STRUCTURE AND SYNTHESIS OF CARDIAC GENINS[1, 2]

RICHARD B. TURNER

Department of Chemistry, Harvard University, Cambridge, Massachusetts

Received December 9, 1947

CONTENTS

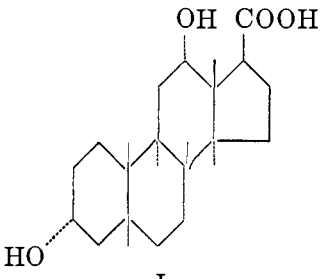
I. Introduction

The stereochemistry and partial synthesis of the cardiac genins have been actively investigated during the past several years. In the course of this work a number of genins have been degraded to derivatives of etiodesoxycholic acid (I), the spatial configuration of which has been firmly established by the recent work of Reichstein (158), Gallagher (25), and Kendall (84).[3] In many instances,

[1] This paper was presented at the Symposium on Steroidal Compounds which was held under the auspices of the Division of Medicinal Chemistry at the 112th Meeting of the American Chemical Society, New York City, September 18, 1947.

[2] Previous reviews are included in references 15, 39, 49, 71, 124, 145, 151, 175, and 176.

[3] The stereochemistry of desoxycholic acid and its derivatives has recently been reviewed by Shoppee (152). The dotted and solid lines in formula I refer respectively to α- and β-orientations, which are used in the sense suggested by Fieser (19c).



I
Etiodesoxycholic acid

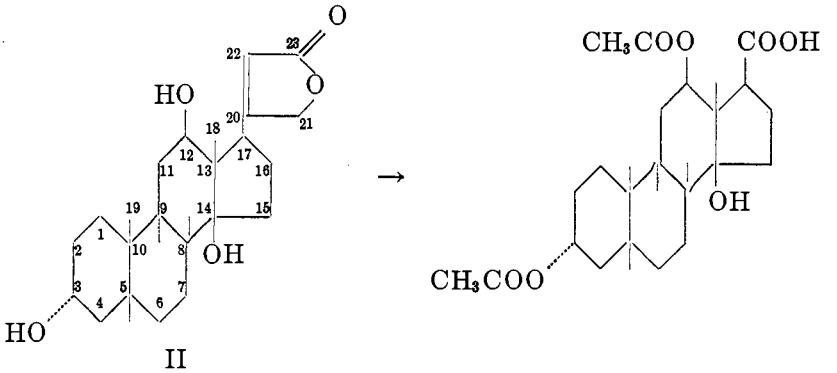
therefore, it is now possible to relate the cardiac genins to known reference compounds. The synthesis of important degradation products in this series has confirmed formulas assigned on the basis of other evidence, and in addition several compounds have been prepared that are closely related to the natural aglycones.

The pertinent literature up to and including that for the year 1947 is reviewed in the present paper. A few allied topics of special interest have also been included.

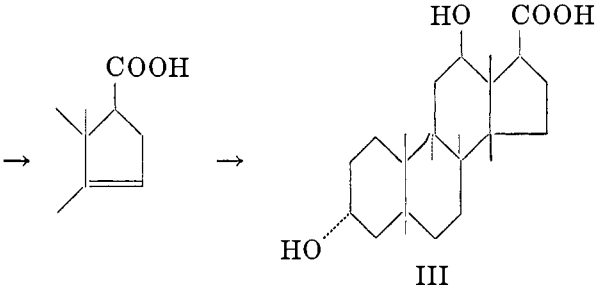
II. Stereochemistry

A. Digoxigenin and digitoxigenin

In 1938 Steiger and Reichstein (163) degraded the aglycone digoxigenin (II) by a series of steps involving acetylation, oxidation with potassium perman-



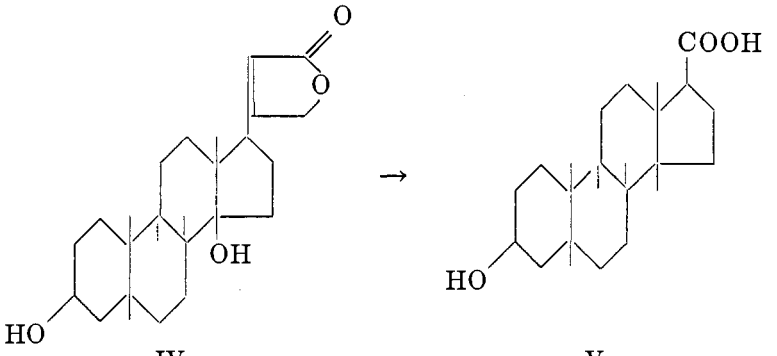
II
Digoxigenin



III
12-Epietiodesoxycholic acid

ganate, dehydration, and hydrogenation to an acid that was subsequently identified as 12-epietiodesoxycholic acid (III) by Mason and Hoehn (83). This evidence establishes the location and orientation (3(α), 12(β)) of the two secondary hydroxyl groups of the genin, the configuration of the nucleus at all centers of asymmetry except that at C_{14}, and the position (C_{17}) and configuration (β) of the lactone side chain.[4]

In a similar manner Hunziker and Reichstein (36) converted digitoxigenin (IV) into 3(β)-hydroxyetiocholanic acid (V), and thereby proved the incorrectness of the view held previously that digitoxigenin was a 3(α)-hydroxy compound (19a, 76).



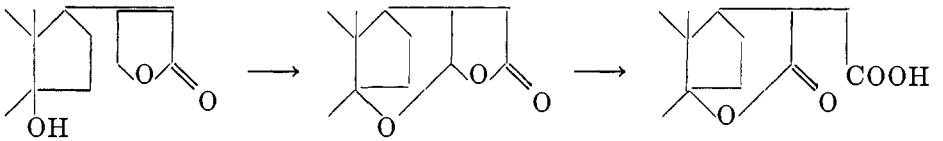
IV
Digitoxigenin

V
3(β)-Hydroxyetiocholanic acid

Inasmuch as the degradations just described offer an opportunity for inversion of the configuration at C_{14}, it cannot be inferred that the genins, like the bile acids, possess a *trans* junction between rings C and D. It can in fact be shown that in this respect the cardiac aglycones are unique and differ from other naturally occurring steroids in the presence of a *cis* C/D ring fusion.

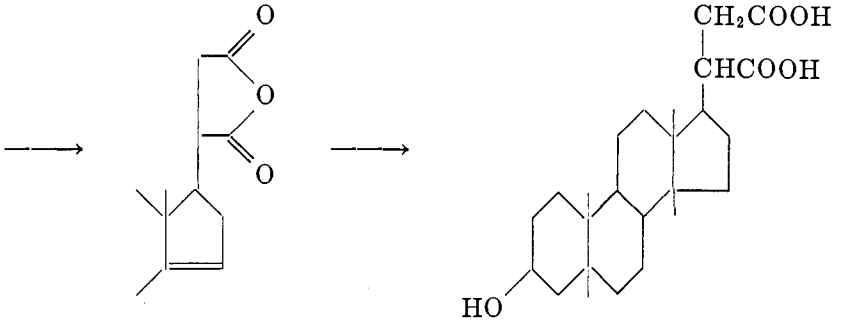
When digitoxigenin is allowed to stand with cold methanolic potassium hydroxide, it undergoes an irreversible isomerization to isodigitoxigenin (VI) (55). This reaction is typical of the cardiac glycosides as well as of the aglycones. It is evident that the formation of the bicyclic isogenin VI is possible only if the hydroxyl group at C_{14} and the lactone side chain at C_{17} are *cis* in digitoxigenin (IV), or if a *cis* arrangement can be attained by inversion at either of these points under the conditions of the reaction. By a series of transformations (VI $\rightarrow$ VIII) not involving the asymmetric center at C_{17}, the isogenin has been converted into etiocholanic acid (VIII) (49, 57), for which the 17(β)-configuration has been established. The identity of digitoxigenin and isodigitoxigenin with respect to their orientations at C_{17} therefore excludes the possibility of an inversion at this point, which might result from an intermediate β,γ-shift of the lactone double bond to C_{17}:C_{20}. Under the mild conditions employed for the

[4] The double bond of the lactone ring was originally assigned to the β,γ-position by Jacobs (60) on the basis of analogous color reactions (Legal test) obtained with the genins and with the β,γ-angelica lactones. More recent evidence derived by Elderfield (96) from ultraviolet absorption studies and hydrogenation experiments indicates that the substances are α,β-unsaturated lactones.



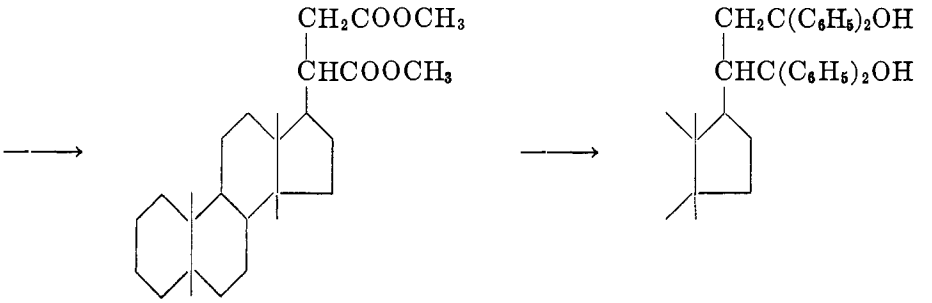
IV VI

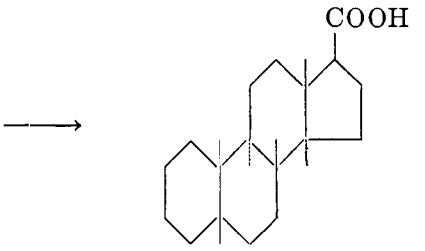
Isodigitoxigenin



VII

Digitoxanol diacid





VIII

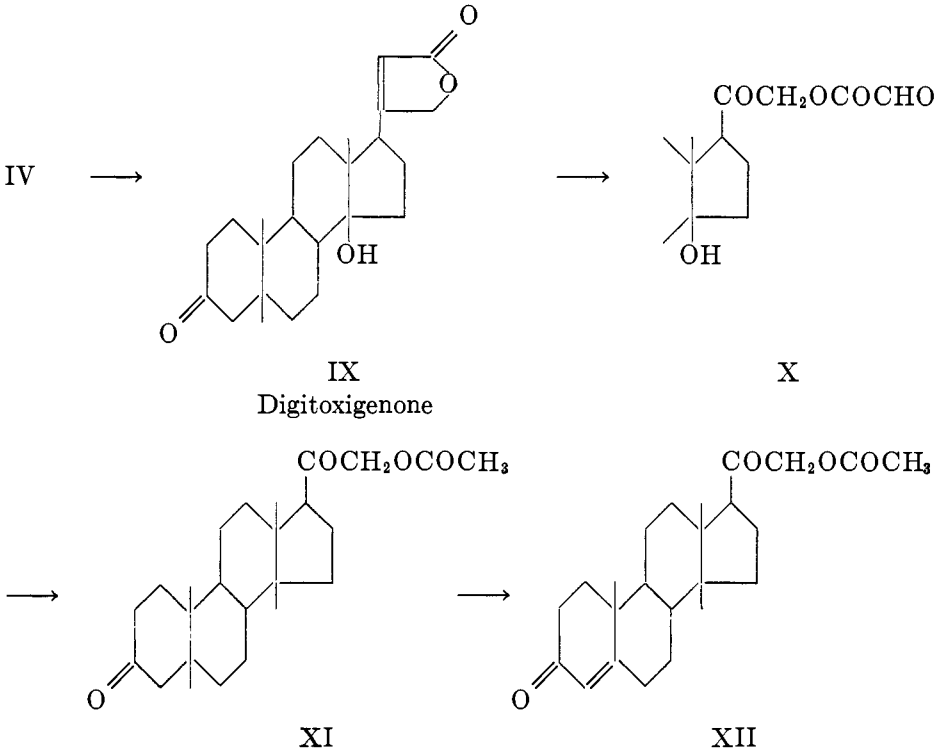
Etiocholanic acid

preparation of VI, epimerization at C_{14} is unlikely, and on the basis of this assumption it follows that the groups in question are *cis* in digitoxigenin. The β-configuration can, therefore, be assigned to the C_{14}-hydroxyl group, an arrangement which requires a *cis* junction between rings C and D. This conclusion is supported by the formation of cyclic keto lactones ($C_{21} \rightarrow C_{14}$) from strophan-

thidin (5, 12, 16, 37) and digitoxigenin (36, 88),[4a] and by the synthesis of methyl 3(β)-acetoxy-14(β)-hydroxyetiocholanate (CX) (page 33), which has been obtained as a degradation product of digitoxigenin.

Similar arguments can be advanced in the case of other members of the group, and the formulations of digoxigenin (II) and digitoxigenin (IV) are in all probability correct.

The interesting conversion of digitoxigenin into desoxycorticosterone acetate (XII) has recently been reported by Meyer and Reichstein (90). Careful oxidation of IV with chromic acid gives digitoxigenone (IX), which on ozonolysis



IX
Digitoxigenone

X

XI

XII

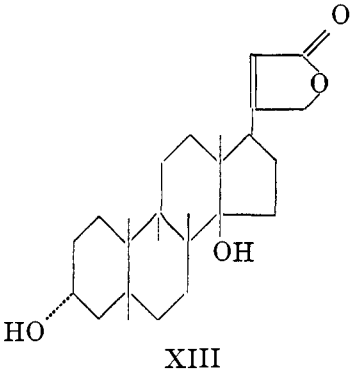
suffers a rupture of the lactone ring. The intermediate glyoxalate (X) has not been isolated in this case but is converted by standard procedures (hydrolysis, acetylation, etc.) into 21-acetoxypregnane-3,20-dione (XI), identified by comparison with an authentic sample. The transformation of XI into desoxycorticosterone acetate was accomplished earlier by Reichstein and Fuchs (120).

B. THEVETIGENIN AND UZARIGENIN

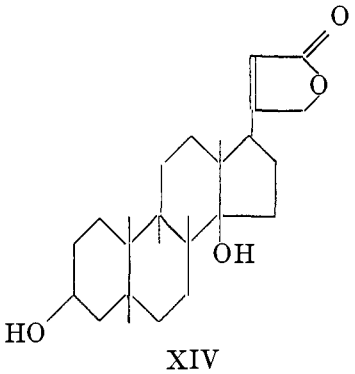
A crystalline glycoside, thevetin, was isolated from natural sources in 1933 by Chen and Chen (7). On treatment with alkali the glycoside gives an iso compound analogous to that derived from digitoxigenin, and for this reason the

[4a] *Added to proof, July 17, 1948:* This evidence is discussed in a recent paper by Buzas and Reichstein (5a), which appeared too late for inclusion in the present manuscript.

presence of a hydroxyl group at C_{14} in thevetin is assumed. The hydrolytic conditions required for the preparation of the aglycone, however, are so severe that this hydroxyl group is lost, and only a dehydration product, anhydrothevetigenin, can be isolated. The anhydro compound differs from Δ^{14}-anhydrodigitoxigenin, but on oxidation both compounds yield the same 3-keto derivative, anhydrodigitoxigenone (179). The true aglycone thevetigenin is therefore probably the C_3-epimer of digitoxigenin and is assigned formula XIII.[5]



XIII
Thevetigenin



XIV
Uzarigenin

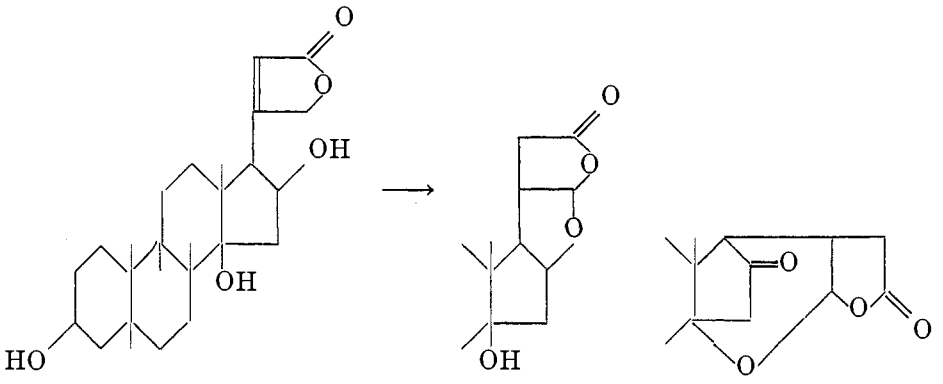
Isomeric with digitoxigenin and thevetigenin is the aglycone uzarigenin, which occurs in combination with two molecules of glucose as the glucoside uzarin (195). As in the case of thevetin, hydrolytic elimination of the sugar component is accompanied by loss of the tertiary hydroxyl group, and only anhydro derivatives of the true aglycone have been obtained (174, 178). In addition to the main product, α-anhydrouzarigenin, to which a Δ^{14}-structure has been assigned, a small amount of an isomer, β-anhydrouzarigenin, has been isolated for which Tschesche proposed the alternate $\Delta^{8,14}$-formulation. The latter point has not been established. Hydrogenation of the α-isomer proceeds smoothly with the absorption of 2 moles of hydrogen, and gives a mixture from which two completely saturated lactones can be isolated. These compounds, α_1- and α_2-tetrahydroanhydrouzarigenins, differ with respect to their configurations at the new center of asymmetry C_{20}, but both on degradation furnish etioallocholanic acid in which the A/B ring fusion is of the *trans* type (175, 176, 177). Uzarigenin, therefore, belongs to the cholestane series and it is the only genin thus far encountered for which this arrangement has been demonstrated. The β-orientation of the C_3-hydroxyl group is probable (see page 25) and formula XIV has accordingly been proposed for the unaltered genin (*cf.* 133).

