THE CHEMISTRY OF THE CARDIAC GLYCOSIDES

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I. INTRODUCTION

Within the past few years our knowledge of the chemical structure of the more important cardiac glycosides has been developed to such an extent that it is now possible to bring order out of the confusion in which these substances have hitherto been enveloped. Strikingly enough, they are now

found to fall into the growing list of substances related to the sterols and bile acids, but the fact that this exact relationship has been demonstrated subsequent to the more recent reviews of the latter substances, taken together with the occurrence of several individual features characteristic of the cardiac drugs, would seem to warrant a somewhat fuller treatment of their chemistry independent of the sterol group.

The cardiac glycosides comprise a group of naturally occurring substances placed together because of their common characteristic action on the heart. The present treatment will be restricted to a discussion of their chemistry with but passing attention given to their physiological and pharmacological properties, a phase which has already been competently presented by Cushny (19). Suffice it to say that the pharmacological study of these substances had kept far ahead of the accumulation of strictly chemical information available. This was doubtless due to a variety of factors. The plants in which these materials occur not infrequently contain several active constituents which are responsible for the physiological action. The active principles, in addition to possessing at times very similar physical properties, often occur in relatively small amounts—a combination of circumstances which has rendered the isolation of chemical individuals exceedingly difficult. Furthermore, such substances are of a very complex structural character and often quite labile, so that their isolation in crystalline form has been hindered. In many cases where crystalline compounds have been reported, these have later been shown to be mixtures. The presence of relatively large quantities of saponins and other inert substances which are extracted along with the cardiac principles frequently alters the solubilities of the active substances, a circumstance which contributes in no small measure to the difficulty of their isolation.

II. OCCURRENCE AND FORMULATION OF THE CARDIAC GLYCOSIDES

The use of infusions of certain plants for the treatment of cardiac diseases in medicine has been known to a number of peoples for centuries. However, until the latter part of the eighteenth century this use was based on purely empirical knowledge. The geographical distribution of plants containing cardiac principles is rather wide, many species being found in tropical regions where the principal use made of them seems to have been in the preparation of arrow and ordeal poisons. There also seems to be no rule governing their distribution in the vegetable kingdom for, while the majority of the plants appear to be included in the order of *Apocynaceae*, others have been found in the *Scrophulariaceae*, *Liliaceae*, and *Ranunculaceae*.

The classical review of Schmiedeberg (151) and the more recent treat-

ment of these substances by Jacobs (47) have described the better known glycosides of the group. However, inasmuch as new information has accumulated since the latter appeared, it will be necessary to treat this phase of the subject in some detail in order that the present discussion may be complete. With the exception of the toad venoms and the nitrogenous principle of *Erythrophloeum guineense*, the active principles are glycosides consisting entirely of carbon, hydrogen, and oxygen. The glycosides on treatment with acids are hydrolyzed more or less easily with formation of the component sugars and the non-carbohydrate constituents, termed the aglycones or "genins." The sugars so obtained have proved in a number of instances to contain certain unusual structural features. Several of them fall in the class of 2-deoxysugars, which fact renders the glycosides extremely labile and easily hydrolyzed. Others belong to the normal type of sugars with hydrolysis in consequence being more difficult, a circumstance which has occasioned difficulty in obtaining the aglycones without extensive alteration produced by the necessarily severe hydrolytic conditions. Tables 1 and 2 list the important members of the group.

A. Digitalis glycosides

Digitalis purpurea. In 1875 Withering published his epoch-making treatise on the therapeutic powers of the foxglove in the treatment of dropsy. Subsequent workers traced these effects to a specific and characteristic action on the heart, and as a result a large amount of work with this drug has been recorded which far exceeds that with any other cardiac drug. The search for the active principle in the early days proved so unfruitful that in order to stimulate activity La Société de Pharmacie de Paris in 1835 offered a prize of 500 francs for its isolation. Five years later this was increased to 1000 francs. In 1841 Homolle and Quevenne (43) isolated a highly active, partly crystalline mixture from the leaves of *Digitalis purpurea*, called by them "digitaline;" to this work was awarded the prize. This was apparently the first successful isolation of even a crude cardiac glycoside. Since then, work on this subject has been prolific.

The seeds and especially the leaves of the plant have been the source of therapeutic preparations. The active glycosides preponderating in the former, while closely related to those in the latter, are substances distinctly different. Extraction of the seeds with water removes all the glycosides (162) and yields a complex mixture of active and inactive glycosides; one of the former has been recognized as a chemical individual, the digitalin of Schmiedeberg (150), who also noted its glycosidic nature. Kiliani (98), using commercial *Digitalinum pur. pulv. germanic* (Merck), obtained it practically pure, renamed it digitalinum verum, and developed a practical method of separating it from other substances (98). He also adopted a

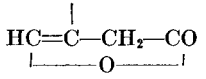
TABLE 1

Cardiac glycosides of definitely determined structure

GLYCOSIDE	FORMULA	FUNCTIONS AND POSITIONS IN THE AGLYCONES	SUGAR COMPONENTS	PRINCIPAL SOURCE
Digitoxin	$C_{41}H_{64}O_{13}$	Normal side chain;* OH on carbon atoms 3 and 14; CH_3 on carbon atom 10	3 digitoxose	Leaves of *Digitalis purpurea*
Gitoxin	$C_{41}H_{64}O_{14}$	Normal side chain; OH on carbon atoms 3, 14, and 16; CH_3 on carbon atom 10	3 digitoxose	Leaves of *Digitalis purpurea*
Purpurea glycoside A (deacetyldigilanide A)	$C_{47}H_{74}O_{18}$	Same as digitoxin	3 digitoxose; 1 glucose	Leaves of *Digitalis purpurea*
Purpurea glycoside B (deacetyldigilanide B)	$C_{47}H_{74}O_{19}$	Same as gitoxin	3 digitoxose; 1 glucose	Leaves of *Digitalis purpurea*
Digilanide A	$C_{49}H_{76}O_{19}$	Same as digitoxin	3 digitoxose, one of which contains an acetyl group; 1 glucose	Leaves of *Digitalis lanata*
Digilanide B	$C_{49}H_{76}O_{20}$	Same as gitoxin	3 digitoxose, one of which contains an acetyl group; 1 glucose	Leaves of *Digitalis lanata*

Cymarin	$C_{30}H_{44}O_9$	Normal side chain; OH on carbon atoms 3, 5, and 14; CHO on carbon atom 10	1 cymarose	Various species of *Apocynum* Enzymatic hydrolysis of certain strophanthus glycosides
Allocymarin	$C_{30}H_{44}O_9$	Same as cymarin, but stereoisomeric	1 cymarose	Enzymatic isomerization of k-strophanthus glycosides
k-Strophanthin-β	$C_{36}H_{54}O_{14}$	Same as cymarin	Strophanthobiose (1 cymarose and 1 glucose)	k-Strophanthus seeds
Periplocymarin	$C_{30}H_{46}O_8$	Normal side chain; OH on carbon atoms 3, 5, and 14; CH_3 on carbon atom 10	1 cymarose	Wood and bark of *Periploca graeca*

* The normal side chain is considered to be attached at carbon atom 17, and to have the structure



All the aglycones have a CH_3 group on carbon atom 13.

