reoxidized according to Butenandt (14).

21-Benzalpregnanol-3(β)-one-20 (*XII*) was prepared essentially according to Marker and Wittle (13), except that the method of working up the reaction mixture has been materially improved. After the reaction mixture had stood for 24 hrs., the crystalline benzal compound was filtered off and washed successively with small amounts of 95% alcohol, progressively more dilute alcohol, and finally with water to remove the alkali. In this manner

TABLE I

PHYSICAL CONSTANTS OF HEXAHYDRODIANHYDROTHEVETIGENIN

SOURCE	M.P., °C.	$[\alpha]_D$
Digitoxigenin	185 (9); 188–189 (10)	33.7° (9)
Sarmentogenin	189 (10)	35.4° (10)
Digoxigenin	185 (11)	34.5° (11)
Etiocholanic acid	187–189	33.0°

1.1 g. of pregnanolone yielded 900 mg. of the benzal compound, which melted at 169–174°. Another 350 mg. of less pure material was obtained by dilution of the mother liquors.

The *acetate* of the above compound was prepared by refluxing 1.92 g. of the substance with 18 cc. of acetic anhydride for 30 min. On cooling, the acetate crystallized readily as prismatic needles, which were collected and washed with a small amount of acetic anhydride. The yield of material melting at 172–174° was 1.72 g. An additional 150 mg. was obtained from the mother liquors.

3(β)-Acetoxyetiocholanic acid (*XIV*). To a solution of 1.2 g. of the above acetate in 150 cc. of glacial acetic acid, was added dropwise and with stirring, a solution of 2.25 g. of chromic acid in the minimum amount of water and 75 cc. of glacial acetic acid. The temperature was held at 50° until all the chromic acid had been added, after which it was raised to 60–70° and held at that point for 5 hrs. After destroying the excess chromic acid by addition of 5–10 cc. of alcohol, the solution was concentrated to a small volume *in vacuo*. The residual solution was diluted, acidified with dilute sulfuric acid, and extracted with ether. The ether extract was washed carefully with dilute potassium bicarbonate solution until no more acidic material could be detected in the aqueous washes. This treatment removes acetic, benzoic, and probably some phenylacetic acid. The remaining ether solution was washed with water, dried, and the solvent was removed. Crystallization of the residue from dilute methanol yielded 400 mg. of 3(β)-acetoxyetiocholanic acid as shining platelets which melted at 177–179°. Reichstein and Fuchs (12), who prepared the substance by catalytic reduction of methyl 3-keto-$\Delta^{4,5}$-etiocholenate, report the melting point 162–174°. An additional 50 mg. of slightly impure acid was obtained from the mother liquors.

3(β)-Acetoxy-21-diazopregnanone-20 (*XVI*) was prepared as described above. It was obtained as a yellow syrup (12).

3(β)-Acetoxy-21-chloropregnanone-20. To a solution of diazoketone, prepared from 80 mg. of 3(β)-acetoxyetiocholanic acid, in 10 cc. of dry ether, was added at 0° 10 cc. of a saturated solution of hydrogen chloride in dry ether. After standing 10 min. the ether was evaporated and the residual light brown oil was taken up in 10 cc. of a 1:1 mixture of benzene and isopentane. This solution was passed through a column of 2 g. of aluminum oxide (Brockmann), and the column was washed with 20 cc. of the same solvent. The colorless solution which passed through the column was concentrated, leaving the chloro ketone as a crystalline residue. After recrystallization from methanol it formed stout prisms which melted at 115–116°. The yield was 45 mg.

Anal. Calc'd for $C_{23}H_{35}ClO_3$: C, 69.9; H, 8.9; Cl, 9.0.
Found: C, 69.9; H, 8.8; Cl, 9.2.

3(β)-Acetoxy-21-bromopregnanone-20 (*XVII*) was prepared in a similar manner, except that a benzene solution of the diazoketone was used. The bromo ketone crystallized readily from methanol as stout prisms without chromatographing, and melted at 139–141°. Marker, Crooks, and Wagner (16), who prepared the substance by catalytic reduction of 3(β)-acetoxy-21-bromo-Δ^{16}-pregneneone-20, report it as crystallizing as fine needles from methanol and melting at 145–147°. No rotation was observed by these workers. For our substance: $[\alpha]_D^{29}$ 100° ± 5 [*c* = 0.100 in chloroform].

Anal. Calc'd for $C_{23}H_{35}BrO_3$: C, 62.9; H, 8.0.
Found: C, 62.9; H, 8.1.

Pregnanediol-3(β),21-one-20 diacetate (*XVIII*). The oily diazo ketone prepared from 500 mg. of 3(β)-acetoxyetiocholanic acid was heated on the steam-bath with 8 cc. of glacial acetic acid until evolution of nitrogen ceased. The solution was then concentrated to dryness under reduced pressure and the residue was dissolved in 5 cc. of methanol. After refrigerating overnight, the yellow solution was decanted from a small amount of amorphous material. The methanol was then completely removed *in vacuo* and the material was taken up in 40 cc. of benzene-isopentane (1:1). This solution was passed through a column of 10 g. of aluminum oxide (Brockmann), after which the column was washed with 40 cc. of the same solvent. After removal of the solvent from the combined effluent from the column, 300 mg. of an almost colorless oil, which was sufficiently pure for use in the next reaction, was obtained. A small part of the product was crystallized from methanol and formed prisms which obviously contained methanol of crystallization. The air-dried crystals softened at 50–60° and melted with evolution of vapors. After drying at 15 mm., first at room temperature and finally for 3 hrs. at 80°, the diacetate melted at 111–112°. The air-dried substance lost 3.64% of its original weight on vacuum drying. The calculated value for loss of 0.5 mole of methanol of crystallization is 3.68%. $[\alpha]_D^{27}$ 91° ± 4° [*c* = 0.138 in chloroform].