C. GITOXIGENIN

The aglycone gitoxigenin (XV) is distinguished from other genins by the presence of a secondary hydroxyl group at C_{16} (48, 56). On treatment with

[5] In this connection it should be mentioned that the behavior of anhydrothevetigenin and digitoxigenin toward digitonin is anomalous. Of the two substances only the 3(α)-derivative, anhydrothevetigenin, affords an insoluble digitonide. This test as applied to the cardiac genins is therefore of doubtful value. Further examples of the reaction of the aglycones with digitonin are listed on page 2256 of reference 178.

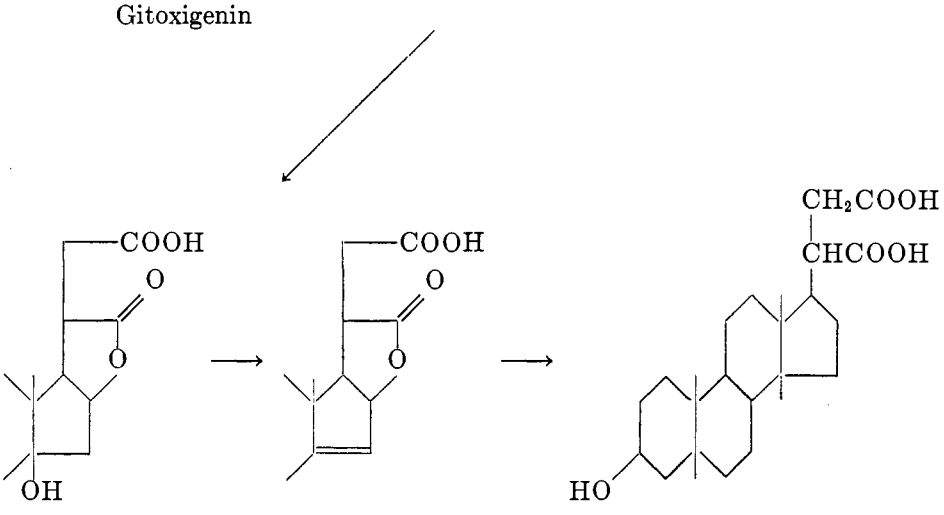
alkali the unsaturated lactone ring reacts with this hydroxyl group in preference to the one at C_{14} and gives an isogenin (XVI) containing a stable five-membered ring. After oxidation of gitoxigenin to a 16-keto derivative, interaction of the lactone side chain with the hydroxyl group at C_{14} is observed. Gitoxigenin, therefore, can form two types of iso compounds (XVI and XVII) that involve the C_{16}-hydroxyl group on the one hand and the C_{14}-hydroxyl group on the other.



XV
Gitoxigenin

XVI

XVII



XVIII

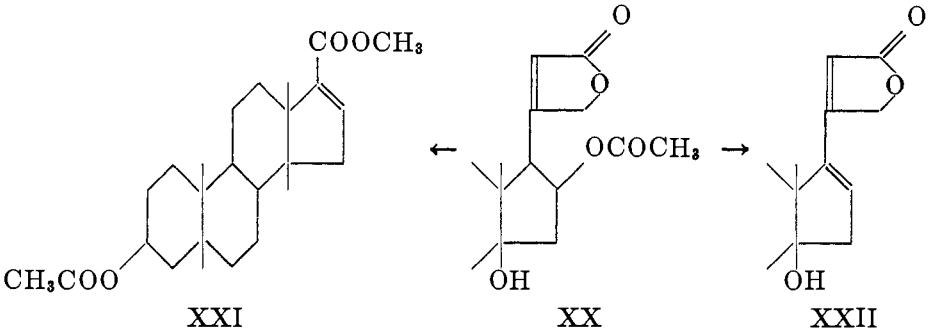
XIX

VII
Digitoxanol diacid

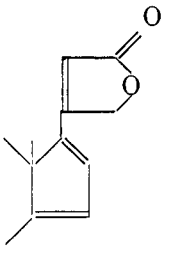
This behavior has been interpreted as evidence for the *cis* arrangement of the substituents at C_{14}, C_{16}, and C_{17} (151).

The β-orientation at C_{17} as well as at C_3 is demonstrated by the conversion of XVI into digitoxanol diacid (VII) (57). This transformation is accomplished by hypobromite oxidation of the isogenin to isogitoxigenic acid (XVIII), which in turn affords the anhydro acid XIX on dehydration. Hydrogenation of the latter compound is accompanied by hydrogenolysis at C_{16} and gives the dicarboxylic acid VII, obtained also from digitoxigenin (57).

Gitoxigenin yields a 3,16-diacetate (XX), which has recently been degraded to methyl 3(β)-acetoxy-Δ^{16}-etiocholenate (XXI) (86). This product was identified by comparison with a sample prepared from 3(β)-acetoxy-17-etiocholanone by way of the cyanohydrin and unsaturated nitrile (86, 122).



XXI XX XXII



XXIII

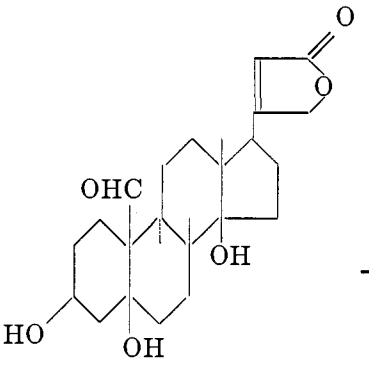
One molecule of acetic acid is easily eliminated from gitoxigenin diacetate (XX) when this substance is chromatographed on alumina. There can be isolated a doubly unsaturated lactone, monoanhydrogitoxigenin (XXII), which shows characteristic absorption in the ultraviolet at 273 mμ, log ε = 4.4 (85). A dianhydro derivative (XXIII) of gitoxigenin has also been prepared (197), which absorbs maximally at about 337 mμ, log ε = 4.7 (180).

D. STROPHANTHIDIN AND PERIPLOGENIN

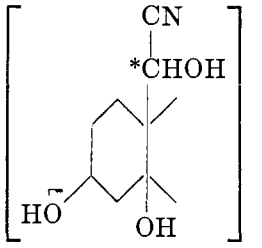
Certain of the cardiac aglycones, e.g., strophanthidin and periplogenin, possess in addition to the tertiary hydroxyl group at C_{14} a second tertiary hydroxyl group at C_5 (53). The *cis* relation between this substituent and the angular group at C_{10} was established by Jacobs and Elderfield (51), and is based on the following facts.

Strophanthidin (XXIV) on catalytic hydrogenation absorbs 1 mole of hydrogen and gives dihydrostrophanthidin containing a saturated lactone side chain. Under the conditions of reduction the C_{10}-aldehyde group is not attacked. The reaction of the dihydro compound with potassium cyanide affords a mixture of isomeric cyanohydrins (XXV) which has not been resolved, but which yields two homolactones (XXVIa and XXVIb) on hydrolysis with acetic acid. These

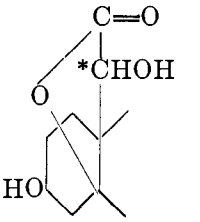
substances are epimeric at C_{19} and on careful oxidation both compounds give the same 3,19-diketohomolactone.



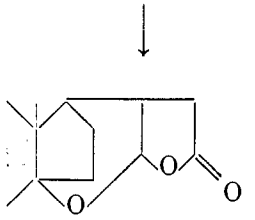
XXIV
Strophanthidin



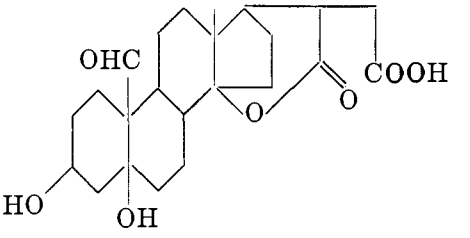
XXV



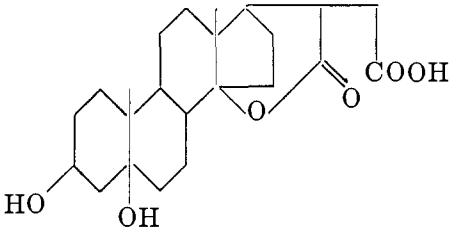
XXVIa and XXVIb



XXVII

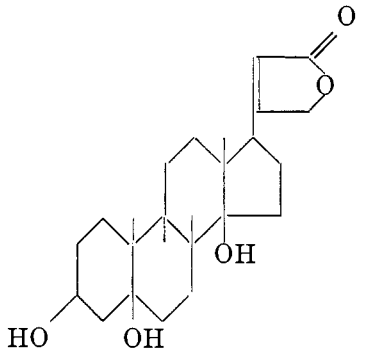


XXVIII
α-Isostrophanthidic acid



XXIX
α-Isoperiplogenic acid

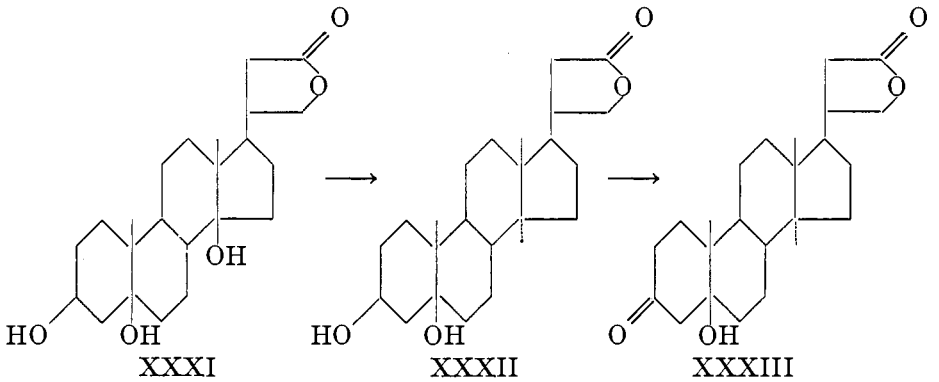
Strophanthidin when treated with alkali forms an iso compound (XXVII), which can be converted into α-isostrophanthidic acid (XXVIII) (44). Reduction of the angular aldehyde group (45) by the Wolff–Kishner procedure affords α-isoperiplogenic acid (XXIX), obtained also from periplogenin (XXX) (52). This iso acid (XXIX) has been related through a common derivative to



XXX

digitoxigenin (46), which in turn has been degraded to etiocholanic acid as noted previously.

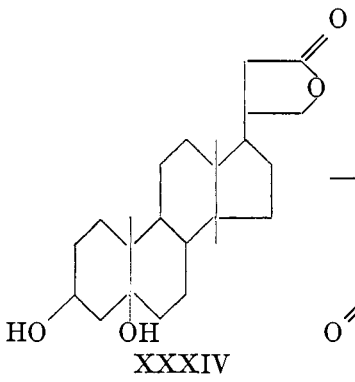
It can be concluded then that the angular groups at C_{10} in strophanthidin and periplogenin occupy the same spatial position (β) as does the C_{10}-methyl group of the bile acids. If the possibility of rearrangements during the Wolff–Kishner reaction is excluded, the two aglycones must be 5(β)-hydroxy compounds differing only in the nature of the groups attached at C_{10}.



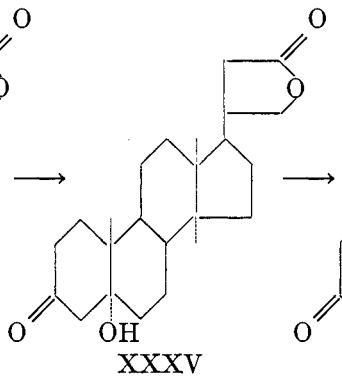
XXXI

XXXII

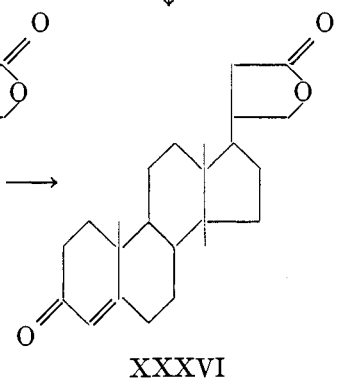
XXXIII



XXXIV



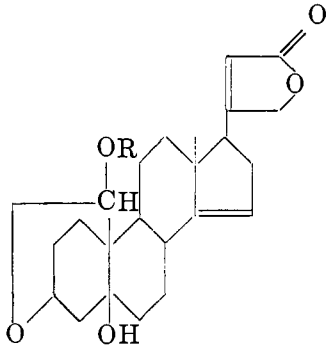
XXXV



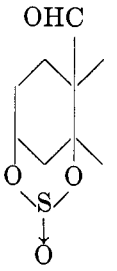
XXXVI

The orientation at C_5 has been explored further by Plattner, Segre, and Ernst (114). Dihydrostrophanthidin was subjected to Wolff–Kishner reduction, and a product was obtained that is identical in all respects with dihydroperiplogenin (XXXI). Dehydration and hydrogenation of XXXI furnish a 14-desoxy derivative (XXXII) that differs from a related synthetic product (XXXIV) of known structure (see page 37). The difference persists in the 3-keto derivatives XXXIII and XXXV, but on elimination of the asymmetry at C_5 both compounds give the same α,β-unsaturated ketone (XXXVI). It follows that XXXIII and XXXV are C_5-epimers and since the $5(\alpha)$-configuration of the latter is known, the corresponding $5(\beta)$-orientation can be assigned to XXXIII.

For the C_3-hydroxyl groups in strophanthidin and periplogenin, the β-configuration is inferred from the following observations. Strophanthidin with alcoholic hydrogen chloride yields an anhydro derivative, which no longer possesses the aldehydic or secondary hydroxylic functions of the original aglycone. For this substance the cyclic lactol structure XXXVII has been proposed (43). Such a transformation would appear to be possible only if the hydroxyl group at C_3 is in the β-position (i.e., *cis* to the C_{10} aldehyde group).[6]

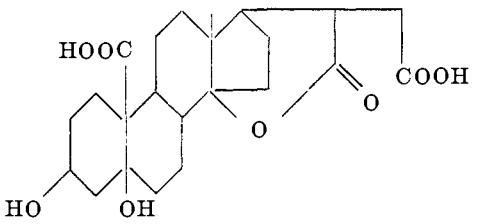


XXXVII



XXXIX

[6] Certain abnormalities in the behavior of α-isostrophanthic acid (XXXVIII) were



XXXVIII

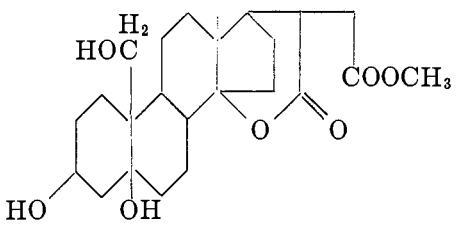
noted by Jacobs and Gustus (54), who reported that the substance does not form a lactone. A lactone is readily obtained, however, from an isomeride, β-isostrophanthic acid, which has been variously described as the C_3- or C_5-epimer of XXXVIII (15, 182). No adequate explanation of these results has been advanced.

A *cis* relation between the hydroxyl substituents at C_3 and C_5 is likewise implied by the formation of neutral sulfites (partial formula XXXIX) from α-isostrophanthidic acid (52; *cf.* 47, 50) and from strophanthidin (73, 114) by the action of thionyl chloride. A neutral carbonate of similar constitution has also been obtained from a degradation product of periplogenin by treatment with phosgene and pyridine (159).

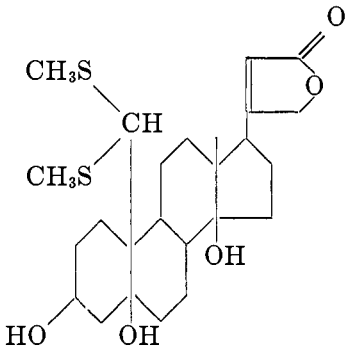
Conclusive evidence for the β-orientation at C_3 in periplogenin has recently been provided by Speiser and Reichstein (159), who succeeded in degrading periplogenin acetate to methyl 3(β)-acetoxyetioallocholanate (128, 161). On the basis of this work and in view of the evidence that has already been given, a similar arrangement at C_3 is probable in strophanthidin.

Arguments for the configuration of strophanthidin based on its conversion into derivatives of periplogenin are weakened to some extent by the fact that rearrangements under the conditions of the Wolff–Kishner reduction are not rigorously excluded. The classical conversions of coprosterol into epicoprosterol and of epidihydrocholesterol into dihydrocholesterol under the influence of sodium alkoxides at high temperature (198) serve to illustrate this point. A search has therefore been made for milder conditions for the conversion of strophanthidin into products of known structure.