TABLE 2

Cardiac glycosides in which rigid proof for some particulars is still lacking

GLYCOSIDE	FORMULA	FUNCTIONS AND POSITIONS IN THE AGLYCONE	SUGAR COMPONENTS	PRINCIPAL SOURCE
Digitalinum verum.....	$C_{36}H_{56}O_{14}$	Normal side chain; OH on carbon atom 3; two double bonds from removal of two OH groups during hydrolysis;* CH_3 on carbon atom 10	1 digitalose; 1 glucose	Seeds of *Digitalis purpurea*
Digoxin.....	$C_{41}H_{64}O_{14}$	Presumably normal side chain; presumably OH on carbon atom 3; presumably CH_3 on carbon atom 10; two OH groups, position unknown	3 digitoxose	Leaves of *Digitalis lanata*
Digilanide C.....	$C_{49}H_{76}O_{20}$	Same as digoxin	3 digitoxose, one of which contains an acetyl group; 1 glucose	Leaves of *Digitalis lanata*
Ouabain.....	$C_{29}H_{44}O_{12}$	Normal side chain; presumably OH on carbon atoms 3 and 14; three OH groups which are removed during hydrolysis;* substituent on carbon atom 10 may be CH_2OH	Rhamnose	Seeds of *Strophanthus gratus* Bark of ouabaio tree
Sarmentocymarin.....	$C_{30}H_{46}O_8$	Normal side chain; presumably OH group on carbon atoms 3 and 14; one OH group, position unknown; presumably CH_3 on carbon atom 10	Sarmentose	Seeds of *Strophanthus sarmentosus*

Oleandrin.....	$C_{31}H_{48}O_9$	Same as digitalinum verum	Probably digitalose	*Nerium oleander*
Uzarin.....	$C_{35}H_{56}O_{16}$	Presumably normal side chain; OH group on carbon atom 3; two OH groups, position unknown, which are removed during hydrolysis;* CH_3 on carbon atom 10	2 glucose	Wood of *Uzara*
Scillaren A.....	$C_{36}H_{52}O_{13}$	Side chain:  attached to carbon atom 17; presumably OH group on carbon atom 14;* one OH group and one double bond, position not certain; CH_3 on carbon atom 10	1 rhamnose; 1 glucose	*Scilla maritima*
Proscillaridin A.....	$C_{30}H_{42}O_8$	Same as scillaren A	1 rhamnose	Enzymatic hydrolysis of scillaren A

* The aglycone has never been isolated as such, anhydro derivatives formed by loss of hydroxyl groups during the hydrolysis being obtained. Double bonds mentioned in this table are in addition to the side chain one.

formula, $C_{35}H_{56}O_{14}$, for the substance and showed that it yielded on hydrolysis an aglycone, digitaligenin, and two sugars, digitalose and glucose. More recent work of Windaus and coworkers (188) has shown that Kiliani's formula must be revised to $C_{36}H_{56}O_{14}$. Further, the digitaligenin of Kiliani is not the primary aglycone, but a dehydration product of the original aglycone, $C_{23}H_{34}O_5$, probably gitoxigenin, produced by removal of two molecules of water under the comparatively severe hydrolytic conditions necessary. Hence the hydrolysis of digitalinum verum is represented by:

$$C_{36}H_{56}O_{14} + 2H_2O = C_{23}H_{34}O_5 + C_6H_{12}O_6 + C_7H_{14}O_5$$
$$C_{23}H_{34}O_5 - 2H_2O = C_{23}H_{30}O_3$$

In digitalinum verum it appears likely that digitalose is the sugar joined directly to the aglycone, and that glucose is attached to one of the remaining hydroxyl groups of the digitalose molecule. This is suggested by the fact that crystalline oleandrin (to be treated later, p. 201) has been shown by Windaus and Westphal (196) to be a glycoside of an aglycone, probably gitoxigenin, and of a sugar, probably digitalose. Addition of a glucose molecule to oleandrin could possibly give digitalinum verum.

Little is known of other active glycosides of the seeds, which are undoubtedly present. Among them may be mentioned the digitalein of Schmiedeberg (150), a mixture concerning the chemistry of which nothing is known (110).

Turning to the leaves, one is confronted with a much more complicated picture. After a long period of confusion certain facts have emerged which can be regarded as definitely proved. Roughly two-thirds of the active glycosides can be extracted with water, the remainder by 50 per cent alcohol. From this extract two glycosides have been isolated in pure crystalline form by numerous workers,—digitoxin and gitoxin. A third glycoside, gitalinum cristallisatum, has been described by Cloetta.

Digitoxin occurs mainly in the alcoholic extract of the leaves after preliminary exhaustion with water, and it is isolated by careful fractionation of the material removed from this extract with ether. Digitoxin in a highly impure state was probably the substance in the hands of Homolle and Quevenne (43), as well as the main constituent of the original "digitaline crystallisée" of Nativelle (135), who apparently was the first to have isolated it in a state approaching purity. Schmiedeberg, in the course of a study of it, adopted the name digitoxin (150, 151). Arnaud (5) appears to have been the first to have prepared it in a high state of purity. He recognized its glycosidic character as well as the presence of a saponifiable group (lactone). Others who have investigated it are Kiliani (100), Krafft (117), and, more recently, Cloetta (17) and Windaus and coworkers (186, 191c). Kiliani showed that it is easily cleaved on acid hydrolysis to

digitoxigenin and digitoxose. The correct formulation has been shown by Windaus (187) to be $C_{41}H_{64}O_{13}$, or digitoxigenin, $C_{23}H_{34}O_4$, joined in glycosidic union with three molecules of digitoxose. Its hydrolysis is represented by the following equation:

$$C_{41}H_{64}O_{13} + 3H_2O = C_{23}H_{34}O_4 + 3C_6H_{12}O_4$$

Only one of the digitoxose molecules can be directly joined to the aglycone.