Anal. Calc'd for $C_{25}H_{38}O_5$: C, 71.7; H, 9.2.
Found: C, 71.7; H, 9.1.

The same substance is reported by Marker, Crooks, and Wagner (16) as melting at 145–146°. No value for optical rotation is given by these authors.

14-Desoxythevetigenin acetate (*XX*) *and the lactone of 3(β)-acetoxy-20,21-dihydroxynorcholanic acid* (*XIX*). The Reformatzky reaction was carried out essentially as described in the preceding case, using 350 mg. of pure pregnanediol-3(β),21-one-20 diacetate, 0.6 cc. of ethyl bromoacetate, and 500 mg. of 60 mesh zinc in 10 cc. of dry benzene. After 30 min., 0.1 cc. of ethyl bromoacetate and 200 mg. of zinc were added and like amounts of the reagents were added after 40 and 50 min. After 60 min., 0.3 cc. of absolute alcohol was added to decompose the very insoluble zinc complex, and the mixture was refluxed for another

hour. The reaction mixture was worked up in the usual way and the product was extracted with ether. The combined ether and benzene extracts, after washing and drying, were warmed to 50°, and 2.5 cc. of dry pyridine and 1.1 cc. of acetic anhydride were added. After standing overnight at room temperature, the dark brown mixture was concentrated to dryness under reduced pressure, and the residue was dissolved in ether. The ether solution was washed successively with dilute hydrochloric acid and water and dried with sodium sulfate. The residue, after evaporation of the ether, was dissolved in 25 cc. of dry benzene and chromatographed over a column of 7 g. of aluminum oxide (Brockmann), using 50 cc. of dry benzene to wash the column. The effluent solution contained 14-desoxythevetigenin acetate (XX) and was concentrated to dryness under reduced pressure. The residual pale yellow oil crystallized rapidly on the addition of a few drops of alcohol. After several recrystallizations from alcohol, the acetate formed rectangular platelets which melted at 197–198° and gave a strong positive nitroprusside (Legal) color test. $[\alpha]_D^{28}$ 11.3° ± 1 [c = 0.708 in chloroform].

Anal. Calc'd for $C_{25}H_{36}O_4$: C, 75.0; H, 9.1.
Found: C, 75.0; H, 9.3.

The aluminum oxide column was eluted with 100 cc. of dry ether and the eluate was concentrated, leaving a residue which was the lactone of 3(β)-acetoxy-20,21-dihydroxynorcholanic acid (XIX). The substance was recrystallized from benzene, and formed fine needles which melted at 196–200°. The melting point was depressed to 170–185° when this substance was mixed with the unsaturated lactone described above.

Anal. Calc'd for $C_{25}H_{38}O_5$: C, 71.7; H, 9.2.
Found: C, 72.1; H, 9.3.

This hydroxy lactone was converted to 14-desoxythevetigenin acetate as follows (18): Thirty milligrams of the hydroxy lactone was refluxed with 3 cc. of acetic anhydride for 9 hrs. After removal of the acetic anhydride under reduced pressure, the residue was sublimed at 0.2 mm. pressure and a bath temperature of 200–220°. After several recrystallizations from alcohol, 15 mg. of pure 14-desoxythevetigenin acetate was obtained.

14-Desoxythevetigenin (XXI). A mixture of 35 mg. of 14-desoxythevetigenin acetate, 2 cc. of alcohol, 1.6 cc. of water, and 0.4 cc. of conc'd hydrochloric acid was refluxed for 2.5 hrs. Addition of water caused the formation of a crystalline precipitate which was extracted with chloroform. After washing and drying the chloroform extract, the solvent was removed under reduced pressure, leaving an oil which crystallized immediately upon the addition of a few drops of ethyl acetate. The lactone was recrystallized from a mixture of ethyl acetate and isopentane, or from dilute alcohol, and formed fine needles which melted at 220–222°. The substance gave a strong positive Legal test and an emerald green, as well as a blue ring, in the Keller-Kiliani test. $[\alpha]_D$ 11.5° ± 1.5 [c = 0.434 in chloroform].

Anal. Calc'd for $C_{23}H_{34}O_3$: C, 77.1; H, 9.6.
Found: C, 77.1; H, 9.5.

The microanalyses here reported were performed by Mr. Saul Gottlieb of these laboratories.

SUMMARY

1. 14-Desoxythevetigenin and 3,14-bisdesoxythevetigenin have been prepared from 3(β)-hydroxyetiocholanic acid and etiocholanic acid, respectively.

2. A more convenient preparation of 3(β)-hydroxyetiocholanic acid from pregnanediol has been described.

3. Hydrogenation of 3,14-bisdesoxythevetigenin yields a saturated lactone identical with that obtained from digitoxigenin, sarmentogenin, and digoxigenin.

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