In 1931 Jacobs and his associates (52) treated α-isostrophanthidolic acid methyl ester (XL) and its 3-acetate with phosphorus halides, thionyl chloride,



XL



XLI

and various halogen acids in the hope of replacing the primary hydroxyl group at C_{19}. In no case could this be accomplished. More recently Koechlin and Reichstein (69) prepared the methylsulfonyl derivative of methyl 19-hydroxyetioallocholanate, obtained by a series of reactions starting with strophanthidol acetate (*cf.* LXIII). Only products resulting from a rearrangement of the neopentyl system could be obtained from the reaction of this substance with sodium iodide.

By treatment of strophanthidin with methyl mercaptan in the presence of zinc chloride a mercaptol (XLI) is obtained (69), which should be susceptible

to desulfuration with Raney nickel (92). The results of this work are not available at the present time.

A number of other transformations of strophanthidin have been carried out by Ehrenstein (12) and Ehrenstein and Johnson (13).

E. ALLOSTROPHANTHIDIN AND ALLOPERIPLOGENIN

Of considerable stereochemical interest are the inactive glycosides allocymarin (38), alloemicymarin (41, 72), and alloperiplocymarin (64), which have been isolated from plant material after prolonged storage. These substances are isomeric with cymarin (strophanthidin-cymarose), emicymarin (periplogenin-digitalose), and periplocymarin (periplogenin-cymarose), respectively, and are apparently derived from these glycosides by the action of enzymes. Lamb and Smith (72) were able to obtain alloemicymarin by treatment of pure emicymarin with a crude enzyme preparation from the seeds of *Strophanthus emini.*

Jacobs (38) found that the isomerism involves the aglycone portion of cymarin, for cymarose is obtained on hydrolysis of both the active and inactive glycosides. The genin isolated from the latter, however, is not identical with strophanthidin and is therefore called allostrophanthidin. Further investigation of this material by Bloch and Elderfield (2) has revealed the fact that the asymmetric centers at C_3 and C_5 are not responsible for the observed difference; the ultraviolet absorption spectra of alloemicymarin and alloperiplogenin exclude the possibility of isomerism in these compounds based on a shift of the lactone double bond (64).

Allostrophanthidin, in contrast to strophanthidin, does not yield an isogenin on treatment with alkali (38), and Tschesche and his collaborators (184) observed the failure of alloemicymarin to furnish an iso compound under similar conditions. On the basis of this evidence it was proposed that the allogenins differ from their normal counterparts in a *trans* arrangement of the C_{14}-hydroxyl group and the lactone side chain. Such an arrangement could be attained by inversion at C_{14}, an assumption originally favored by Bloch and Elderfield, or by a corresponding inversion at C_{17}. The latter explanation is preferred by Tschesche (184) and by Katz and Reichstein (64), for the isomerism persists in trianhydroalloperiplogenin in which all three hydroxyl groups have been removed by dehydration. The correctness of this view was subsequently established by Speiser and Reichstein (159) by the degradation of alloperiplogenin to methyl 3(β)-acetoxy-14-iso-17-isoetioallocholanate (CI) and to methyl 14-iso-17-isoetioallocholanate, which possess the α-configuration at C_{17} (112).[7]

III. Glycoside Cleavage

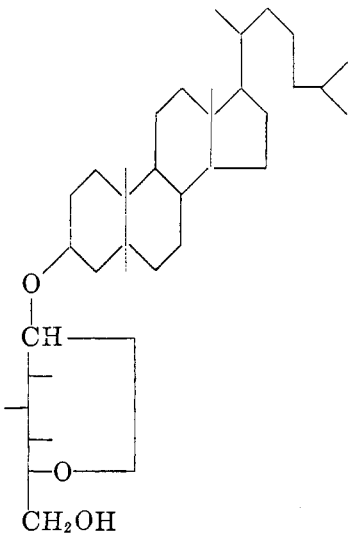
An outstanding difficulty in the elucidation of the structures of certain genins has been presented by the extreme difficulty with which glucose, rhamnose, and digitalose (119) are eliminated when attached directly to the steroid nucleus.

[7] The structure of these compounds is discussed in a later section (page 30).

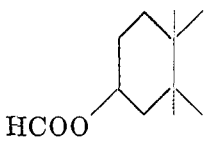
This behavior contrasts with the easy rupture of steroid–carbohydrate linkages that involve the 2-desoxysugars cymarose and digitoxose. Thus thevetin (2 glucose, digitalose?), uzarin (2 glucose), emicymarin (digitalose), alloemicymarin (digitalose), ouabain (rhamnose), and convallatoxin (rhamnose) require hydrolytic conditions so drastic that the aglycones undergo partial anhydrization.

In 1942 Mannich and Siewert (78) introduced a particularly mild method of cleavage and for the first time obtained from ouabain the unaltered aglycone ouabagenin. The method consists in treatment of ouabain with cold acetone containing a small amount of hydrogen chloride, under which conditions the glycoside dissolves as its monoacetone derivative. During the space of 2 or 3 weeks rhamnose is split off as the acetonide of a chloro sugar and ouabagenin acetonide crystallizes in good yield, accompanied by only small amounts of an anhydro derivative. The free genin is readily obtained by treatment of the acetone derivative with very dilute mineral acid. Other ketones, e.g., butanone and cyclohexanone, have been substituted for acetone in this reaction.

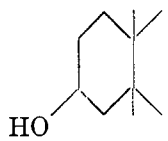
Employing Mannich's procedure, Reichstein and Katz (121) split convallatoxin into rhamnose and strophanthidin. This work provides proof for the formula first suggested for this glycoside by Fieser and Jacobsen (20), who showed that the convallatoxigenin benzoate of Tschesche and Haupt (186) is identical with anhydrostrophanthidin benzoate (43). The splitting of digitalose from emicymarin and alloemicymarin has also been effected by the method of Mannich (65).



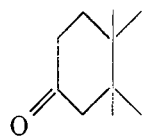
XLII



XLIII



XLIV



XLV

Unfortunately, in certain cases the cleavage employed with such success above gives poor yields or fails completely. This phenomenon has been observed particularly with glycosides that are very difficultly soluble in acetone. Accordingly, Steinegger and Katz (164) investigated the possibility of an oxidative

degradation of the sugar moiety in the hope of obtaining more easily hydrolyzable products. Such an approach was suggested by the conversion of bornyl glucuronide into bornyl formate by the action of periodic acid (35). β-(Cholestanyl)-*d*-glucoside-1,5 (XLII) and the corresponding glucoside of androsterone were used as model compounds, and although periodic acid proved unsatisfactory, it was found that by the use of chromic acid at 18°C. three products can be isolated which have been identified as cholestanyl formate (XLIII), cholestanol (XLIV), and cholestanone (XLV). In addition a small acidic fraction is obtained which yields further amounts of cholestanol on saponification. Analogous results are obtained for the androsterone glucoside, and in this case mild alkaline hydrolysis of the crude oxidation product followed by a second treatment with chromic acid affords a 50 per cent yield of pure 3,17-androstanedione.

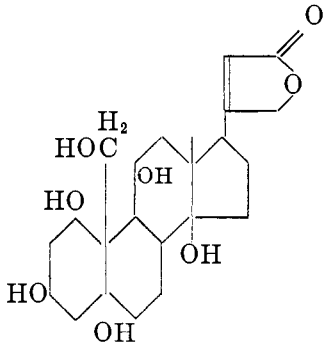
IV. Genins and Glycosides of Indefinite Structure

A. Ouabagenin

The results of the investigation of ouabagenin have been reviewed elsewhere (124) and are reproduced here only in outline.

Of the eight oxygen atoms present in the aglycone, two are presumably associated with the lactone side chain; one is evidently present as a tertiary hydroxyl group at C_{14}, since ouabain on treatment with alkali gives isoöuabain (40). Of the remaining five hydroxyl groups, four are readily acetylated. The fifth is therefore assumed to be tertiary and has been assigned to C_5 by analogy with strophanthidin and periplogenin.

Acetolysis of ouabain proceeds with the loss of formaldehyde (42), and for this reason a hydroxyl group has been assigned to C_{19}. Ouabagenin does not react with lead tetraacetate and thus contains no adjacent hydroxyl groups (78). The monoacetone derivative is therefore assumed to involve α,γ-hydroxyl groups, which must be *cis* on the basis of this interpretation. These groups



XLVI
Ouabagenin
(Mannich and Siewert, 1942)

have been placed provisionally at C_1 and C_3. The remaining hydroxyl group has been tentatively assigned to C_{11} (21, 79, 187) and is probably α-oriented if the proposal regarding its position is correct, since it is readily acetylated (*cf.* 26).

The foregoing evidence led Mannich and Siewert (78) to propose XLVI as the formula of ouabagenin.

B. SARMENTOGENIN

The aglycone sarmentogenin, obtained by Jacobs and Heidelberger (58) from the glycoside sarmentocymarin, is an isomer of digoxigenin. One of the two secondary hydroxyl groups is located at C_3 (183) and the other has been placed at C_{11} by reason of its hindered character. The latter proposal has not been substantiated, however, and is not favored by Reichstein (124) in view of certain abnormalities in specific rotation. The possibility that sarmentogenin might be a 3,12-dihydroxy compound epimeric with digoxigenin has been carefully tested by Katz and Reichstein (64), who prepared the diketo derivative sarmentogenone. This product is not identical with digoxigenone (156), and the structure of sarmentogenin must therefore be regarded as uncertain.[7a]

C. ADYNERIGENIN AND NERIANTOGENIN

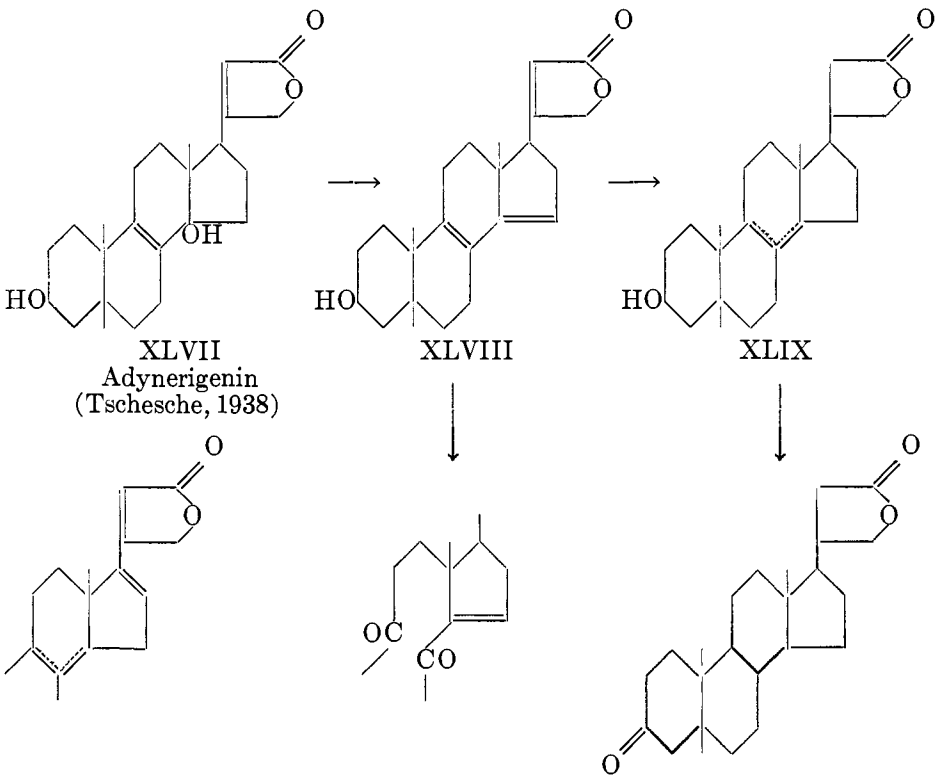
An interesting structural problem is presented by the aglycones adynerigenin and neriantogenin, which have been isolated in small amounts from oleander leaves as the inactive glycosides adynerin (94) and neriantin (146, 185).

Adynerigenin, according to Tschesche (184, 185), possesses two double bonds, of which only the one associated with the lactone grouping is normally susceptible to catalytic hydrogenation. The second unsaturated linkage, because of its inert character, has been assigned provisonally to the 8,9-position. Of the two hydroxyl groups present in the molecule, one is secondary and attached at C_3; the other is presumably located at the tertiary position C_{14}, since adynerin forms an iso compound in alkaline solution.

Adynerigenin when treated with dilute acid affords an anhydro compound with absorption in the ultraviolet region (247 mμ, log ϵ = 4.1) compatible with structure XLVIII (*cf.* 11, 202). This substance is converted by concentrated hydrochloric acid into an isomeric compound with maximum absorption at 280 mμ (log ϵ = 4.4), which Tschesche described as a derivative containing a conjugated system of double bonds in a single ring. This suggestion is not in accord with the observed spectrum, for a shift of the double bonds to ring D should produce maximum absorption at 330–340 mμ as in the case of dianhydrogitoxigenin (XXIII), whereas compounds like ergosterol and $\Delta^{2,4}$-cholestadiene show much lower extinction (log ϵ = 3.8–4.0) than that exhibited by the rearranged anhydro derivative.

The experimental findings are perhaps better accommodated by a structure

[7a] *Added to proof, July 17, 1948:* In a communication that appeared after this manuscript was submitted the conversion of sarmentogenin into methyl 3(β),11(α)-diacetoxyetiocholanate is described (63b). The latter product was identified by comparison with an authentic sample (63a), and the structure of sarmentogenin is thereby established.



XLVII
Adynerigenin
(Tschesche, 1938)

XLVIII

XLIX

LI LII L

of type LI related to monoanhydrogitoxigenin (XXII), for which absorption at 273 mμ (log ϵ = 4.4) has been reported (85).

In contrast to XLVIII the rearrangement product absorbs 3 moles of hydrogen and gives a mixture from which no crystalline material has been isolated. This result can perhaps be explained by a shift of the isolated double bond from either C_8:C_9 or C_8:C_{14} to the conjugated position C_{14}:C_{15} in the presence of the platinum catalyst (*cf.* 160, 192, 200). Hydrogenation of the resulting intermediate might be expected to give a mixture of stereoisomers, as is the case with 20,21-dihydrodianhydrogitoxigenin (199).

Hydrogenation of XLVIII furnishes a tetrahydro derivative XLIX, which can be further reduced by the method of Windaus, Linsert, and Eckhardt (196). Under these conditions the secondary hydroxyl group is lost, but when this group is protected by acetylation a product is obtained after hydrolysis and oxidation that is identical with tetrahydroanhydrodigitoxigenone (L). This evidence establishes C_3 as the location of the secondary hydroxyl group.

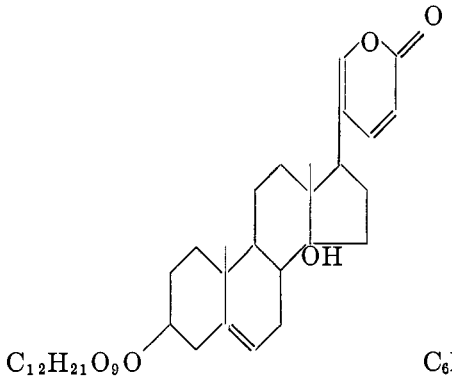
Chromic acid oxidation of XLVIII gives a compound $C_{23}H_{28}O_5$ ($\lambda_{max.}$ = 252 mμ, log ϵ = 4.1) for which Tschesche proposed formula LII. A neutral product of similar nature, but without characteristic absorption in the ultraviolet, is obtained by oxidation of XLIX.

The glycoside neriantin possesses the formula $C_{29}H_{42}O_9$ and like adynerin contains two double bonds, which, however, are both easily reduced (185). Hydrolysis of the glycoside furnishes glucose and neriantogenin and apparently proceeds without the elimination of a hydroxyl group from the aglycone, since the composition of the latter substance corresponds to the formula $C_{23}H_{32}O_4$. The formation of a diacetyl derivative of the genin indicates the presence of two secondary hydroxyl groups, and treatment with strong acid yields an anhydro compound that has been identified as dianhydrogitoxigenin (XXIII). The diacetate is not identical with 3,16-diacetyl-Δ^{14}-anhydrogitoxigenin from oleandrigenin (94, 180), and in the absence of other evidence the formulation of neriantogenin cannot be made with certainty.

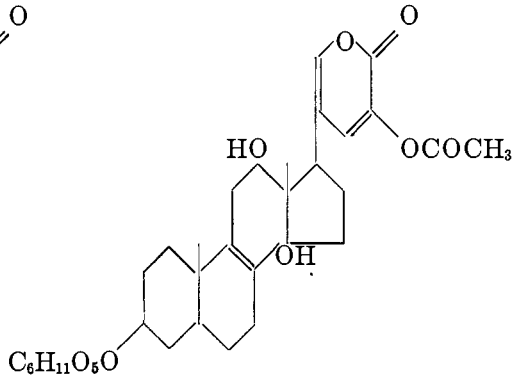
Both neriantin and adynerin are devoid of cardiotonic activity, a fact which suggests the possibility that these substances may be artifacts derived from other glycosides.