Krafft (117) described a water-soluble glycoside, gitalin, extractable from the aqueous extract of the leaves by chloroform. This substance on heating in aqueous solution deposited a sparingly soluble, so-called dehydration product, "anhydrogitalin." Kiliani (109) showed gitalin to be a complex mixture of substances and, more recently, Windaus and Schwarte (194) and Cloetta (18) showed "anhydrogitalin" to be an impure form of a glycoside already present in the leaves, which is characterized by its very sparing solubility in alcohol and chloroform. It appears as the purification of the crude drug proceeds and is collected by taking advantage of its insolubility in chloroform.

The substance has been named gitoxin by Windaus and Schwarte (digitalinum cristallisatum by Cloetta). Windaus, Westphal, and Stein (197) derived the correct formula, $C_{41}H_{64}O_{14}$, and showed the substance to consist of an aglycone, gitoxigenin, $C_{23}H_{34}O_5$, joined to three molecules of digitoxose, which are readily obtained on acid hydrolysis:

$$C_{41}H_{64}O_{14} + 3H_2O = C_{23}H_{34}O_5 + 3C_6H_{12}O_4$$

On treatment with alcoholic hydrochloric acid, gitoxigenin readily loses two molecules of water with the formation of a dianhydro derivative identical with digitaligenin, the product of the hydrolysis of digitalinum verum.

The gitalinum cristallisatum, $C_{17}H_{28}O_6$, of Cloetta (18) was reported to yield on hydrolysis one molecule of digitoxose and one molecule of a new aglycone, gitaligenin, $C_{11}H_{18}O_3$, the close relationship of which to gitoxigenin was apparent from its ready conversion into derivatives of the latter on acylation and dehydration. However, Cloetta's interpretation of gitalinum cristallisatum is open to question. Windaus (190) and Jacobs and Gustus (75a) have discussed this subject and have pointed out that titration of the lactone group indicated the molecular size of the genin to be of the same order of magnitude as that of the other genins. Windaus arrived at the conclusion that gitaligenin may be considered a hydrate of gitoxigenin, and that the glycoside has a formula in the neighborhood of $C_{35}H_{56}O_{12} \cdot 0.5H_2O$. This substance, however, is in need of reinvestigation.

Kiliani (101) reported another crystalline glycoside, which he at first considered to be a distinct substance and designated β-digitoxin. Later he

regarded it as identical with digitoxin. However, he claimed to have obtained on hydrolysis with stronger acid an aglycone derivative, so-called β-anhydrodigitoxigenin, which melted at 223°C., contrasting with ordinary anhydrodigitoxigenin for which melting points of 183°C. and 193°C. have been observed. No other report of such a substance has been recorded.

Very recently Stoll and Kreis (158), following the earlier work of Jacobs and Hoffmann (84) on the enzymatic cleavage of the strophanthus glycosides, have succeeded in isolating a new series of glycosides from the leaves of *Digitalis purpurea*. By a special technique of extraction whereby enzymatic cleavage is hindered, they have obtained two glycosides, corresponding to digitoxin and gitoxin, both of which however contain an additional glucose molecule, the so-called purpurea glycoside A (deacetyldigilanide A first obtained from *Digitalis lanata*, to be discussed later) and purpurea glycoside B (deacetyldigilanide B). These on acid hydrolysis break in accordance with the following scheme:

$C_{47}H_{74}O_{18}$	$+ 3H_2O \rightarrow$	$C_{23}H_{34}O_4$	$+$	$2C_6H_{12}O_4$	$+$	$C_{12}H_{22}O_9$
Purpurea glycoside A		Digitoxigenin		Digitoxose		Digilanidobiose
$C_{47}H_{74}O_{19}$	$+ 3H_2O \rightarrow$	$C_{23}H_{34}O_5$	$+$	$2C_6H_{12}O_4$	$+$	$C_{12}H_{22}O_9$
Purpurea glycoside B		Gitoxigenin		Digitoxose		Digilanidobiose

Digilanidobiose on stronger hydrolytic treatment yields glucose, but the conditions used cause decomposition of the digitoxose component. Its presence has been proved indirectly by an enzymatic method.

If the glycosides be subjected to the action of an enzyme obtained from the leaves, their hydrolysis is represented as follows, and the nature of the second component of digilanidobiose is at once obvious.

$C_{47}H_{74}O_{18}$	$+ H_2O \rightarrow$	$C_{41}H_{64}O_{13}$	$+$	$C_6H_{12}O_6$
Purpurea glycoside A		Digitoxin		Glucose
$C_{47}H_{74}O_{19}$	$+ H_2O \rightarrow$	$C_{41}H_{64}O_{14}$	$+$	$C_6H_{12}O_6$
Purpurea glycoside B		Gitoxin		Glucose

Digitalis lanata. The chemistry of the glycosides contained in the leaves of this species has recently been the subject of investigation. The glycosides contained therein have been shown to be closely related to, and in some cases identical with, those occurring in *D. purpurea*. Smith (154) was the first to describe a new glycoside, digoxin, isomeric with gitoxin, $C_{41}H_{64}O_{14}$. On hydrolysis it yields digoxigenin, $C_{23}H_{34}O_5$, isomeric with

gitoxigenin, and three molecules of digitoxose. In addition, Smith also found gitoxin.

Mannich, Mohs, and Mauss (126) have reported four glycosides, the principal one of which is lanadigin, $C_{41}H_{66}O_7$, and which yielded on hydrolysis an aglycone, $C_{23}H_{34}O_5$, lanadigenin, identical with Smith's digoxigenin. The sugar fraction consisted of digitoxose and a disaccharide, $C_{12}H_{22}O_9$, which was more resistant to acids.

A second glycoside, called by them lanata glycoside II, yielded on hydrolysis two genins—digoxigenin and digitoxigenin—and as sugars, digitoxose and the above disaccharide. This glycoside maintained the appearance of chemical homogeneity in all attempts at fractional crystallization. However, in view of the later observations of Stoll and Kreis (159), who noted the strong tendency of these substances to form mixed crystals, it is not improbable that this substance may be a mixture. A third glycoside was considered to be digitalinum verum. Finally, the fourth glycoside, for which a formula of either $C_{29}H_{48}O_{11}$ or $C_{42}H_{66}O_{16}$ was given, gave on hydrolysis glucose and a genin, either $C_{23}H_{32}O_3$ or $C_{30}H_{42}O_4$.

More recently Stoll and Kreis (159) have applied their technique of extraction to the leaves of this species. Three glycosides were thus obtained: digilanides A, B, and C with formulas $C_{49}H_{76}O_{19}$, $C_{49}H_{76}O_{20}$, and $C_{49}H_{76}O_{20}$, respectively. Digilanide A is composed of one molecule of digitoxigenin, three molecules of digitoxose, and one molecule of glucose. In addition, an acetyl group is present, presumably attached to one of the digitoxose units. Enzymatic cleavage with digilanidase, from the leaves, removes the glucose to give acetyldigitoxin. Saponification of the acetyl group in this with calcium hydroxide gives digitoxin, which on acid hydrolysis behaves in the usual manner. Saponification of the acetyl group in the original digilanide A leads to deacetyldigilanide A, identical with the above-mentioned purpurea glycoside A. However, direct acid hydrolysis of digilanide A gives one molecule of digitoxigenin, two molecules of digitoxose, and one molecule of digilanidobiose apparently identical with Mannich's biose. Digilanide B is composed of one molecule of gitoxigenin, three molecules of digitoxose, one molecule of glucose, and one acetyl group; digilanide C is similar except that the aglycone, gitoxigenin, is replaced by digoxigenin. Both show exactly the same type of hydrolytic reactions as digilanide A. An interesting isomerization occurs under the influence of the enzyme, whereby two glucose-free acetylglycosides are formed. They are physiologically alike in their action, but show different optical properties. Apparently this change involves the arrangement of the acetyl group, since deacetylation gives the same deacetylglycosides from both isomers. In this connection the change is confined to the sugar portion of the molecule in contrast to the allomerization of strophanthidin, to be discussed later, which involves the aglycone.

B. Apocynaceae

Strophanthins. As previously mentioned, the *Apocynaceae* family embraces the largest number of plants containing cardiac poisons. The aborigines of Africa have made use of infusions of the seeds and bark of a number of species of *Strophanthus* in the preparation of arrow poisons. Chemical work on these substances has suffered in the past, owing to incompleteness of botanical data on the seeds from which the glycosides have been prepared. Inasmuch as some forty odd varieties of *Strophanthus* plants have been reported, confusion was almost inevitable. In time, clarification ensued as this difficulty was recognized, and certain easily accessible species were investigated. Thoms (169) made the suggestion that the first letter of the species from which the glycosides were obtained be prefixed to the term "strophanthin"—thus, k-strophanthin from *S. kombé*, h-strophanthin from *S. hispidus*, g-strophanthin from *S. gratus*, etc.

The *kombé* arrow poisons were the subject of classical studies by Fraser (30) and Arnaud (3). Subsequently Feist (29) reinvestigated them. However, the work of these investigators suffered from uncertainty as to the identity of the *Strophanthus* species under examination. They did succeed in showing the glycosidic character of the material from which an aglycone, strophanthidin, was obtained on hydrolysis. The critical reviews of Heffter and Sachs (40) and Brauns and Closson (10) accomplished much to clarify this subject. The former reported on identified *hispidus* seeds and commercial *kombé* seeds, while the latter studied identified *kombé* and commercial *hispidus* seeds. The identical aglycone, strophanthidin, was obtained by both workers from both varieties. *Kombé* seeds were found to yield two strophanthidin glycosides,—a crystalline k-strophanthin, sparingly soluble in water, and an amorphous, water-soluble k-strophanthin.

More recently, the glycosides of *S. kombé* have been given extensive study by Jacobs and Hoffmann. While practically all the glycosides are derivatives of one aglycone, strophanthidin, the nature of the sugar components has been found to vary, and it is certain that none of the glycosides in the hands of the earlier workers was an individual substance. Strophanthidin, as first shown by Jacobs and Heidelberger (80), has the formula $C_{23}H_{32}O_6$. The *kombé* strophanthins appear to be made up of one molecule of cymarin joined with one or more glucose units. Cymarin had previously been found in a number of varieties of *Apocynum* (*cannabinum*, *androsaemifolium*, and *venetum*). It was first isolated by Taub and Fickewirth (24) and subsequently shown by Windaus and Hermanns (193) to possess the formula $C_{30}H_{44}O_9$ and to yield strophanthidin and cymarose, $C_7H_{14}O_4$, on hydrolysis.

The so-called crystalline k-strophanthin of the earlier workers was found by Jacobs and Hoffmann (83) to be resolvable into two components, viz.

chloroform-soluble cymarin and chloroform-insoluble k-strophanthin-β. The latter represents cymarin in combination with one additional glucose unit. On hydrolysis it gives strophanthidin and a biose, strophanthobiose, $C_{13}H_{24}O_9$, which requires such vigorous hydrolytic conditions for its further breaking down that decomposition of the resulting sugars ensues. However, in the *Strophanthus* seeds an enzyme, strophanthobiase, was found which possesses the property of hydrolyzing the union between the two monoses in k-strophanthin-β with the formation of one molecule of cymarin and one molecule of glucose, thus demonstrating the nature of strophanthobiose.

By application of the enzyme method, the mixture of higher water-soluble glycosides, the so-called amorphous k-strophanthin, which predominates in the *kombé* seeds, has been broken down to cymarin. While the exact formulation of these glycosides is a matter of uncertainty, analyses of samples thereof have indicated that the chief component of the mixture is a trioside consisting of cymarin in union with two glucose molecules. However, the production of cymarin from all of them shows that strophanthidin is joined in glycosidic union only with cymarose.

A curious type of enzymatic isomerization of the k-strophanthus glycosides has been noticed by Jacobs (46). After digestion of a suspension of ground and defatted *S. kombé* seeds, which procedure had been attempted in the hope of going directly to the easily isolatable cymarin, the glycoside found proved to be not cymarin but an isomer, allocymarin. It yielded on hydrolysis cymarose and an aglycone, allostrophanthidin, which, while retaining all the functional groups of strophanthidin, nevertheless differed from the latter in its stereo configuration. Thus, there is a second enzyme present in the seeds in addition to strophanthobiase, which possesses the power of isomerizing the strophanthidin molecule while still in glycosidic union. In contrast to the isomerization noted by Stoll and Kreis (159) in the case of the acetyldigilanides, this allomerization involves the aglycone portion of the molecule and the typical cardiac action is almost, if not entirely, destroyed by the change.

Strophanthus hispidus. The glycosides of this species, while closely resembling those of *S. kombé,* nevertheless show certain differences. Jacobs and Hoffmann (87) obtained a complex mixture of water-soluble glycosides from the seeds, from which it was possible to obtain cymarin after enzymatic cleavage of glucose. However, the h-strophanthins differ from the k-strophanthins in being considerably more resistant to hydrolysis.

Very recently Tschesche (173) has reported the isolation of derivatives of two new aglycones from seeds which were considered to be *S. hispidus.* Without isolation of the glycosides he subjected the mixture obtained by methyl alcohol extraction of the seeds directly to hydrolytic cleavage by

acid. To the first of these, α-monoanhydrohispidogenin-A, is ascribed the formula $C_{23}H_{32}O_4$. It is presumably derived from an aglycone, $C_{23}H_{34}O_5$, from which one hydroxyl group has apparently been removed as water during the hydrolysis. The second, dianhydrohispidogenin-B, $C_{23}H_{30}O_6$, is similarly derived from an aglycone, $C_{23}H_{34}O_8$, which loses two hydroxyl groups during the hydrolysis. Tschesche's results are definitely at variance with the experience of earlier workers, since from the seeds studied by him derivatives of aglycones other than strophanthidin were obtained.