D. SCILLAREN A AND SCILLIROSIDE

As a result of the extensive investigations of Stoll and his collaborators (165–171) structures LIII and LIV have been tentatively assigned to scillaren A and to scilliroside. The chemistry of these compounds has been reviewed by



LIII
Scillaren A
(Stoll, 1941)



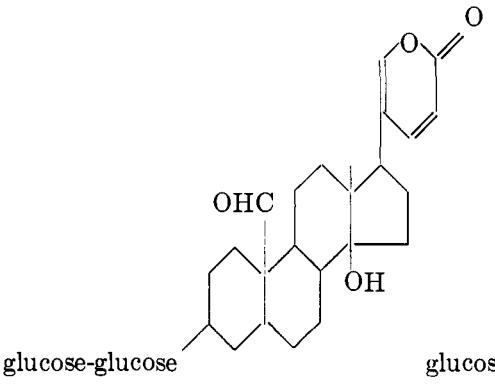
LIV
Scilliroside
(Stoll, 1943)

Reichstein and Reich (124), Shoppee (151), and Fieser (19b).

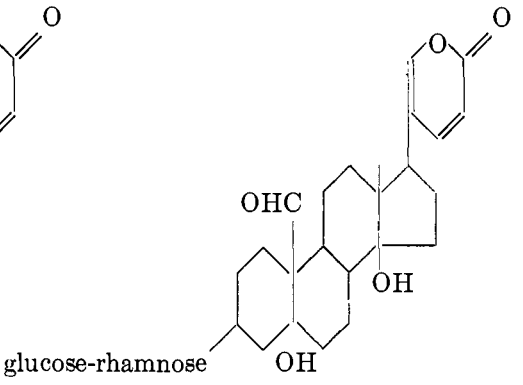
E. HELLEBRIN

For hellebrin, a highly active glycoside obtained from *Helleborus niger* L., W. Karrer has proposed structure LV (62). Like scillaren A and scilliroside, hellebrin shows an absorption band with maximum intensity at 300 mμ ($\log \epsilon = 3.8$) characteristic of the α-pyrone side chain. When the glycoside linkage is split by drastic hydrolysis with aqueous or aqueous-alcoholic sulfuric acid, an amorphous product derived from the aglycone is obtained as well as a sugar fraction from which glucose is the only component that can be isolated.

In the course of an investigation of milder conditions of cleavage, it was observed by Schmutz (147) that although Mannich's procedure (78) fails, owing to the extreme insolubility of hellebrin in acetone, a partial hydrolysis takes place in the presence of the enzyme strophanthobiase (59) from the seeds of *S. kombé*. One molecule of glucose is eliminated and a crystalline glycoside desglucohellebrin is formed, which differs from hellebrin in its relatively greater



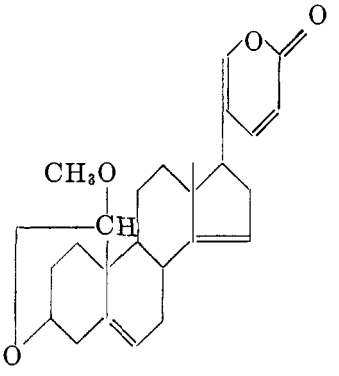
LV
Hellebrin
(W. Karrer, 1943)



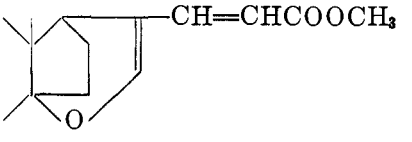
LVI
Hellebrin
(Schmutz, 1947)

solubility in acetone. On treatment with hydrogen chloride in acetone there are obtained two isomeric substances corresponding to the formula $C_{24}H_{32-34}O_6$ and a reducing sugar identified as rhamnose. The carbohydrate fraction therefore contains one oxygen atom less and the aglycone portion one oxygen atom more than Karrer proposed. These facts are expressed in formula LVI, which has been tentatively advanced by Schmutz.

The aldehydic character of hellebrin is inferred from the fact that on mild treatment with methanolic hydrogen chloride according to the method of Voss and Vogt (189) a compound $C_{25}H_{30-32}O_4$ is formed, which possesses a methoxyl group but no free hydroxyl groups and no active hydrogen (62). The



LVII



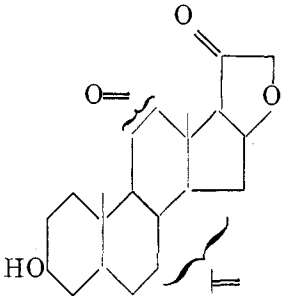
LVIII

ease with which methylation occurs is suggestive of the analogous behavior of strophanthidin (43). The preparation of a crystalline oxime confirms the above view, and the structure of the alcoholysis product is probably correctly represented by LVII.

On reaction with hot methanolic potassium hydroxide hellebrin yields isohellebrinic acid methyl ester, for which structure LVIII was suggested by Karrer.

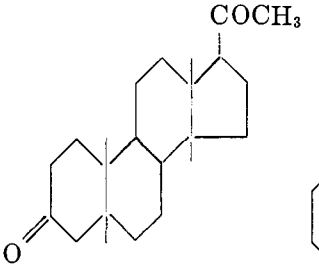
F. DIGININ

The inactive glycoside diginin, isolated from the leaves of *Digitalis purpurea* by W. Karrer (61), is easily hydrolyzed by the action of dilute mineral acid (154) and yields a sugar, diginose, isomeric with cymarose (155). The aglycone, diginigenin, has the formula $C_{21}H_{28}O_4$ and possesses strong reducing properties. Although the substance gives a positive Legal test with sodium nitroprusside, it does not contain the unsaturated lactone side chain characteristic of other cardiac genins, and on the basis of the following evidence the β-ketotetrahydrofuran structure LIX has been proposed for this compound by Shoppee (153).

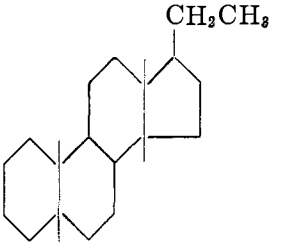


LIX

Diginigenin
(Shoppee, 1944)



LX



LXI

Diginigenin is readily converted into a monoacetate and a monosemicarbazone and thus contains a hydroxyl group and a carbonyl group in reactive positions. The presence of a —$COCH_2$— grouping is indicated by the formation of a piperonylidine derivative. Since diginigenin monoacetate is relatively stable to chromic acid, the presence of other primary or secondary hydroxyl groups is apparently excluded, whereas the absence of tertiary hydroxyl groups is inferred from the stability of the genin to hot aqueous-alcoholic sulfuric acid. Under energetic conditions diginigenin yields a diacetate for which an enol-acetate structure has been suggested.

Evidence for an olefinic linkage is drawn from a positive color reaction with tetranitromethane and from the formation of a tetrahydro derivative from diginigenin on hydrogenation. Tetrahydrodiginigenin gives no response with tetranitromethane, shows no reducing properties, and fails to yield a semicarbazone. Reduction of the reactive carbonyl group therefore accompanies

saturation of the double bond. Lack of selective absorption in the ultraviolet excludes the possibility that the double bond is located in a conjugated position.

Although tetrahydrodiginigenin does not form a semicarbazone, an oxime can be obtained under forcing conditions, and on the basis of this and other evidence the presence of a hindered carbonyl group has been postulated.

Neither diginigenin nor its tetrahydro derivative is attacked by periodic acid.

Wolff-Kishner reduction of diginigenin followed by hydrogenation affords a mixture from which three products can be isolated (153). One of these substances on oxidation with chromic acid yields 14-iso-17-isoallopregnane-3,20-dione (LX), identified by synthesis from methyl 3(β)-acetoxy-14-iso-17-iso-etioallocholanate (CI) (117). Further reduction of LX by the Wolff-Kishner procedure furnishes the hydrocarbon diginane (LXI), which is formulated as 14-iso-17-isoallopregnane (117; *cf.* 89). In view of the possibility of rearrangement in the Wolff-Kishner reaction the orientation assigned to C_{17} in this compound must be regarded as tentative, although certain observations of Meyer (88) would appear to indicate that the 17(α)-configuration is correct.

V. Miscellaneous Glycosides

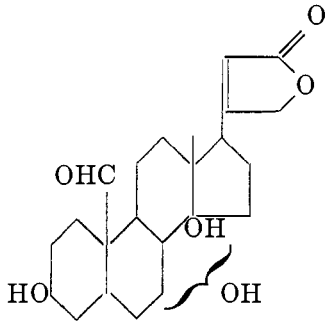
A. NEW GLYCOSIDES

1. Somalin

In 1940 Hartmann and Schlittler (30) isolated from the dried roots of *Adenium somalense* Balf. fil. an active principle which they called somalin. The substance was shown by hydrolysis to be a glycoside of cymarose and digitoxigenin.

2. Adonitoxin

Two new crystalline compounds have been obtained from *Adonis vernalis* (61, 125, 126). Of these substances one is only slightly active and appears to be an aglycone. The other is the glycoside adonitoxin, melting at 262–265°C. (dec.) and possessing activity intermediate between that of cymarin and convallatoxin. The substance shows maximum absorption at 218 mμ (log ϵ = 4.1), forms an oxime, and yields a carboxylic acid derivative on oxidation with chromic acid.



LXII

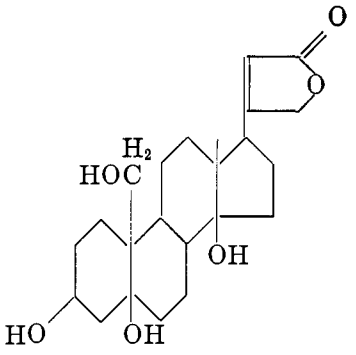
Adonitoxigenin

(Katz and Reichstein, 1947)

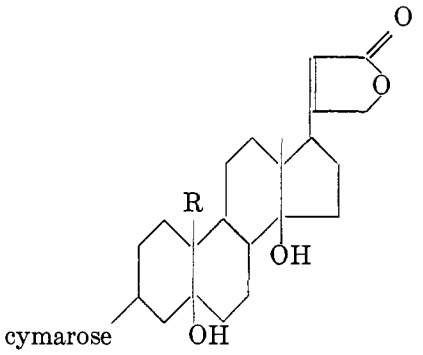
The glycoside linkage when split by hydrogen chloride in acetone yields rhamnose and, in poor yield, adonitoxigenin. The aglycone appears to contain one tertiary and two secondary hydroxyl groups. Formula LXII has been proposed for this genin by Katz and Reichstein (66).

3. Cymarol

A further investigation of the glycosides from *S. kombé* has disclosed the presence of a new substance, cymarol, isolated by chromatographic methods (3). Hydrolysis of the glycoside gives cymarose and a crystalline genin that has been identified as strophanthidol (LXIII), obtained also from strophanthidin by reduction with aluminum isopropoxide (118) or aluminum amalgam (69, 118).



LXIII



LXIV

LXIVa: R = CH_3 Periplocymarin

LXIVb: R = CH_2OH Cymarol

LXIVc: R = CHO Cymarin

LXIVd: R = COOH Cymarylic acid

Cymarol (LXIVb) is intermediate between periplocymarin (LXIVa) and cymarin (LXIVc) with respect to oxygenation at C_{19}, and it was of interest to prepare the fourth member of the series, cymarylic acid (LXIVd), in which carbon atom 19 is present as a carboxyl group. This product is obtained by chromic acid oxidation of cymarin acetate (4), and is considerably less active than the other three glycosides. Convallatoxin acetate has likewise been converted into convallatoxin acid (63), but no data on its physiological activity are available.

4. Convalloside

Related to convallatoxin is a new glycoside, convalloside, isolated from *Convallariae majalis* L. by Schmutz and Reichstein (149). The substance is converted into convallatoxin and glucose by the action of strophanthobiase.

5. *Cheirotoxin*

Schwarz, Katz, and Reichstein (150) have recently obtained a diglycoside of strophanthidin from *Cheiranthus cheiri* L. to which they give the name cheirotoxin. One of the sugar groups is glucose; the other may be a pentose or a methylpentose.

6. *Sarmentoside A and sarmentoside B*

Two substances, sarmentoside A and sarmentoside B, have been isolated from the seeds of *S. sarmentosus* by Schmutz and Reichstein (148). The aglycone obtained from sarmentoside A by cleavage with acetone and hydrogen chloride is probably isomeric with strophanthidin. The sugar component appears to be fucose. In addition to sarmentosigenin A there has been isolated a small amount of a compound, "substance C," that is perhaps an acetone derivative.

Mannich splitting of sarmentoside B gives sarmentosigenin B, which, on the basis of its elementary analysis, is isomeric with strophanthidol (LXIII). The sugar fraction consists of two components, glucose and digitalose.

B. SYNTHETIC GLYCOSIDES FROM NATURAL GENINS

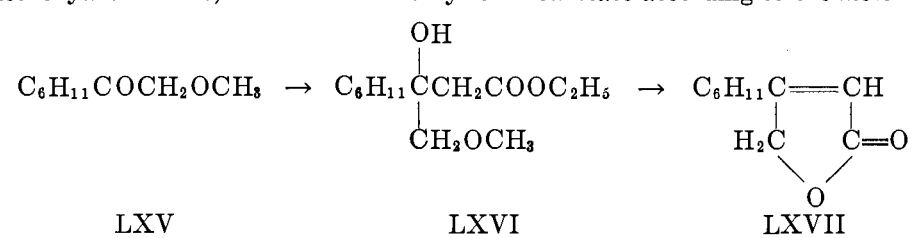
In addition to the synthetic glycosides of strophanthidin (188) that have been tabulated by Reichstein and Reich (124), three new synthetic glycosides derived from other genins have been reported (17). These are digitoxigenin-(3)-β-*d*-glucoside, digoxigenin-(3)-β-*d*-glucoside, and periplogenin-(3)-β-*d*-glucoside. All are prepared by condensation of the aglycones with acetobromoglucose (70), a procedure that is known to give β-glucosides. Attachment of the sugar residue at C_3 is likely, owing to the low reactivity of the hydroxyl groups at C_5, C_{12}, and C_{14}.

The three substances thus obtained are more potent than the natural glycosides digitoxin, digoxin, and periplocymarin (10).[8]

IV. Synthesis of Cardiac Genins

A. LACTONE SIDE CHAIN

In 1940 a method for the preparation of β-substituted α,β-butenolides was reported by Elderfield and his collaborators (23). Methoxymethyl cyclohexyl ketone (LXV), obtained by the reaction of cyclohexylmagnesium bromide and methoxyacetonitrile, is treated with ethyl bromoacetate according to the Refor-

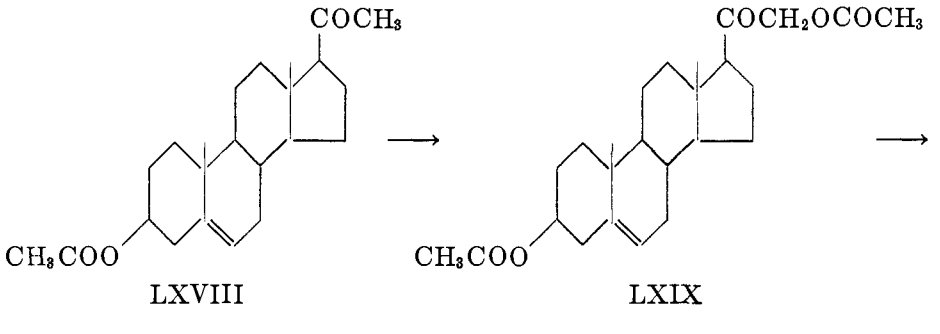


LXV LXVI LXVII

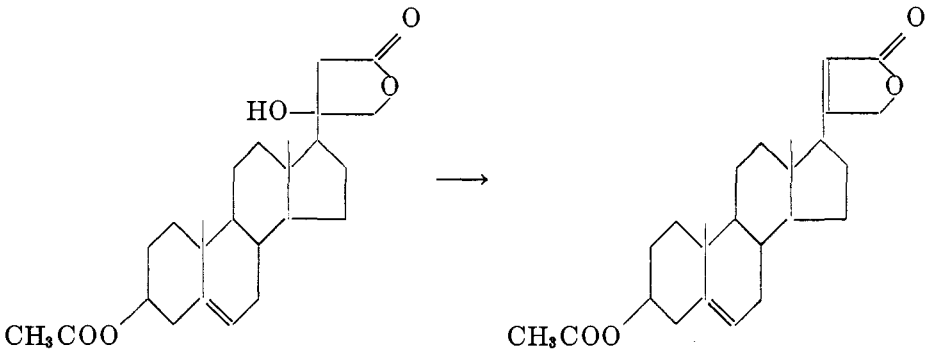
[8] Pharmacological data for a number of glycosides and genins are to be found in references 6, 8, 9, 10, and 93.

matsky procedure and gives the β-hydroxy ester LXVI. This product when heated with hydrogen bromide undergoes cyclization and dehydration and furnishes the unsaturated lactone LXVII.

Inasmuch as the halides required as starting material for this synthesis are not readily available in the steroid series, the use of acetoxymethyl or benzoxymethyl alkyl ketones rather than the methoxymethyl ketones was investigated (75, 76, 144). These substances are easily obtained from the appropriate etio acids through the acid chlorides and diazoketones (162), or in certain cases from the corresponding methyl ketones with lead tetraacetate (14, 123). Thus 3(β)-acetoxy-Δ⁵-pregnen-20-one (LXVIII) with lead tetraacetate yields 3(β), 21-diacetoxy-Δ⁵-pregnen-20-one (LXIX) (123), which has been converted into 3(β)-acetoxy-Δ⁵-etiocholenylbutenolide (LXXI) by Ruzicka, Reichstein, and



LXVIII LXIX

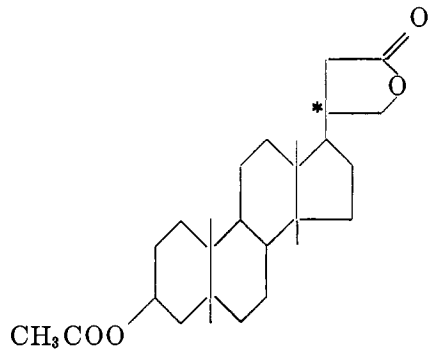


LXX LXXI

Fürst (144). Small amounts of the intermediate hydroxy lactone (LXX) can be isolated from the reaction mixture, but the main product is LXXI.

Starting with etiocholanic acid and 3(β)-hydroxyetiocholanic acid, the Columbia investigators (76) prepared the corresponding ketol acetates by the acid chloride-diazoketone route. After treatment with bromoacetic ester and zinc these compounds give, respectively, etiocholanylbutenolide and 3(β)-hydroxyetiocholanylbutenolide. The strong positive nitroprusside (Legal) test and the ultraviolet absorption ($\lambda_{max.} = 217$ mμ, $\log \epsilon = 4.3$) exhibited by these lactones are characteristic of the natural cardiac aglycones.

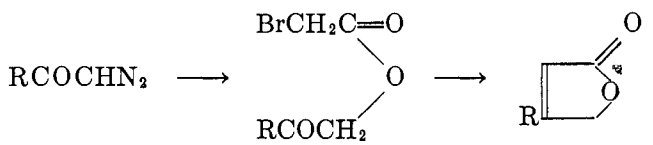
Hydrogenation of LXXI with a platinum catalyst in acetic acid solution gives a mixture from which two isomeric saturated lactones (LXXIIa and LXXIIb) have been isolated (131). Inasmuch as hydrogenation of Δ^5-compounds with few exceptions leads exclusively to compounds of the cholestane series (122, 193), the isomerism must involve the new center of asymmetry at C_{20}. The



LXXIIa: m.p. 203–204°C., $[\alpha]_D = +19°$
LXXIIb: m.p. 243°C., $[\alpha]_D = +5.9°$

lactones closely resemble in melting point and specific rotation two saturated lactones obtained by hydrogenation of α-anhydrouzarigenin acetate (174), for which formulas LXXIIa and LXXIIb have also been suggested. These are α_1-tetrahydroanhydrouzarigenin acetate (m.p. 205°C., $[\alpha]_D = +20.2°$) and α_2-tetrahydroanhydrouzarigenin acetate (m.p. 248°C., $[\alpha]_D = +3.9°$), and although a direct comparison between the two series was unfortunately not possible, the identity of the compounds in question is probable. With this reservation the above observations provide evidence for the β-configuration assigned to the C_3-hydroxyl group of uzarigenin (133) (see page 6).

A recent modification of the Reformatsky synthesis was introduced by Plattner and Heusser (99), who treated the intermediate diazoketone LXXIII with bromoacetic acid and obtained the bromoacetate LXXIV. This substance fails to give the unsaturated lactone (LXXV) in the presence of zinc alone, but does

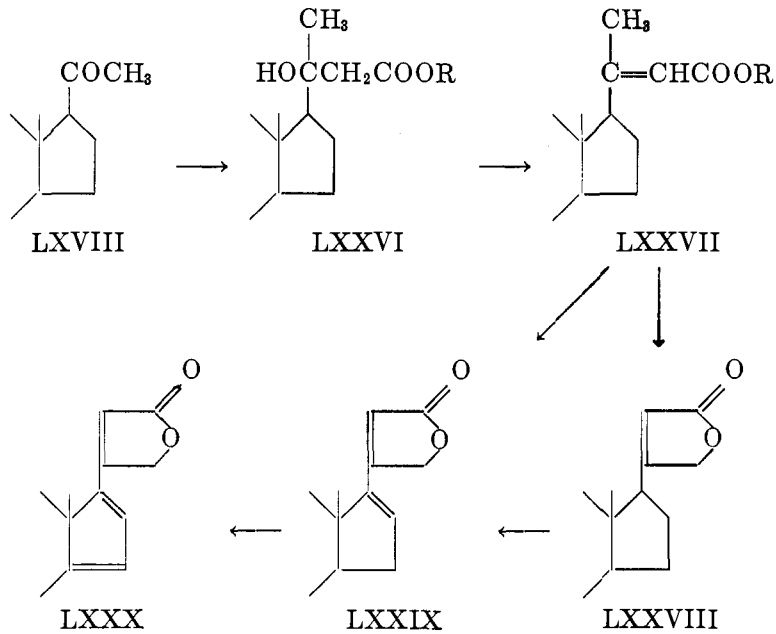


so on the addition of ethyl bromoacetate. The mechanism of the conversion of LXXIV into LXXV is therefore not necessarily intramolecular.

The modified procedure gives a somewhat better yield of a more easily purified product than that obtained by the usual method.

Another approach to the preparation of the unsaturated lactone side chain was developed on model compounds by Torrey, Kuck, and Elderfield (173), and was

later extended by Ruzicka, Plattner, and Pataki (141) to the preparation of certain unsaturated steroid lactones. 3(β)-Acetoxy-Δ⁵-pregnen-20-one (LXVIII) was treated with ethyl bromoacetate and zinc, and the resulting 20-hydroxy norester LXXVI, after saturation of the double bond at C_5:C_6, was dehydrated with acetic anhydride. The product (LXXVII) when heated



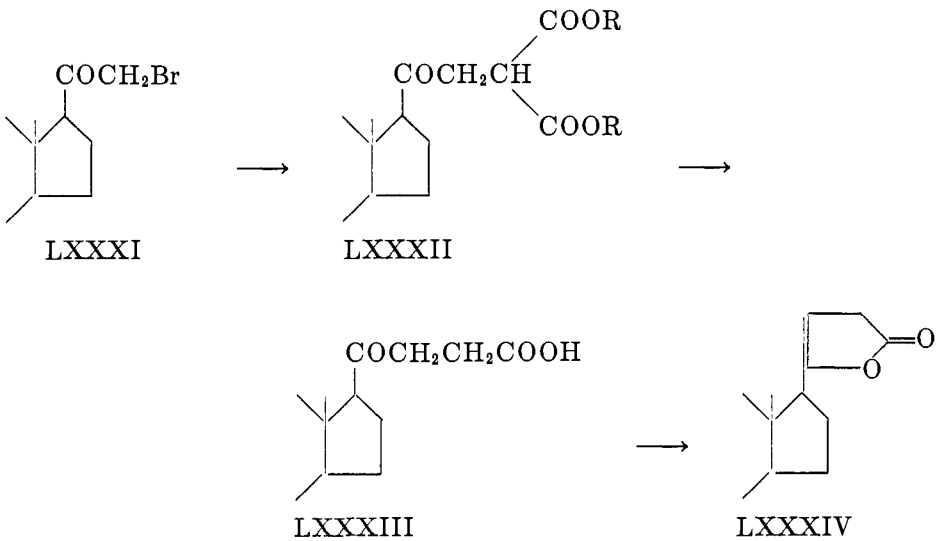
with selenium dioxide gives 3(β)-acetoxyetioallocholanylbutenolide (LXXVIII) directly, though in rather poor yield. If *N*-bromosuccinimide is used in place of selenium dioxide (143), LXXVIII cannot be isolated, but instead there is obtained in about 10 per cent yield a new product ($\lambda_{max.}$ = 273 mμ, log ε = 4.4) that has been identified as LXXIX by an independent synthesis from 3(β), 21-diacetoxy-Δ¹⁶-allopregnen-20-one (101, 137). The reaction of LXXVIII with *N*-bromosuccinimide also furnishes LXXIX (137), which on further treatment with this reagent is converted into the triply unsaturated lactone LXXX (100). The ultraviolet absorption of the latter product ($\lambda_{max.}$ = 333 mμ, log ε = 4.32) corresponds to that of dianhydrogitoxigenin (XXIII) (180).

By application of the methods described above α,β-butenolides substituted in the β-position by the following groups have been prepared: cyclohexyl (75), 4-hydroxycyclohexyl (28, 29), 3,4-dihydroxycyclohexyl (27), phenyl (75, 173), *p*-hydroxyphenyl (82), *m*-hydroxyphenyl (82), decahydro-β-naphthyl (68), 6-hydroxy-2-naphthyl (68), 1-hydrindanyl (68), etiocholanyl (76), 3(α)-hydroxyetiocholanyl (130), 3(β)-hydroxyetiocholanyl (76), 3(α),12(α)-dihydroxyetiocholanyl (142), 3(α),7(α),12(α)-trihydroxyetiocholanyl (34), etioallocholanyl (106), Δ²-etioallocholenyl (97), 3(α)-hydroxyetioallocholanyl (106), 3(β)-hydroxyetioallocholanyl (106, 132, 137), 3(β)-hydroxy-Δ¹⁶-etioallocholenyl

(137, 143), 2,3-dihydroxyetioallocholanyl (97), 3(β),5-dihydroxyetioallocholanyl (138), 3(β)-hydroxy-Δ⁵-etiocholenyl (99, 131, 144), 3(β)-hydroxyetioallocholanylmethyl (98), 3(β)-hydroxy-Δ⁵-etioallocholenylmethyl (98), norcholanyl (67), 3(β)-hydroxy-Δ⁵-norcholenyl (99, 134), and 3(α),7(α),12(α)-trihydroxynorcholanyl (67, 135). Allouzarigenin has also been prepared (109).

Two derivatives that are further substituted by a methyl group in the α-position of the lactone ring have been synthesized (136). These are β′-(3(β)-hydroxy-Δ⁵-etiocholenyl-17)-α′-methyl-$\Delta^{\alpha',\beta'}$-butenolide and β′-(3(β)-hydroxy-Δ⁵-norcholenyl-23)-α′-methyl-$\Delta^{\alpha',\beta'}$-butenolide.

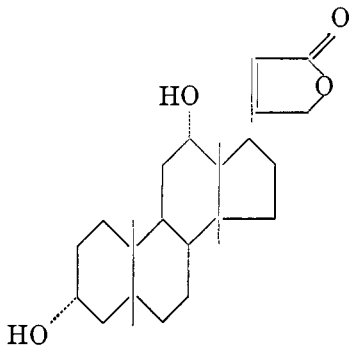
The synthesis of two γ-substituted β,γ-butenolides has also been carried out (102) for which purpose 3(β)-hydroxy-21-bromo-Δ⁵-pregnen-20-one (LXXXI) serves as the starting material. Treatment of this substance with sodiomalonic



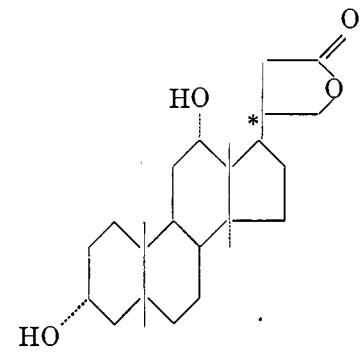
ester gives LXXXII, which on decarboxylation is converted into the γ-keto acid LXXXIII. The latter compound yields the β,γ-unsaturated lactone LXXXIV with acetic anhydride and acetyl chloride. Hydrogenation of the 5,6-double bond of LXXXIII prior to lactonization affords the corresponding γ′-(3(β)-hydroxyetioallocholanyl)-β′,γ′-butenolide. The ultraviolet absorption spectra of both products are consistent with the proposed structures.

Two of the synthetic lactones listed above, 3(β)-hydroxy-Δ⁵-etiocholenylbutenolide and 3(β)-hydroxy-Δ⁵-norcholenylbutenolide, have been converted into the corresponding glucosides (91, 115). The maltoside of the former has likewise been prepared (91).

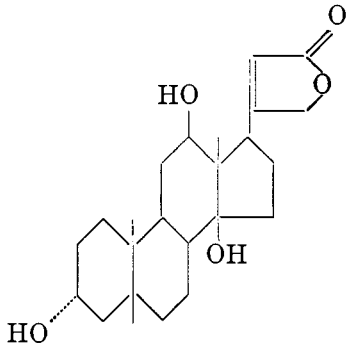
The following reactions have been carried out with etiodesoxycholylbutenolide (LXXXV) and provide additional correlation of the synthetic lactones with those of natural origin (113). Hydrogenation of LXXXV gives a mixture from which two epimeric saturated lactones (LXXXVIa and LXXXVIb) have been



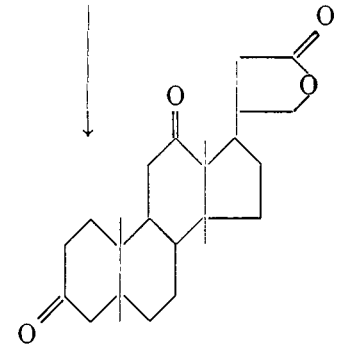
LXXXV



LXXXVI (a and b)



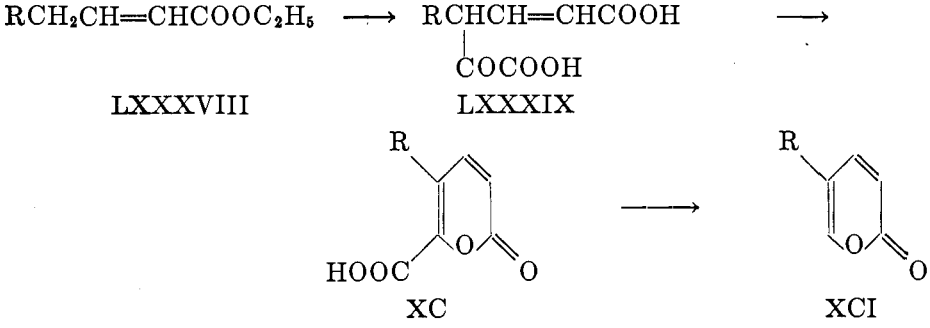
II



LXXXVII

isolated. One of these on oxidation yields a diketone identical with tetrahydroanhydrodigoxigenone (LXXXVII) from digoxigenin (II) (181).

The synthesis of certain 5-substituted α-pyrones analogous to the side-chain structures of scillaren A and hellebrin has been investigated by Fried and Elderfield (22; *cf.* 23). The alkyl crotonic ester (LXXXVIII) is condensed with ethyl oxalate, and after hydrolysis the ketodicarboxylic acid (LXXXIX) is obtained. Cyclization of LXXXIX takes place under the influence of hydrogen bromide in acetic acid, and the resulting 5-alkyl-6-carboxy-α-pyrone (XC) affords the 5-alkyl-α-pyrone (XCI) on decarboxylation with copper powder.



LXXXVIII LXXXIX

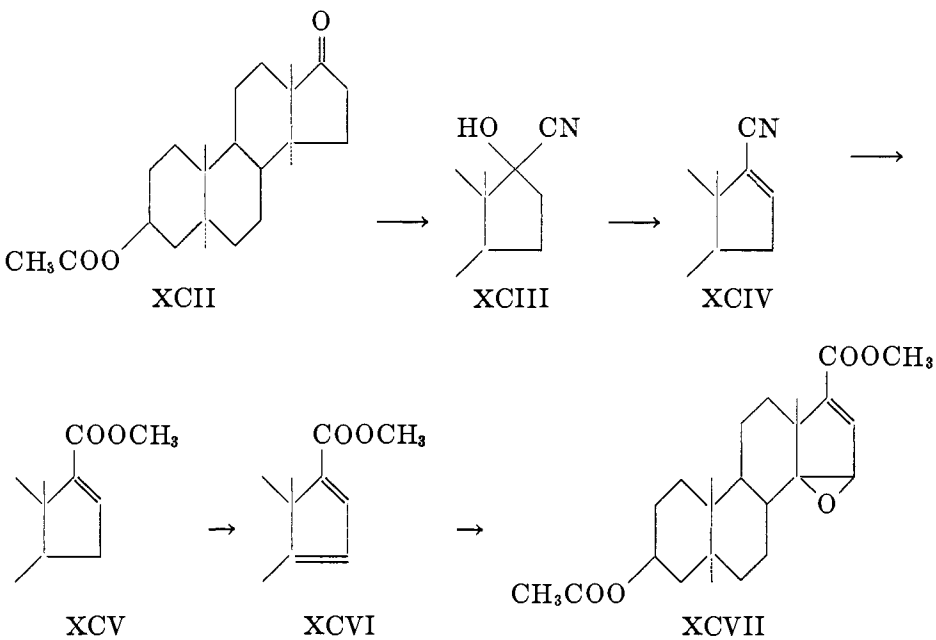
XC XCI

The synthesis is successful when R is a methyl or ethyl group, and the absorption spectra of these products ($\lambda_{max.}$ = 300 mμ, log ϵ = 3.7) closely resemble those of scillaren A and hellebrin. The method fails, however, when R is a cyclohexyl group. Attempts to effect suitable conversions from α-pyrone-5-carboxylic acid have likewise been unsuccessful.