Strophanthus gratus. The Pahouins have long made use of an arrow poison prepared from the seeds of this species. The principal glycosidic constituent thereof is ouabain, a material which possesses an unusually strong power of crystallization, thus rendering its isolation and purification comparatively simple. For this reason it has been used as a standard in the assay of commercial digitalis and strophanthus preparations. Arnaud (2) first discovered ouabain in the roots and bark of the ouabaio tree, a species of *Acocanthera* used as an arrow poison by the Somalis of East Africa. He assigned to it the formula $C_{30}H_{46}O_{12}$, showed it to be a rhamnose glycoside of an hypothetical aglycone, $C_{23}H_{36}O_8$, and noted the presence of a saponifiable group through the formation of a crystalline barium salt. Ouabain, in contrast to the easily hydrolyzable deoxysugar glycosides, shows such resistance to hydrolytic cleavage that under the conditions necessary, dehydration of the aglycone with possible polymerization ensues. As a result, the latter has never been isolated, and its chemistrv has until recently remained obscure. Jacobs and Bigelow (50) revised Arnaud's formulas for ouabain and its aglycone to $C_{29}H_{44}O_{12}$ and $C_{23}H_{34}O_8$, respectively. They have also succeeded in securing crystalline derivatives of the aglycone by the use of special procedures. These will be discussed later. *S. gratus* seeds also contain a mixture of amorphous glycosides concerning which nothing is known.

In addition to crystalline ouabain, several other crystalline or amorphous glycosides have been reported from various species of *Acocanthera.* Among these may be mentioned the rhamnose glycoside, acocantherin, of Fraser and Tillie (31) and Faust (28), to which the formula $C_{32}H_{50}O_{12}$ has been assigned, and several arrow poisons such as the crystalline glucose glycoside ukambin of Paschkis (137), and the glycosides of the Wakamba arrow poison studied by Brieger (11). Some of these on further study may be found to be identical with, or closely related to, ouabain. For a detailed ethnological study of the arrow poisons the reader is referred to Lewin (123).

Strophanthus sarmentosus. Jacobs and Heidelberger (81) have investigated the glycosides obtained from *S. sarmentosus.* The seeds contain a mixture of glycosides, from which especially after the use of the enzymatic

method a crystalline substance, sarmentocymarin, $C_{30}H_{46}O_8$, was extracted with chloroform. The glycoside is easily hydrolyzed and yields an aglycone, sarmentogenin, $C_{23}H_{34}O_5$, and a sugar, sarmentose, $C_7H_{14}O_4$, which is isomeric with cymarose. The nature of the more complex water-soluble but chloroform-insoluble glycosides has also been explained by the enzyme method and shown to consist of sarmentocymarin united with one or more molecules of glucose.

Strophanthus eminii. Jacobs and Bigelow (48) have found the seeds of this species to yield a mixture of easily hydrolyzable and more stable glycosides. By extraction of the concentrate obtained by alcoholic extraction of the seeds, a chloroform-soluble monoside, $C_{30}H_{46}O_9$, was obtained. On hydrolysis, which required severe conditions, a trianhydro derivative, $C_{23}H_{28}O_2$, of an hypothetical aglycone, $C_{23}H_{34}O_5$, was obtained. This substance was isomeric with trianhydroperiplogenin, a fact which would seem to indicate that the aglycone is not periplogenin (to be discussed later). However, it is not excluded that periplogenin in glycosidic union might yield such a trianhydro derivative. The sugar of this monoside is a methyl ether sugar either identical or isomeric with digitalose. On mild hydrolysis of the chloroform-insoluble glycoside mixture, a mixture of aglycones was obtained which consisted of strophanthidin and periplogenin, which can be taken to indicate the presence of deoxysugar glycosides. In addition, the characteristic Keller–Kiliani test was also given by the crude glycoside mixture. From the mother liquors from this hydrolysis a bioside, $C_{36}H_{56}O_{14}$, was isolated. This probably consists of the above monoside with an additional hexose, possibly glucose, attached in a manner analogous to the conditions found in the case of k-strophanthin-β. On hydrolysis it gave the same trianhydroaglycone as did the monoside.

Nerium oleander. This member of the family *Apocynaceae* is a plant the poisonous properties of which were known in antiquity, Hippocrates having recorded observations thereon. The leaves contain a mixture of glycosides, the chemistry of which was given a superficial study by Schmiedeberg (151) and by Pieszczek (141). A crystalline oleandrin has been prepared in very poor yield from the leaves of the plant in the laboratories of C. F. Boehringer and Söhne at Mannheim-Waldhof. This was shown by Windaus and Westphal (196) to be a glycoside, $C_{31}H_{48}O_9$, which on hydrolysis yields digitaligenin and a sugar, probably digitalose. Its possible close relationship with digitalinum verum has already been noted (p. 194). In addition to crystalline oleandrin, a number of other glycosides, some crystalline and some amorphous, have been reported by Leulier (121), Tauber and Zellner (167), and Tanret (165). The chemistry of these is still obscure. *Nerium odorum* has been stated by Greenish (36) to yield

two amorphous glycosides, neriodorin and neriodorein, the latter of which is soluble in water. On hydrolysis a reducing sugar and a crystalline aglycone were obtained.

Thevetia neriifolia. The nuts of this plant, known as "be-still nuts," have long been used in the Malayan archipelago as one of the chief sources of arrow poisons. A beginning has recently been made on their chemistry. De Vry (23) and Blas (7) obtained a water-soluble glycoside to which was given the name thevetin. More recently Weitz and Boulay (178) corroborated the presence of thevetin, and Ayyar (6) succeeded in isolating a crystalline glycoside. Ghatak (35) has reported two crystalline glycosides, thevetin and thevetoxin. Chen and Chen (13) have subjected the nuts to careful reëxamination and fully characterized one cardiac glycoside, thevetin, to which the formula $C_{29}H_{46}O_{13}\cdot 2H_2O$ has been given. The sugar component has not been determined, and the aglycone has been obtained only in amorphous form. Herrara (42) has described a crystalline glycoside, thevetosin, yielding glucose and an amorphous aglycone, obtained from *Thevetia ycottli*, the joyote of Mexico, a plant the medicinal properties of which were made use of by the ancient Aztecs.