B. C_{14}-HYDROXYL GROUP

For the introduction of a hydroxyl group at C_{14}, compounds possessing a conjugated system extending to this point (*cf.* LXXX) have been employed by Ruzicka *et al.* (111, 140). Preliminary experiments were carried out with methyl 3(β)-acetoxy-$\Delta^{14,16}$-etioallocholadienate (XCVI), which is readily available from 3(β)-acetoxyandrostan-17-one (190) by the transformations XCII → XCVI. The last step in this series is accomplished with *N*-bromosuccinimide and furnishes the doubly unsaturated ester XCVI ($\lambda_{max.}$=292 mμ, log ϵ = 4.2) in 77 per cent yield.

XCVI reacts with only 1 mole of perbenzoic acid and gives an oxide to which the 14,15-epoxy structure XCVII is assigned on the basis of an absorption maximum at 233 mμ, log ϵ = 3.86 (*cf.* 172). Hydrogenation of this product in the presence of palladium yields a saturated epoxy ester (XCVIII), but when

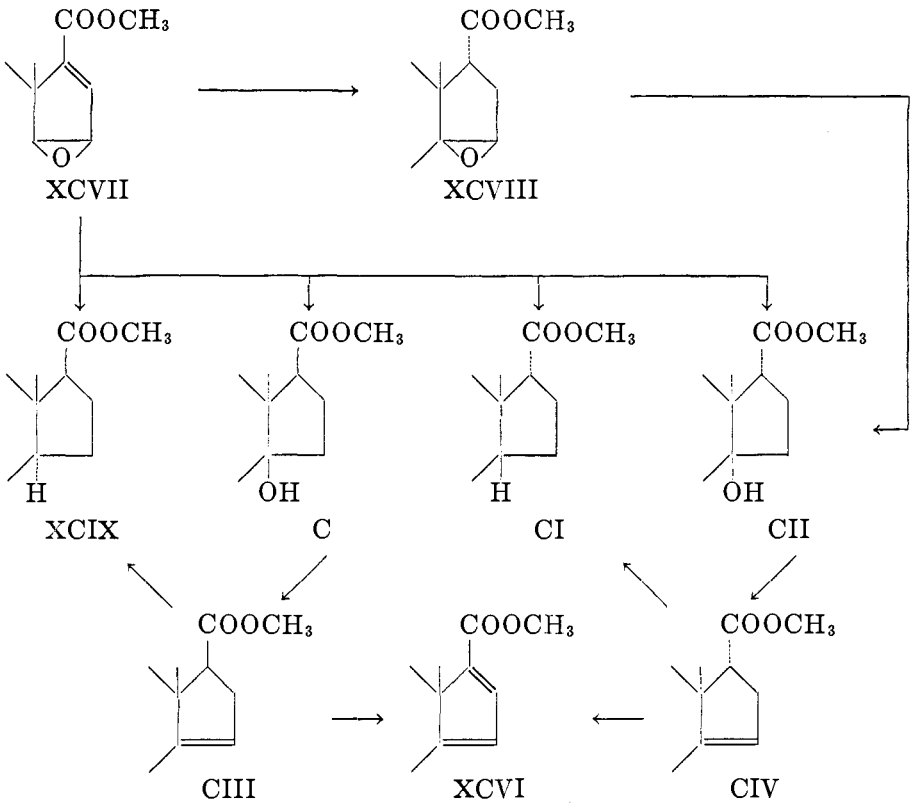


XCII XCIII XCIV XCV XCVI XCVII

platinum is used as the catalyst, rupture of the oxide ring takes place in addition to reduction of the olefinic linkage. From the hydrogenation mixture four products, XCIX, C, CI, and CII, have been separated by chromatographic methods. Two of these products, C and CII, contain hydroxyl groups that are resistant to acetylation and to oxidation with chromic acid and must therefore be located at

the tertiary position C_{14}. The two remaining products, XCIX and CI, were shown by analysis to have suffered the loss of an oxygen atom, and of these XCIX was identified as methyl 3(β)-acetoxyetioallocholanate.

In order to establish the configurations of the hydroxy esters C and CII, the centers of asymmetry at C_{14} were first eliminated by dehydration with phosphorus oxychloride and pyridine. The unsaturated esters CIII and CIV formed in this way are isomers, and since both are easily reduced it is assumed that in each the unsaturated linkage occupies the 14,15-position. The isomerism must therefore involve the configuration at C_{17}, a view that is confirmed by the conversion of

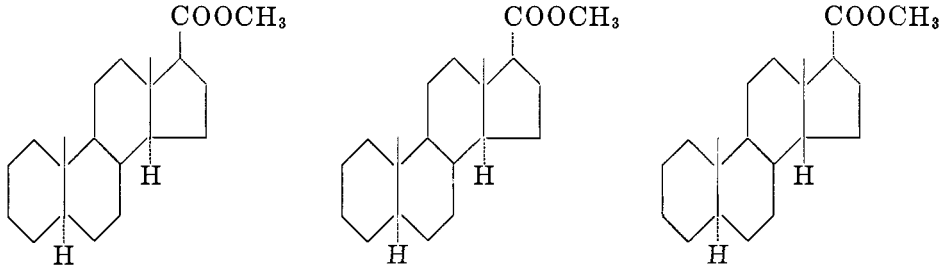


both substances into XCVI, in which asymmetry at this point is absent. Inasmuch as hydrogenation of CIII gives XCIX, for which the β-arrangement at C_{17} is established, assignment of the β-configuration to CIII and C and the α-configuration to CIV and CII as well as to CI, the reduction product of CIV, is undoubtedly correct.

CI, however, differs from the known methyl 3(β)-acetoxy-17-isoetioallocholanate (157), and replacement of the oxygen at C_3 by hydrogen affords an ester (CVII) that is not identical with methyl 17-isoetioallocholanate (CVI) (18). It is therefore concluded that the non-identity of the compounds in question is the

result of a difference in spatial arrangement at C_{14}, and that CI has a *cis* C/D ring fusion.[9]

Evidence that may support the above formulation has been drawn from a study of the rates of hydrolysis of the three known methyl etioallocholanates CV (176, 177), CVI (18), and CVII (112), of which the latter is derived from CI. From an inspection of molecular models, it can be predicted (33) that, in terms



CV CVI CVII

of hindrance of the ester groups, the 14-iso-17-iso compound (CVII) should resemble methyl etioallocholanate (CV), which is less hindered than methyl 17-isoetioallocholanate (CVI). This prediction has been tested experimentally by measurements of the per cent of ester hydrolyzed in 1 hr. under standardized conditions. The results obtained with the three esters are as follows: CV, 48.3 per cent and 44.1 per cent; CVI, 17.8 per cent; CVII, 48.6 per cent. The fourth member of the series, methyl 14-isoetioallocholanate, is unknown but should be comparable to CV and CVII rather than to CVI.

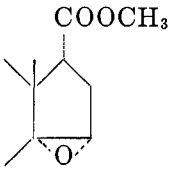
With regard to the configurations of the tertiary hydroxyl groups in C and CII, it can be assumed that these groups are similarly oriented since both are derived from the same oxide (XCVII). In each case dehydration can be accomplished with phosphorus oxychloride in pyridine, but whereas this reaction occurs quantitatively at room temperature with CII, higher temperatures are required for the elimination of water from C (110). It is apparent from a study of models of the four possible hydroxy esters epimeric at C_{14} and C_{17} that hindrance of the hydroxyl group is considerably greater in the two products in which this group is *cis* to the side chain at C_{17} than in the corresponding *trans* compounds. It follows from this argument that the arrangement of the substituents at C_{14} and C_{17} is *trans* in CII and *cis* in C. Since for the latter compound the β-orientation of the side chain has been established by the conversion of C into XCIX, the β-configuration must likewise be assigned to the hydroxyl group at C_{14}. This evidence also implies the β-configuration for the tertiary hydroxyl group in CII.

Although no lactone has been obtained from the free acid of C, such a lactonization would appear to be excluded by the blocking effect of the angular methyl

[9] The influence of the configuration at C_{17} on the steric course of hydrogenation of the 14,15-double bond has also been observed by Speiser and Reichstein (159) and by Meyer (85, 87). It is of interest in this connection that hydrogenation of XCVI yields both XCIX and CI, but not the corresponding 14-normal-17-iso or 14-iso-17-normal compounds, a result that suggests a 1,4-*cis* addition of hydrogen at C_{14} and C_{17} (*cf.* 74).

group at C_{13}. A similar phenomenon has been observed by Sorkin and Reichstein (158) in the case of 12-epietiodesoxycholic acid (III).

Inasmuch as hydrogenation of other steroid oxides gives rise to hydroxy compounds of the same stereochemical series as the oxides from which they are derived (25, 77, 95, 103, 116), the oxide bridge of XCVII is undoubtedly β-oriented. The formation of α-oxides, however, has been observed in the reaction of peracids with Δ^{14}-monounsaturated esters. CIV, for example, gives a product CVIII that differs from XCVIII and that is not subject to hydrogenolysis with platinum in either alcohol or acetic acid (110).



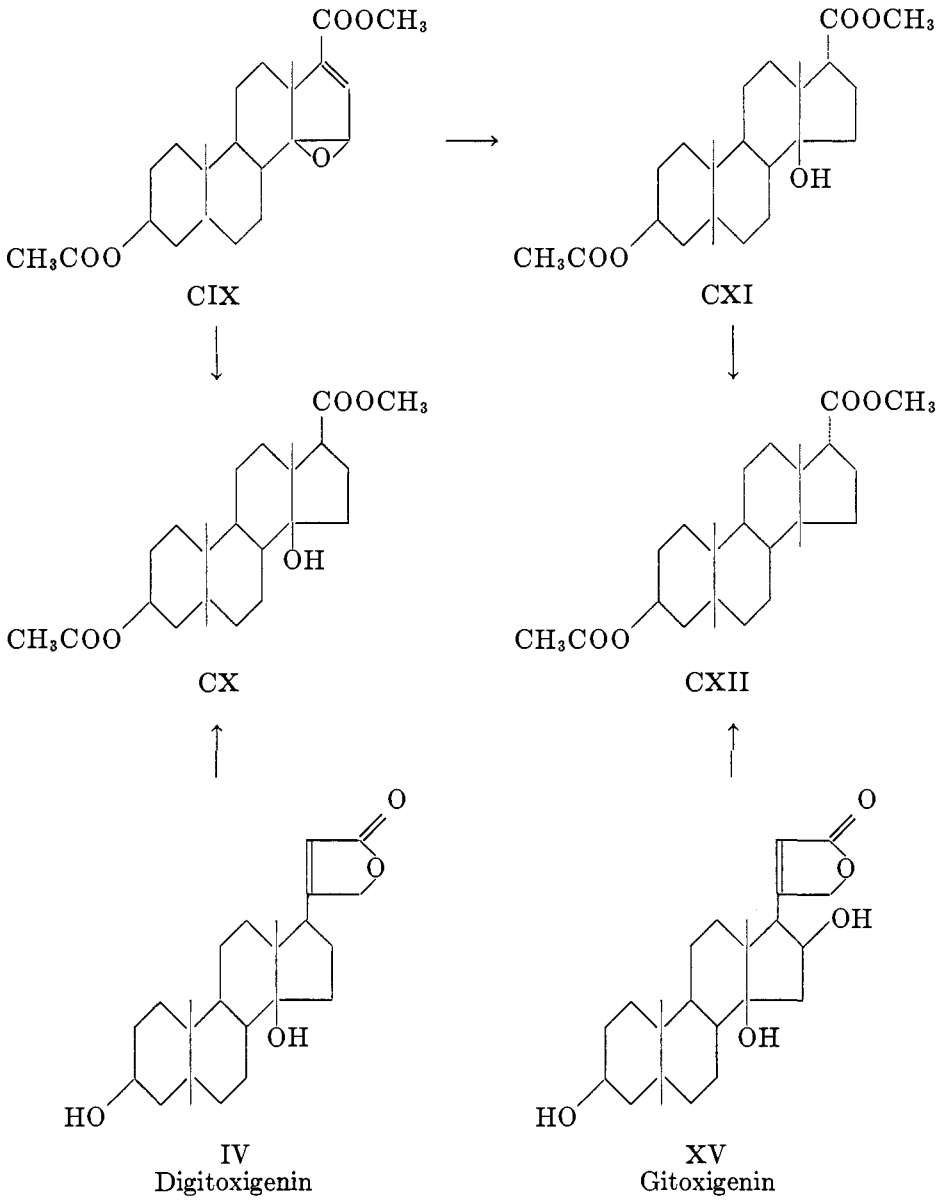
CVIII

It was shown in an earlier section (page 4) that the C_{14}-hydroxyl group of the natural cardiac aglycones has the β-configuration. By hydrogenation of methyl 3(β)-acetoxy-14(β),15(β)-epoxy-Δ^{16}-etiocholanate (CIX) Ruzicka and his associates (139) have for the first time obtained by synthetic means an aglycone derivative possessing an intact hydroxyl group at C_{14}. From the mixture of products obtained by hydrogenation of CIX, two hydroxy esters (CX and CXI) have been isolated whose structures are analogous to those of C and CII. Of these, CX is identical with a degradation product of digitoxigenin (IV) (36). The other product (CXI) on dehydration and hydrogenation gives the ester CXII, that differs from methyl 3(β)-acetoxyetiocholanate (120) and methyl 3(β)-acetoxy-17-isoetiocholanate (157), but is identical with the reduction product of dianhydrogitoxigenin acetate from gitoxigenin (XV).

The methods described above for the introduction of a hydroxyl group at C_{14} have been extended to the preparation of certain 14-hydroxy-20-keto derivatives for which the unsaturated ketone CXIII serves as starting material (107). This substance is obtained by the conversion of 3(β)-acetoxyallopregnan-20-one into 3(β)-acetoxy-Δ^{16}-allopregnen-20-one (80), followed by treatment of the latter compound with *N*-bromosuccinimide (107). A better method consists in the reaction of the unsaturated nitrile XCIV with *N*-bromosuccinimide and conversion of the doubly unsaturated product into CXIII by the action of methylmagnesium bromide (104).

CXIII with monoperphthalic acid furnishes the oxide CXIV, which, after hydrogenation with a palladium catalyst and regeneration of the C_{20}-keto group by chromic acid oxidation, gives the hydroxy ketone CXV in about 50 per cent yield. Small amounts of an isomer (CXVIII) and of the two desoxy compounds CXVI and CXVII[10] are also obtained.

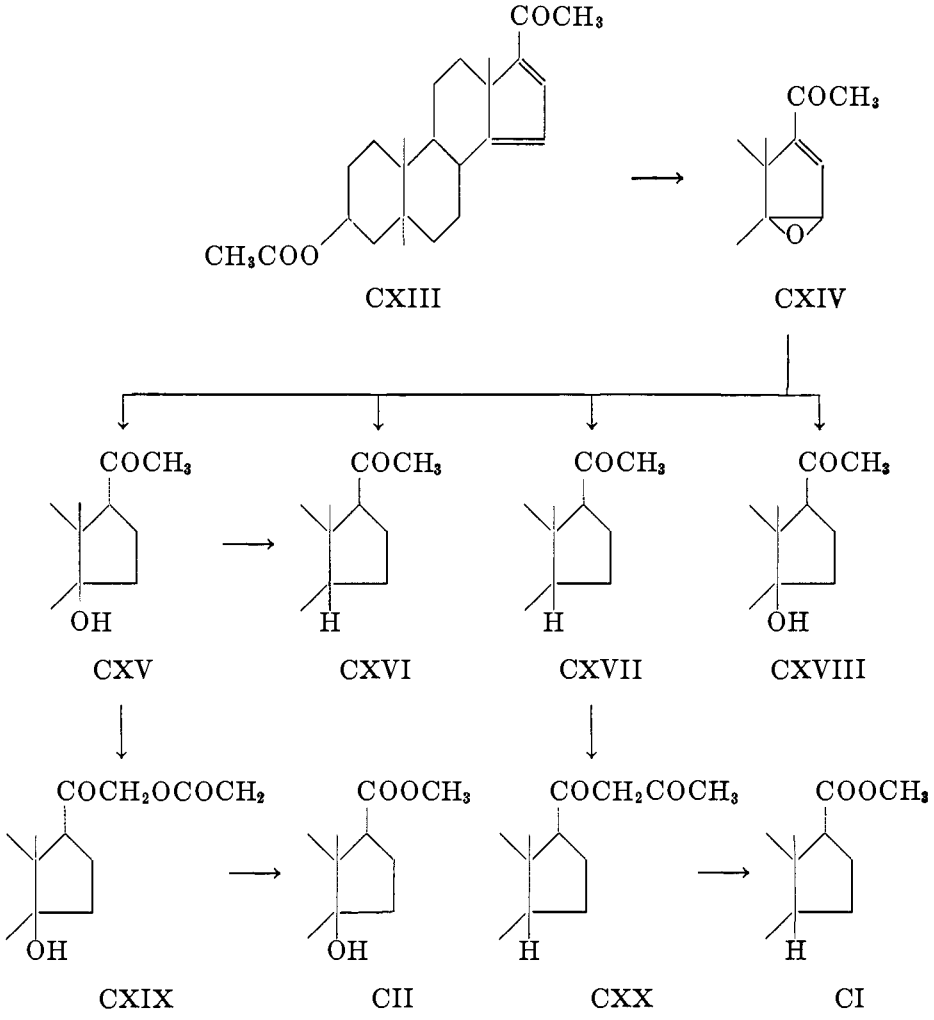
[10] This substance is the main product when the hydrogenation of CXIV is carried out with platinum instead of palladium.