Miscellaneous Apocynaceae. Various other members of the family have been reported to yield active glycosides. Among these may be mentioned tanghinine, a crystalline glycoside from the nuts of *Tanghinia Madagascariensis*, first isolated by Olivier and Henry (136). Arnaud (4) assigned to it the formula $C_{27}H_{44}O_{10}$ or $C_{27}H_{40}O_8$, and noted the presence of a saponifiable group by the formation of a crystalline barium salt. His description of its properties as closely resembling digitoxin makes a revision of his formula for tanghinine seem desirable. Bowrey (9) has described two crystalline glycosides from the leaves and green tips of *Urechites suberecta*, a native of the West Indies. Urechitoxin, for which the formula $C_{13}H_{20}O_5$, or a multiple thereof, is proposed, yields on hydrolysis a crystalline aglycone, urechitotoxin, and reducing sugar. Urechitin, $C_{28}H_{42}O_8$, also yields a crystalline aglycone. In addition, a mixture of amorphous glycosides was found. These substances have also been studied by Minkiewicz (131). Various species of *Adenium* have been used as arrow poisons in Africa, some of which have been given study. Boehm (8) isolated a crystalline glycoside, $(C_5H_8O_2)_n$, echugin (or echujiin), from the milk sap of *Adenium boehmianum*. On hydrolysis glucose and a crystalline aglycone, echujetin, were obtained. Krausse (118) has described an amorphous glycoside from *Adenium coetaneum*, used as an arrow poison by the Watindigas, and Perrot and Leprince (139) attribute the poison used by the natives of the French Sudan to a glycoside, $C_{20}H_{31}O_8$, from *Adenium hongkel*. Plugge (142) confirmed the presence of a crystalline glycoside, cerberin, isolated by de Vry (21) from the seeds of *Cerbera odallam*, which closely resembles tanghinine

and thevetin in many respects. He assigned to it the formula $C_{27}H_{40}O_8$; upon hydrolysis he obtained amorphous cerberetin and glucose. However, the chemistry of these substances requires careful and discreet reinvestigation.

C. Asclepiadaceae

Periplocin. Lehmann (120) isolated a crystalline glycoside from the stems of *Periploca graeca*, to which he gave the name periplocin. He also showed its glycosidic nature by hydrolysis to an aglycone, periplogenin, and reducing sugar. However, this substance is but one of a mixture which occurs in the plant. Jacobs and Hoffmann (86) showed this mixture to contain a monoside, periplocymarin, which is present in relatively small quantities as such, being mostly in combination with one or more molecules of glucose. By digestion of the purified extract of the plant with strophanthobiase, the crude mixture of glycosides can be hydrolyzed to glucose and periplocymarin, which can then be extracted from the complex mixture with toluene. Periplocymarin possesses the formula $C_{30}H_{46}O_8$, and on hydrolysis gives periplogenin and cymarose.

The water-soluble periplocin of Lehmann was probably a bioside consisting of periplogenin in combination with cymarose, which is in turn combined with glucose. Its formula, therefore, should perhaps be $C_{36}H_{56}O_{13}$ and not $C_{30}H_{48}O_{12}$, as provisionally adopted by Lehmann at a time when the nature of the sugar component had not been determined.

Uzarin. In Africa certain tribes have made use of a species of *Gomphocarpus* called uzara, the root of which has furnished a drug "uzaron." Gürber (37) isolated a crystalline glycoside, uzarin, to which the drug owes its activity. Hennig (41) and Wolff (198) showed that it yielded a crystalline aglycone, uzarigenin, and glucose on hydrolysis. Windaus and Haack (192) assigned the formula $C_{35}H_{56}O_{16}$ to uzarin and showed it to be a glycoside of a hypothetical aglycone, $C_{23}H_{34}O_5$, combined with two molecules of glucose. However, the conditions required for the hydrolysis of uzarin cause decomposition of the aglycone which, therefore, has not been isolated. The product obtained by Windaus and Haack was a dianhydrouzarigenin, $C_{23}H_{30}O_3$.

Karsten (95) has described an amorphous glycoside, dregein, from the seeds of *Dregea rubicunda*. It is very easily hydrolyzed to yield reducing sugar and a crystalline aglycone, which might indicate the presence of a deoxysugar. It resembles strophanthin very closely.

D. Liliaceae

Scilla maritima. Following several previous investigators Stoll and coworkers (161) succeeded in isolating a crystalline glycoside—scillaren

A—from the bulbs of the squill. An excellent historical review of earlier work is given in Stoll's paper. Scillaren A has been assigned the formula $C_{37}H_{54}O_{13}$; on direct acid hydrolysis it yields scillaridin A, $C_{25}H_{32}O_3$, and scillabiose, $C_{12}H_{22}O_{16}$, a rhamnose–glucose biose.[1] During the hydrolysis one molecule of water is removed from the aglycone, so that the scillaridin A obtained is in reality a dehydration product of a hypothetical genin, $C_{25}H_{34}O_4$, which has never been obtained as such. The nature of scillabiose has been determined by the enzymatic method similar to that used by Jacobs and Hoffmann in the investigation of the strophanthins. Scillarenase (160), present in the bulbs, hydrolyzes scillaren A to give glucose and proscillaridin A, $C_{31}H_{44}O_8$, which on acid hydrolysis gives rhamnose and scillaridin A. The amorphous squill glycosides, grouped under the name scillaren B, have not as yet been resolved into pure constituents. However, on hydrolysis of the mixture a small crystalline aglycone fraction was obtained—scillaridin B—to which the tentative formula $C_{15}H_{18}O_3$ was given.

Convallaria majalis. From various parts of this plant highly active glycosides have been prepared. Walz (177) isolated a crystalline substance to which he gave the name convallarin. He recognized its glycosidic character and on its hydrolysis obtained a crystalline aglycone to which he assigned the formula $C_{28}H_{52}O_6$. However, it is quite certain that Walz's substance was a mixture. Tanret (164) obtained a crystalline glycoside, convallamarin, from the roots. On hydrolysis he obtained crystalline convallamaretin, $C_{20}H_{36}O_8$. Votacek and Vondracek (176) reported the sugars obtained by hydrolysis of convallamarin to be glucose, galactose, and a hexomethylose, which were also obtained from convallarin. More recently Lindner (124), by fractional crystallization of Merck's commercial convallarin, obtained an amorphous glycoside which he regarded as uniform, to which he gave the formula $C_{25}H_{38}O_9$. On hydrolysis it gave crystalline convallaretin, $C_{19}H_{28}O_4$, and a sugar, probably glucose. In a later paper (125) the isolation of three different amorphous glycosides from Merck's convallarin was reported. Jacobs and Hoffmann (85) isolated from the roots a crude, highly active, amorphous substance which, however, did not give the nitroprusside reaction. Finally, Karrer (94) isolated from the flowers a highly active crystalline glycoside, convallatoxin, which appeared to be different from any of those previously reported. It is definitely placed in the category of the cardiac glycosides by giving a positive nitroprusside reaction. A negative Keller–Kiliani test indi-

[1] In view of the recent conversion of scillaridin A into allocholanic acid by Stoll, Hoffmann, and Helfenstein (155), a revision of the formulas for scillaren A to $C_{36}H_{52}O_{13}$, for proscillaridin A to $C_{30}H_{42}O_8$, and for scillaridin A to $C_{24}H_{30}O_3$ is strongly indicated.

cated that it was not a glycoside of a deoxysugar. The compounds previously reported should be accepted with caution until a thorough reinvestigation of the convallaria glycosides is made.

E. Ranunculaceae

Adonis vernalis. An extract of this plant has been used since the time of Hieronymus Tragus as a remedy in various ailments of the heart. Cervello (12) isolated an amorphous glycoside to which he gave the name adonidin. Mordagne (133) obtained the substance in a partly crystalline form. Fuckelmann (33) in an exhaustive investigation isolated two amorphous glycosides, the so-called neutral adonidin and adonidinic acid. Both gave a Keller–Kiliani test indicating the presence of deoxysugars. Fromherz (32) corroborated Fuckelmann and definitely placed the glycosides in the digitaloid category by observing a positive nitroprusside reaction.. Mercier and Mercier (128, 129) in an extensive study confirmed Fuckelmann's adonidin and renamed it adonidoside. However, they concluded that his adonidinic acid was a mixture of a new glycoside, adonivernoside, and an acid decomposition product of the latter. The two glycosides were separated by taking advantage of the relative solubility of adonidoside in water. Thus the existence of two glycosides in *Adonis vernalis* seems to have been definitely demonstrated. No crystalline aglycone has been obtained from either.

Kromer (119) obtained from *Adonis aestivalis* a glycoside which showed many similarities to adonidin. He named it adonin and suggested the formula $C_{25}H_{40}O_{10}$. On hydrolysis, which was accomplished by a mere trace of acid, an amorphous aglycone and glucose were obtained. However, it would appear likely that in view of the easy hydrolysis, plus the positive Keller–Kiliani test, a deoxysugar may be a part of the molecule. Kiefer (97) has also investigated adonin.

Tahara (163) and Inoko (45) have described a glycoside obtained from *Adonis amurensis,* which appeared from pharmacological data to be different from the previously reported adonidin or adonin. Analysis indicated a formula $C_{24}H_{10}O_9$ and the ease of hydrolysis was again noted.

Helleborus species. Infusions of hellebore have been used in medicine since the time of Hippocrates. Despite this, the chemistry of the active principle remains obscure. Von Huseman and Marmé (44) isolated from the roots of *Helleborus niger, H. viridis,* and *H. foetidus* two glycosides, helleborein and helleborin. However, helleborein was subsequently shown to be responsible for the cardiac action, while helleborin possessed only the usual hemolytic properties of saponins. They assigned to helleborein the formula $C_{26}H_{44}O_{15}$. On hydrolysis of helleborein they obtained an amorphous aglycone, helleboretin, $C_{14}H_{20}O_3$, and two molecules of a

hexose, probably glucose. Thaeter (168) obtained helleborein in the crystalline state, revised the formula to $C_{37}H_{56}O_{18}$, and upon hydrolysis obtained amorphous helleboretin, $C_{19}H_{30}O_5$, two molecules of glucose, and three molecules of acetic acid. Keller (96) confirmed the presence of both glycosides, and Delattre (20) confirmed Thaeter's formulation. Sieburg (153) made a more exhaustive study of the substance. He readjusted the formula to $[C_{19}H_{31}O_8 \cdot OOCCH_3]_3$. On hydrolysis his products were glucose, arabinose, acetic acid, and two amorphous aglycones,—an acid helleboretin, derived perhaps by saponification of a labile lactone during manipulation, and a neutral helleboretin, $C_{15}H_{24}O_3$. He made the further interesting observation that in addition to its digitaloid properties, helleborein also possesses weak saponin-like characteristics. Thus, it is weakly hemolytic and shows a tendency to foam in solution and to be precipitated by cholesterol.

From the *Anemone hepatica*, another *Ranunculacea*, Delattre (20) has reported a crystalline glycoside. However, nothing is known of its composition.

F. Miscellaneous species

In the East Indies there grows the stately upas tree, or *Antiaris toxicaria*, of the family *Moraceae*, which towers above its neighbors, and which has long been held in awe by the natives because of the poisonous properties of its sap. The latter is the chief constituent of the Ipooh arrow poison. Pelletier and Caventou (138) gave the material a superficial study, and Mulder (134) isolated the active principle in crystalline form and named it antiarin. De Vry and Ludwig recognized its glycosidic character (22). Later Kiliani (99) in a series of studies reported the isolation of two crystalline glycosides, α- and β-antiarin, to both of which he assigned the formula $C_{27}H_{40}O_{10}$. On hydrolysis both yielded in small amounts the same crystalline aglycone, antiarigenin, to which Kiliani gave the formula $C_{21}H_{28}O_5$. The sugar component of the two was found to be different. Neither sugar is a deoxyhexose, which accounts for the small yield of aglycone. β-Antiarin yielded rhamnose, and the α-glycoside gave an isomer of rhamnose, antiarose. However, it seems probable that when a more careful investigation of antiarin can be performed, the formulas of the glycosides and genin will require revision.

Schlagdenhauffen and Reeb (148) isolated an amorphous glycoside, coronillin $(C_7H_{12}O_5)_n$, from the seeds of *Coronilla scorpioides*, *C. glauca*, and *C. montana* of the family of *Leguminosae*. On hydrolysis, glucose and an amorphous coronillëine $(C_8H_{18}O_7)_n$ were obtained. Tanret (166) obtained the same glycoside and reported that it gave a positive Keller–Kiliani test, but upon hydrolysis he obtained only glucose and an amorphous genin.

He suggested the formulas $C_{23}H_{33}O_{10}$ and $C_{17}H_{23}O_5$ for the glycoside and genin, respectively. Hydrolysis with *Aspergillus niger* gave a crystalline aglycone, $C_{14}H_{18}O_3$, and glucose.

Two members of the *Celastraceae* family have yielded active glycosides. Plugge (143) obtained a crystalline glycosidic substance, rabelaisin, from *Rabelaisia philippinensis*. Prescott (145) confirmed Wenzell's isolation of an amorphous glycoside from the root of *Euonymus atropurpurea* (179). Rogerson (147) failed to obtain the glycoside but did get a crystalline substance, $C_{21}H_{30}O_4$, which possessed an intensely bitter taste and gave a positive Liebermann–Burchard reaction. In the aqueous mother liquors from this, glucose was found. This might be taken to suggest that Rogerson's substance was in reality the aglycone, particularly in view of the rather drastic conditions to which he subjected the crude extract which might have caused hydrolysis of the glycoside.