The structures assigned to CXV and CXVII are based on the reactions of these compounds with lead tetraacetate to give the ketol acetates CXIX and CXX, which yield methyl 3(β)-acetoxy-14(β)-hydroxy-17-isoetioallocholanate (CII) and methyl 3(β)-acetoxy-14-iso-17-isoetioallocholanate (CI), respectively, on further oxidation with periodic acid (107, 108).

Although dehydration and hydrogenation of CXV give 3(β)-acetoxyallopreg-

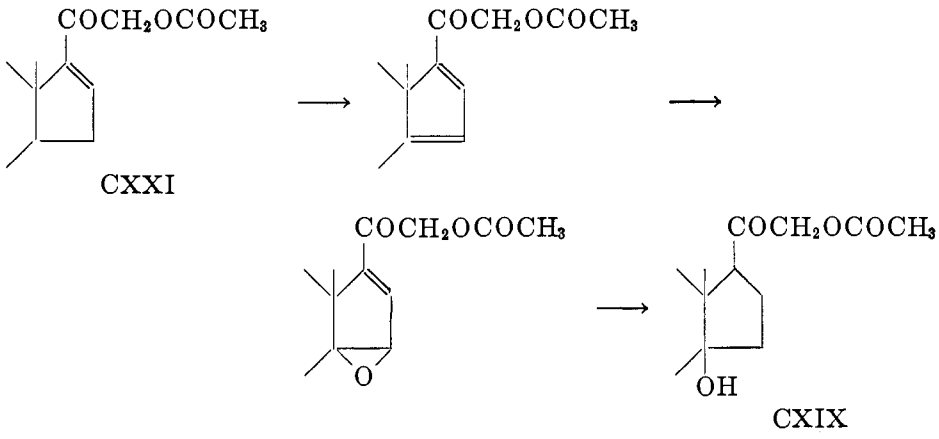
nan-20-one (CXVI) in which the β-configuration at C_{17} is known, it is unlikely that CXV can have a β-arrangement at this point, in view of its conversion under



mild conditions into the 17(α)-ester CII. The 17(α)-orientation of CXV is further supported by evidence drawn from a comparison of specific rotations, and it must therefore be assumed that a rearrangement of the side chain of CXV occurs during the dehydration.

Only very small amounts of CXVIII are obtained by the procedure described above, and its formulation is based on analogy to the earlier work (110).

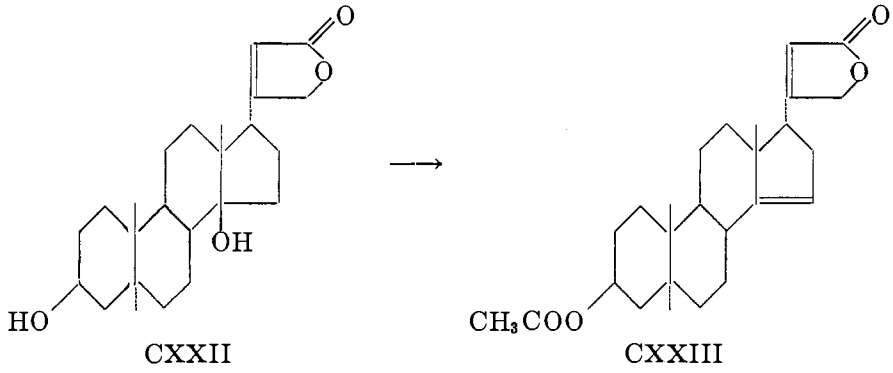
The ketol acetate CXIX, which has also been obtained by the transformations CXXI $\rightarrow$ CXIX (108), possesses the grouping at C_{17} required for elaboration of



CXXI

CXIX

an unsaturated lactone side chain. Treatment of this substance with bromoacetic ester in the presence of zinc affords the lactone CXXII in a yield of about 17 per cent (109). The product possesses all the structural features that have



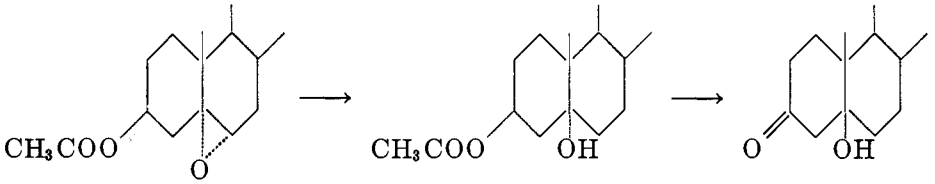
CXXII CXXIII

been attributed to the aglycone uzarigenin, with the exception of the α-orientated side chain at C_{17}. In this respect CXXII resembles allostrophanthidin and alloperiplogenin, and it is therefore called allouzarigenin. Since uzarigenin itself is not known, the synthetic lactone (CXXII) was dehydrated for comparison with material of natural origin. The anhydro derivative (CXXIII), as expected, differs from both the α- and β-anhydrouzarigenins of Tschesche (174). The difference in specific rotation between CXXII and CXXIII is similar to that observed between the allogenins and their anhydro derivatives and is considerably greater than the corresponding rotational differences in the normal series.

c. C_5-HYDROXYL GROUP

In strophanthidin, periplogenin and other related aglycones the presence of a 5(β)-hydroxyl group has been established (page 9). The hydrogenation of 4,5- and 5,6-oxides has been explored as a route to compounds of this type.

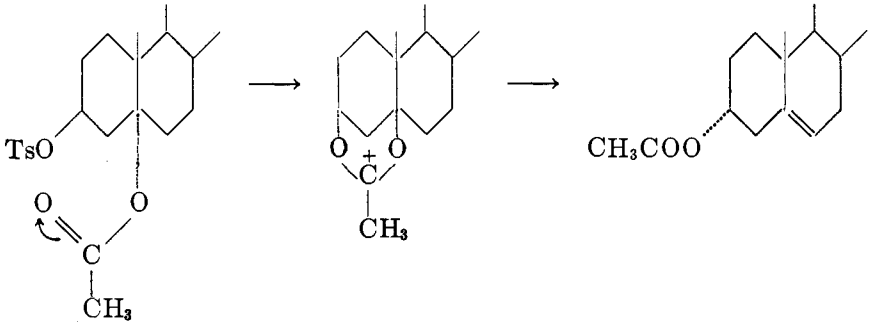
Reduction of α-cholesterol oxide acetate (CXXIV)[11] with platinum in acetic acid proceeds with the absorption of 1 mole of hydrogen and gives in good yield a compound (CXXV) that contains a free hydroxyl group (105). In the presence of chromic acid CXXV forms a golden yellow chromate ester but is otherwise unattacked, whereas saponification and oxidation of CXXV gives the hydroxy ketone CXXVI, which is easily converted into Δ^4-cholesten-3-one by loss of water. This evidence establishes C_5 as the location of the new hydroxyl group, and since a rupture of the bond from C_5 to oxygen is not involved in the



CXXIV CXXV CXXVI

formation of CXXV from CXXIV, the α-configuration is assigned to the C_5-hydroxyl group. This arrangement is opposite to that found in the natural cardiac genins.

Under energetic conditions (dimethylaniline–acetyl chloride) CXXV yields a 3,5-diacetate, which gives 3(β)-hydroxy-5-acetoxycholestane on partial saponification. The transformation of the latter compound into epicholesteryl acetate (CXXVIII) on heating with *p*-toluenesulfonyl chloride and pyridine provides additional evidence for the α-orientation of the hydroxyl group at C_5, in view of the steric requirements of the intermediate CXXVII.[12]



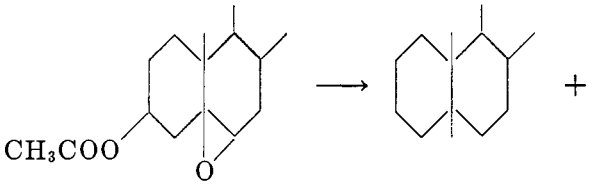
CXXVII CXXVIII

The hydrogenation of β-cholesterol oxide acetate (CXXIX)[11] takes place with the absorption of somewhat more than 1 mole of hydrogen and follows a different

[11] The trivial indices α and β were applied to the two oxides of cholesterol by Westphalen (191). It was subsequently shown that the so-called "β-oxide" is a molecular compound of a true β-oxide and the α-isomer (1, 31). The correctness of the designations α and β as applied to the true oxides has been established by Plattner and Lang (103). Of interest in this connection is the conversion of β-cholesterol oxide into the α-isomer by Fürst and Koller (24).

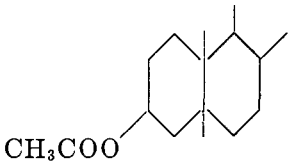
[12] The mechanism suggested for this reaction is based on the work of Winstein, Hess, and Buckles (201).

course from that observed for the α-oxide (CXXIV). A mixture of products is obtained from which cholestane (CXXX), cholestanyl acetate (CXXXI), and 3(β)-acetoxy-6(β)-hydroxycholestane (CXXXII) have been isolated. The lat-

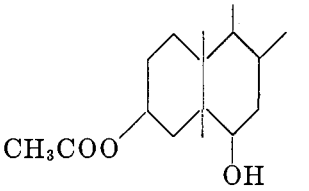


CXXIX

CXXX

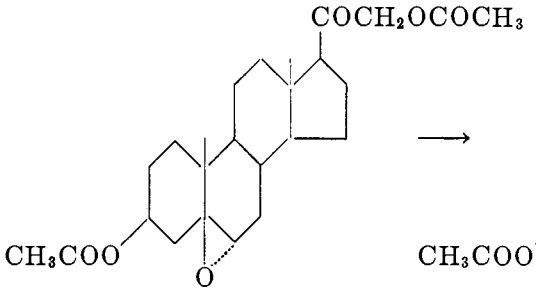


CXXXI

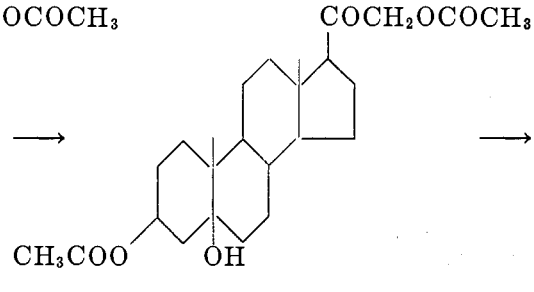


CXXXII

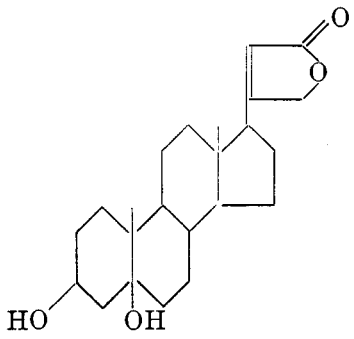
ter substance was converted into the known compounds 3(β),6(β)-diacetoxycholestane (81) and 6-ketocholestanyl acetate (194). The inversion of configuration at C_5 that accompanies the hydrogenation can perhaps be attributed to adsorption of the α-face of CXXIX on the catalyst surface (*cf.* 74).



CXXXIII



CXXXIV



CXXXV

Results similar to those described for the cholesterol oxides have been observed by Ruzicka and Muhr (129) in the reduction of the α- and β-oxides of dehydroisoandrosterone acetate. In addition, the reduction of two oxides of uncertain configuration has been investigated by Plattner, Petrzilka, and Lang (105). These compounds are 5,6-epoxycholestane, m.p. 80°C. (127), and 4,5-epoxycholestane, m.p. 96°C. (32). In both instances hydrogenation leads to a mixture of products from which a 5-hydroxy derivative has been isolated. The configuration of the hydroxyl group in this compound is unknown.

By the hydrogenation of 3(β),21-diacetoxy-5,6-epoxyallopregnan-20-one (CXXXIII) a 5(α)-hydroxy compound (CXXXIV) is obtained that on treatment with zinc and bromoacetic ester yields a lactone (CXXXV) with maximum absorption at 219 mμ, log ϵ = 4.3 (138). This substance differs from periplogenin in the absence of a hydroxyl group at C_{14} and in the configuration at C_5.

VII. References

(1) Baxter and Spring: J. Chem. Soc. **1943**, 613.
(2) Bloch and Elderfield: J. Org. Chem. **4**, 289 (1939).
(3) Blome, Katz, and Reichstein: Pharm. Acta Helv. **21**, 325 (1946).
(4) Blome and Reichstein: Pharm. Acta Helv. **22**, 235 (1947).
(5) Butenandt and Gallagher: Ber. **72**, 1866 (1939).
(5a) Buzas and Reichstein: Helv. Chim. Acta **31**, 84 (1948).
(6) Chen: Ann. Rev. Physiol. **7**, 681 (1945).
(7) Chen and Chen: J. Pharmacol. Exptl. Therap. **49**, 561 (1933); **51**, 23 (1934); J. Biol. Chem. **105**, 231 (1934).
(8) Chen, Chen, and Anderson: J. Am. Pharm. Assoc. **25**, 579 (1936).
(9) Chen and Elderfield: J. Pharmacol. Exptl. Therap. **76**, 81 (1942).
(10) Chen, Elderfield, Uhle, and Fried: J. Pharmacol. Exptl. Therap. **77**, 401 (1943).
(11) Dannenberg: *Über die Ultraviolettabsorption der Steroide.* Verlag der Akademie der Wissenschaften, Berlin (1940).
(12) Ehrenstein: J. Org. Chem. **9**, 435 (1944).
(13) Ehrenstein and Johnson: J. Org. Chem. **11**, 823 (1946).
(14) Ehrhardt, Ruschig, and Aumüller: Z. angew. Chem. **52**, 363 (1939); Ber. **72**, 2035 (1939).
(15) Elderfield: Chem. Revs. **17**, 187 (1935).
(16) Elderfield: J. Biol. Chem. **113**, 631 (1936).
(17) Elderfield, Uhle, and Fried: J. Am. Chem. Soc. **69**, 2235 (1947).
(18) Von Euw and Reichstein: Helv. Chim. Acta **27**, 1851 (1944).
(19) Fieser: *Chemistry of Natural Products Related to Phenanthrene*, 2nd edition, Reinhold Publishing Corporation, New York (1937): (a) p. 285; (b) p. 295; (c) p. 398.
(20) Fieser and Jacobsen: J. Am. Chem. Soc. **59**, 2335 (1937).
(21) Fieser and Newman: J. Biol. Chem. **114**, 705 (1936).
(22) Fried and Elderfield: J. Org. Chem. **6**, 566, 577 (1941).
(23) Fried, Rubin, Paist, and Elderfield: Science **91**, 435 (1940).
Rubin, Paist, and Elderfield: J. Org. Chem. **6**, 260 (1941).
Cf. Ranganathan: Current Sci. **9**, 458 (1940).
(24) Fürst and Koller: Helv. Chim. Acta **30**, 1454 (1947).
(25) Gallagher and Long: J. Biol. Chem. **162**, 495 (1946).
(26) Gallagher and Long: J. Biol. Chem. **162**, 521 (1946).
(27) Hardegger: Helv. Chim. Acta **29**, 1195 (1946).
(28) Hardegger, Heusser, and Blank: Helv. Chim. Acta **29**, 477 (1946).

(29) HARDEGGER, PLATTNER, AND BLANK: Helv. Chim. Acta **27,** 793 (1944).
(30) HARTMANN AND SCHLITTLER: Helv. Chim. Acta **23,** 548 (1940).
(31) HATTORI: J. Pharm. Soc. Japan **60,** 334 (1940); Chem. Abstracts **34,** 7294 (1940).
(32) HEILBRON, SHAW, AND SPRING: Rec. trav. chim. **57,** 529 (1938).
(33) HEUSSER, MEIER, AND RUZICKA: Helv. Chim. Acta **29,** 1250 (1946).
(34) HEUSSER AND WUTHIER: Helv. Chim. Acta **30,** 1460 (1947).
(35) HUEBNER, LOHMAR, DIMLER, MOORE, AND LINK: J. Biol. Chem. **159,** 503 (1945).
(36) HUNZIKER AND REICHSTEIN: Experientia **1,** 90 (1945); Helv. Chim. Acta **28,** 1472 (1945).
(37) JACOBS: J. Biol. Chem. **57,** 553 (1923).
(38) JACOBS: J. Biol. Chem. **88,** 519 (1930).
(39) JACOBS: Physiol. Revs. **13,** 222 (1933).
(40) JACOBS AND BIGELOW: J. Biol. Chem. **96,** 647 (1932).
(41) JACOBS AND BIGELOW: J. Biol. Chem. **99,** 521 (1933).
(42) JACOBS AND BIGELOW: J. Biol. Chem. **101,** 15 (1933).
(43) JACOBS AND COLLINS: J. Biol. Chem. **59,** 713 (1924).
(44) JACOBS AND COLLINS: J. Biol. Chem. **61,** 387 (1924).
(45) JACOBS AND COLLINS: J. Biol. Chem. **65,** 491 (1925).
(46) JACOBS AND ELDERFIELD: J. Biol. Chem. **92,** 313 (1931).
(47) JACOBS AND ELDERFIELD: J. Biol. Chem. **97,** 727 (1932).
(48) JACOBS AND ELDERFIELD: J. Biol. Chem. **100,** 671 (1933).
(49) JACOBS AND ELDERFIELD: J. Biol. Chem. **108,** 497 (1935).
(50) JACOBS AND ELDERFIELD: J. Biol. Chem. **113,** 611 (1936).
(51) JACOBS AND ELDERFIELD: J. Biol. Chem. **113,** 625 (1936).
(52) JACOBS, ELDERFIELD, GRAVE, AND WIGNALL: J. Biol. Chem. **91,** 617 (1931).
JACOBS AND ELDERFIELD: J. Biol. Chem. **91,** 625 (1931).
(53) JACOBS AND GUSTUS: J. Biol. Chem. **74,** 795 (1927).
(54) JACOBS AND GUSTUS: J. Biol. Chem. **74,** 829 (1927).
(55) JACOBS AND GUSTUS: J. Biol. Chem. **78,** 573 (1928).
(56) JACOBS AND GUSTUS: J. Biol. Chem. **79,** 553 (1928); **82,** 403 (1929); **88,** 531 (1930).
(57) JACOBS AND GUSTUS: J. Biol. Chem. **86,** 199 (1930).
(58) JACOBS AND HEIDELBERGER: J. Biol. Chem. **81,** 765 (1929).
(59) JACOBS AND HOFFMAN: J. Biol. Chem. **69,** 153 (1926); *cf.* STOLL AND RENZ: Enzymologia **7,** 362 (1939).
(60) JACOBS, HOFFMANN, AND GUSTUS: J. Biol. Chem. **70,** 1 (1926).
(61) KARRER, W.: Festschrift für E. C. Barell, p. 238 (Basel, 1936).
(62) KARRER, W.: Helv. Chim. Acta **26,** 1353 (1943).
(63) KATZ: Pharm. Acta Helv. **22,** 244 (1947).
(63a) KATZ: Helv. Chim. Acta **30,** 883 (1947).
(63b) KATZ: Helv. Chim. Acta **31,** 993 (1948).
(64) KATZ AND REICHSTEIN: Pharm. Acta Helv. **19,** 231 (1944).
(65) KATZ AND REICHSTEIN: Helv. Chim. Acta **28,** 476 (1945).
(66) KATZ AND REICHSTEIN: Pharm. Acta Helv. **22,** 437 (1947).
(67) KNOWLES, FRIED, AND ELDERFIELD: J. Org. Chem. **7,** 383 (1942).
(68) KNOWLES, KUCK, AND ELDERFIELD: J. Org. Chem. **7,** 374 (1942).
(69) KOECHLIN AND REICHSTEIN: Helv. Chim. Acta **30,** 1673 (1947).
(70) KOENIGS AND KNORR: Sitzber. math-naturw. Abt. bayer Akad. Wiss. München **30,** 103 (1900).
(71) KON: Chemistry & Industry **53,** 593, 956 (1934); Ann. Repts. Progress Chem. (Chem. Soc. London) **31,** 218 (1934).
(72) LAMB AND SMITH: J. Chem. Soc. **1936,** 442.
(73) LANG: Dissertation, Zurich, 1946.
(74) LINSTEAD, DOERING, DAVIS, LEVINE, AND WHETSTONE: J. Am. Chem. Soc. **64,** 1985 (1942).

(75) LINVILLE AND ELDERFIELD: J. Org. Chem. **6**, 270 (1941).
(76) LINVILLE, FRIED, AND ELDERFIELD: Science **94**, 284 (1941).
FRIED, LINVILLE, AND ELDERFIELD: J. Org. Chem. **7**, 362 (1942).
(77) MCKENZIE, MCGUCKIN, AND KENDALL: J. Biol. Chem. **162**, 555 (1946).
(78) MANNICH AND SIEWERT: Ber. **75**, 737 (1942).
(79) MANNICH AND SIEWERT: Ber. **75**, 750 (1942).
(80) MARKER, CROOKS, WAGNER, AND WITTBECKER: J. Am. Chem. Soc. **64**, 2089 (1942).
(81) MARKER AND KRUEGER: J. Am. Chem. Soc. **62**, 79 (1940).
(82) MARSHALL, KUCK, AND ELDERFIELD: J. Org. Chem. **7**, 444 (1942).
(83) MASON AND HOEHN: J. Am. Chem. Soc. **60**, 2824 (1938); **61**, 1614 (1939).
(84) MATTOX, TURNER, ENGEL, MCKENZIE, MCGUCKIN, AND KENDALL: J. Biol. Chem. **164**, 569 (1946).
(85) MEYER: Helv. Chim. Acta **29**, 718 (1946).
(86) MEYER: Helv. Chim. Acta **29**, 1580 (1946).
(87) MEYER: Helv. Chim. Acta **29**, 1908 (1946).
(88) MEYER: Helv. Chim. Acta **30**, 1976, (1947).
(89) MEYER: Helv. Chim. Acta **30**, 2024 (1947).
(90) MEYER AND REICHSTEIN: Helv. Chim. Acta **30**, 1508 (1947).
(91) MEYSTRE AND MIESCHER: Helv. Chim. Acta **27**, 1153 (1944).
(92) MOZINGO, WOLF, HARRIS, AND FOLKERS: J. Am. Chem. Soc. **65**, 1013 (1943).
(93) NEUMANN: Arch. exptl. Path. Pharmakol. **185**, 329 (1937).
(94) NEUMANN: Ber. **70**, 1547 (1937).
(95) OTT AND REICHSTEIN: Helv. Chim. Acta **26**, 1799 (1943).
(96) PAIST, BLOUT, UHLE, AND ELDERFIELD: J. Org. Chem. **6**, 273 (1941).
(97) PLATTNER AND FÜRST: Helv. Chim. Acta **28**, 173 (1945).
(98) PLATTNER, HARDEGGER, AND BUCHER: Helv. Chim. Acta **28**, 167 (1945).
(99) PLATTNER AND HEUSSER: Helv. Chim. Acta **28**, 1044 (1945).
(100) PLATTNER AND HEUSSER: Helv. Chim. Acta **29**, 727 (1946).
(101) PLATTNER, HEUSSER, AND ANGLIKER: Helv. Chim. Acta **29**, 468 (1946).
(102) PLATTNER, HEUSSER, AND OESCHGER: Helv. Chim. Acta **29**, 253 (1946).
(103) PLATTNER AND LANG: Helv. Chim. Acta **27**, 1872 (1944).
(104) PLATTNER, MEIER, AND HEUSSER: Helv. Chim. Acta **30**, 905 (1947).
(105) PLATTNER, PETRZILKA, AND LANG: Helv. Chim. Acta **27**, 513 (1944).
(106) PLATTNER, RUZICKA, AND FÜRST: Helv. Chim. Acta **26**, 2274 (1943).
(107) PLATTNER, RUZICKA, HEUSSER, AND ANGLIKER: Helv. Chim. Acta **30**, 385 (1947).
(108) PLATTNER, RUZICKA, HEUSSER, AND ANGLIKER: Helv. Chim. Acta **30**, 395 (1947).
(109) PLATTNER, RUZICKA, HEUSSER, AND ANGLIKER: Helv. Chim. Acta **30**, 1073 (1947).
(110) PLATTNER, RUZICKA, HEUSSER, AND MEIER: Helv. Chim. Acta **29**, 2023 (1946).
(111) PLATTNER, RUZICKA, HEUSSER, PATAKI, AND MEIER: Helv. Chim. Acta **29**, 942 (1946).
(112) PLATTNER, RUZICKA, HEUSSER, PATAKI, AND MEIER: Helv. Chim. Acta **29**, 949 (1946).
(113) PLATTNER, RUZICKA, AND PATAKI: Helv. Chim. Acta **28**, 389 (1945).
(114) PLATTNER, SEGRE, AND ERNST: Helv. Chim. Acta **30**, 1432 (1947).
(115) PLATTNER AND UFFER: Helv. Chim. Acta **28**, 1049 (1945).
(116) PRESS AND REICHSTEIN: Helv. Chim. Acta **25**, 878 (1942).
(117) PRESS AND REICHSTEIN: Helv. Chim. Acta **30**, 2127 (1947).
(118) RABALD AND KRAUS: Z. physiol. Chem. **265**, 39 (1940); German patent 723,224; Chem. Zentr. **1943, I**, 60.
(119) REBER AND REICHSTEIN: Helv. Chim. Acta **29**, 343 (1946).
(120) REICHSTEIN AND FUCHS: Helv. Chim. Acta **23**, 658 (1940).
(121) REICHSTEIN AND KATZ: Pharm. Acta Helv. **18**, 521 (1943).
(122) REICHSTEIN AND LARDON: Helv. Chim. Acta **24**, 955 (1941).
(123) REICHSTEIN AND MONTIGEL: Helv. Chim. Acta **22**, 1212 (1939).
(124) REICHSTEIN AND REICH: Ann. Rev. Biochem. **15**, 164 (1946).
(125) REICHSTEIN AND ROSENMUND: Pharm. Acta Helv. **15**, 150 (1940).

(126) ROSENMUND AND REICHSTEIN: Pharm. Acta Helv. **17**, 176 (1942).
(127) RUZICKA, FURTER, AND THOMANN: Helv. Chim. Acta **16**, 333 (1933).
(128) RUZICKA, HARDEGGER, AND KAUTER: Helv. Chim. Acta **27**, 1164 (1944).
(129) RUZICKA AND MUHR: Helv. Chim. Acta **27**, 503 (1944).
(130) RUZICKA, PLATTNER, AND BALLA: Helv. Chim. Acta **25**, 65 (1942).
(131) RUZICKA, PLATTNER, AND FÜRST: Helv. Chim. Acta **24**, 716 (1941).
(132) RUZICKA, PLATTNER, AND FÜRST: Helv. Chim. Acta **25**, 79 (1942).
(133) RUZICKA, PLATTNER, FÜRST, AND HEUSSER: Helv. Chim. Acta **30**, 694 (1947).
(134) RUZICKA, PLATTNER, AND HEUSSER: Helv. Chim. Acta **25**, 435 (1942).
(135) RUZICKA, PLATTNER, AND HEUSSER: Helv. Chim. Acta **27**, 186 (1944).
(136) RUZICKA, PLATTNER, AND HEUSSER: Helv. Chim. Acta **27**, 1173 (1944).
(137) RUZICKA, PLATTNER, AND HEUSSER: Helv. Chim. Acta **29**, 473 (1946).
(138) RUZICKA, PLATTNER, HEUSSER, AND ERNST: Helv. Chim. Acta **29**, 248 (1946).
(139) RUZICKA, PLATTNER, HEUSSER, AND MEIER: Helv. Chim. Acta **30**, 1342 (1947).
(140) RUZICKA, PLATTNER, HEUSSER, AND PATAKI: Helv. Chim. Acta **29**, 936 (1946).
(141) RUZICKA, PLATTNER, AND PATAKI: Helv. Chim. Acta **25**, 425 (1942).
(142) RUZICKA, PLATTNER, AND PATAKI: Helv. Chim. Acta **27**, 988 (1944).
(143) RUZICKA, PLATTNER, AND PATAKI: Helv. Chim. Acta **28**, 1360 (1945).
(144) RUZICKA, REICHSTEIN, AND FÜRST: Helv. Chim. Acta **24**, 76 (1941).
(145) SARRETT AND WALLIS: Ann. Rev. Biochem. **16**, 675 (1947).
(146) SCHMIEDEBERG: Arch. exptl. Path. Pharmakol. **16**, 162 (1883); Ber. **16**, 253 (1883).
(147) SCHMUTZ: Pharm. Acta Helv. **22**, 373 (1947).
(148) SCHMUTZ AND REICHSTEIN: Pharm. Acta Helv. **22**, 167 (1947).
(149) SCHMUTZ AND REICHSTEIN: Pharm. Acta Helv. **22**, 359 (1947).
(150) SCHWARTZ, KATZ, AND REICHSTEIN: Pharm. Acta Helv. **21**, 250 (1946).
(151) SHOPPEE: Ann. Rev. Biochem. **11**, 123 (1942); Ann. Repts. Progress Chem. (Chem. Soc. London) **43**, 225 (1946).
(152) SHOPPEE: Chemistry & Industry **1947**, 109.
(153) SHOPPEE: Helv. Chim. Acta **27**, 246, 426 (1944).
(154) SHOPPEE AND REICHSTEIN: Helv. Chim. Acta **23**, 975 (1940).
(155) SHOPPEE AND REICHSTEIN: Helv. Chim. Acta **25**, 1611 (1942).
(156) SMITH: J. Chem. Soc. **1935**, 1305.
(157) SORKIN AND REICHSTEIN: Helv. Chim. Acta **29**, 1209 (1946).
(158) SORKIN AND REICHSTEIN: Helv. Chim. Acta **29**, 1218 (1946).
(159) SPEISER AND REICHSTEIN: Experientia **3**, 281 (1947); Helv. Chim. Acta **30**, 2143 (1947); **31**, 622 (1948).
(160) STAVELY AND BOLLENBACK: J. Am. Chem. Soc. **65**, 1285, 1290, 1600 (1943).
(161) STEIGER AND REICHSTEIN: Helv. Chim. Acta **20**, 1040 (1937).
(162) STEIGER AND REICHSTEIN: Helv. Chim. Acta **20**, 1164 (1937).
(163) STEIGER AND REICHSTEIN: Helv. Chim. Acta **21**, 828 (1938).
(164) STEINEGGER AND KATZ: Pharm. Acta Helv. **22**, 1 (1947).
(165) STOLL *et al.*: Helv. Chim. Acta **17**, 641, 1334 (1934); **18**, 82, 120, 401, 644, 1247 (1935).
(166) STOLL, KREIS, AND HOFMANN: Z. physiol. Chem. **222**, 24 (1933).
(167) STOLL AND RENZ: Helv. Chim. Acta **24**, 1380 (1941).
(168) STOLL AND RENZ: Helv. Chim. Acta **25**, 43 (1942).
(169) STOLL AND RENZ: Helv. Chim. Acta **25**, 377 (1942).
(170) STOLL, RENZ, AND HELFENSTEIN: Helv. Chim. Acta **26**, 648 (1943).
(171) STOLL, SUTER, KREIS, BUSSEMAKER, AND HOFMANN: Helv. Chim. Acta **16**, 703 (1933).
(172) SWERN: J. Am. Chem. Soc. **69**, 1692 (1947).
(173) TORREY, KUCK, AND ELDERFIELD: J. Org. Chem. **6**, 289 (1941).
(174) TSCHESCHE: Z. physiol. Chem. **222**, 50 (1933).
(175) TSCHESCHE: Z. angew. Chem. **47**, 729 (1934).
(176) TSCHESCHE: Z. physiol. Chem. **229**, 219 (1934).
(177) TSCHESCHE: Ber. **68**, 7 (1935).

(178) TSCHESCHE: Ber. **68,** 2252 (1935).
(179) TSCHESCHE: Ber. **69,** 2368 (1936).
(180) TSCHESCHE: Ber. **70,** 1554 (1937).
(181) TSCHESCHE AND BOHLE: Ber. **69,** 793 (1936).
(182) TSCHESCHE AND BOHLE: Ber. **69,** 2443 (1936).
(183) TSCHESCHE AND BOHLE: Ber. **69,** 2497 (1936).
(184) TSCHESCHE AND BOHLE: Ber. **71,** 654 (1938).
(185) TSCHESCHE, BOHLE, AND NEUMANN: Ber. **71,** 1927 (1938).
(186) TSCHESCHE AND HAUPT: Ber. **69,** 459 (1936).
(187) TSCHESCHE AND HAUPT: Ber. **70,** 43 (1937).
(188) UHLE AND ELDERFIELD: J. Org. Chem. **8,** 162 (1943).
(189) VOSS AND VOGT: Ber. **69,** 2333 (1936).
(190) WENNER AND REICHSTEIN: Helv. Chim. Acta **27,** 24 (1944).
(191) WESTPHALEN: Ber. **48,** 1064 (1915).
(192) WIELAND AND BENEND: Ann. **554,** 1 (1943).
(193) WILLSTÄTTER AND MAYER: Ber. **41,** 2199 (1908).
(194) WINDAUS: Ber. **36,** 3752 (1903).
(195) WINDAUS AND HAACK: Ber. **63,** 1377 (1930).
(196) WINDAUS, LINSERT, AND ECKHARDT: Ann. **534,** 22 (1938).
(197) WINDAUS AND SCHWARTE: Ber. **58,** 1515 (1925).
(198) WINDAUS AND UIBRIG: Ber. **48,** 857 (1915).
WINDAUS: Ber. **49,** 1724 (1916).
(199) WINDAUS, WESTPHAL, AND STEIN: Ber. **61,** 1847 (1928).
(200) WINDAUS AND ZÜHLSDORFF: Ann. **536,** 204 (1938).
(201) WINSTEIN, HESS, AND BUCKLES: J. Am. Chem. Soc. **64,** 2796 (1942).
(202) WOODWARD: J. Am. Chem. Soc. **64,** 72 (1942).