A glycoside reported to possess both hemolytic and digitaloid properties is the mowrin isolated from the seeds of *Bassia longifolia* of the *Sapotaceae* family (132). It was given the formula $C_{51}H_{84}O_{32}$; on hydrolysis it gave glucose and amorphous mowric acid.

III. THE AGLYCONES

The structures of strophanthidin, periplogenin, digitoxigenin, and gitoxigenin have been determined with a reasonable degree of certainty. A beginning has been made in the study of scillaridin A and the toad venoms. Inasmuch as the latter substances show some differences from the four former ones, they will be treated separately. The structure of uzarigenin is known except for the positions occupied by two of the hydroxyl groups, the two which are removed during the hydrolysis of uzarin. The structures of the remaining members of the group are at present unknown, although it is probably safe to predict that, in general, they will be found to conform to the same pattern as those which have been studied in detail.

Certain structural features are common to the members of the strophanthidin group of aglycones and are highly characteristic of these substances. These will be discussed first, then the ring system will be treated, and finally the characteristic features of the individual aglycones will be taken up.

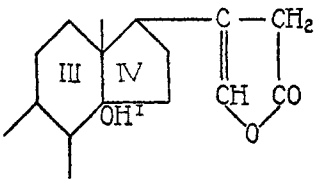
A. The lactone group

The presence of a saponifiable group in certain of the aglycones was first noted by Arnaud (2, 4, 5). He succeeded in isolating crystalline barium salts after saponification of digitoxin, ouabain, and tanghinine, and considered that an anhydride was present in the molecule. Feist (29) showed

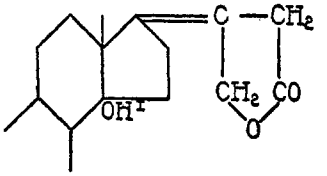
the saponifiable group to be a lactone. The more recent investigations of Jacobs and coworkers have demonstrated that the lactone consists of a side chain of four carbon atoms which constitutes the most characteristic portion of the molecule. The double bond (the only ethylenic unsaturation in the aglycones aside from that produced by secondary cleavage of hydroxyl groups during hydrolysis of certain glycosides such as ouabain and uzarin) is found in this side chain. This double bond can be catalytically reduced to form the dihydroaglycones. The whole side chain can be directly oxidized to a carboxyl group with loss of three carbon atoms in trianhydrostrophanthidin, and in certain other derivatives by a more indirect process. Moreover, the intensity of the physiological activity of the glycosides appears to depend to a large extent on the presence of this unsaturated side chain (117, 191b). Many of the typical reactions of the aglycones, such as the production of a red color with alkaline nitroprusside solution (the Legal reaction), reduction of Tollen's reagent, and the presence of an "active" hydrogen as shown by the Zerewitinoff test, have been shown to be conditioned by the presence of this unsaturated lactone side chain (82). These properties are all characteristics of $\Delta^{\beta,\gamma}$ unsaturated lactones; they vanish in the dihydroderivatives, which also display a marked diminution in physiological activity.

The nature of this side chain has been shown to be that of a lactone of an enolized γ-aldehydo acid attached by its β-carbon atom to the main tetracyclic ring system. This has been elucidated by the results of a study by Jacobs and coworkers of the influence of alkali on the aglycones whereby the highly characteristic isoaglycones are formed. Isostrophanthidin will be taken as typical for the present discussion. When strophanthidin is dissolved in alcoholic alkali and the solution, after standing, acidified, an isomeric substance, isostrophanthidin, is produced (29, 54, 193). This compound, while still possessing a lactone group, no longer gives the Legal reaction, nor can it be catalytically hydrogenated. Furthermore, dihydrostrophanthidin does not undergo this change, hence the isomerization must be conditioned by the presence of the double bond. The explanation of the nature of the transformation to isostrophanthidin furnished the key to the interrelationship of the lactone group, the double bond, and one of the hydroxyl groups of strophanthidin. This is shown in formulas I to III. The change is not due to liberation of the free aldehyde group on saponification of strophanthidin followed by subsequent lactal formation, since preliminary saponification is not necessary in order that the strophanthidin–isostrophanthidin change may occur. Rather the explanation would seem to lie in a rearrangement involving a shift of the double bond. By assuming that in the original aglycone the unsaturated lactone side chain is *trans* to the hydroxyl group involved in the new oxidic bridge (OH^1) (I), a shift of the $\Delta^{\beta,\gamma}$ double bond to the position shown in formula II would

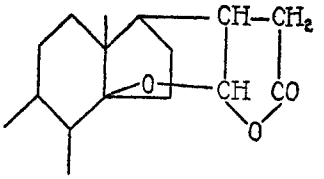
permit a *cis* rearrangement with consequent formation of the new oxidic bridge as shown in formula III. The new oxidic bridge involving OH^1 restricts the position of the latter group to one either γ or δ to the enolized aldehyde group of strophanthidin. As will be shown later, the δ-oxidic structure is preferable. After saponification of the lactone group of isostrophanthidin, the hydroxyaldehyde may exist in either the lactal (formula IV) or free aldehydic form. The existence of a free aldehyde group in saponified isostrophanthidin has been directly demonstrated by the formation of a semicarbazone in the case of an appropriate derivative of isostrophanthidin (70). A derivative of isodigitoxigenin has also yielded a similar semicarbazone (73). The lactal character of saponified isostrophanthidin (IV) is clearly demonstrated by its oxidation, with consumption of one atom of oxygen, to a new lactone as in α-isostrophan-



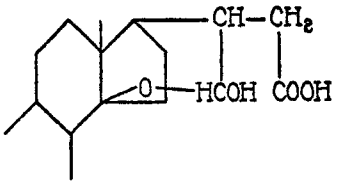
I



II



III
Isostrophanthidin



IV

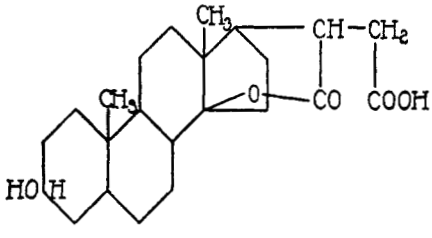
thidic acid (70). Further, the existence of the hydroxyaldehyde in both the free aldehydic and lactal forms is strikingly shown by the selective action of permanganate and hypobromite. The former acts specifically on the aldehyde group, whereas the latter oxidizes the lactal directly to the lactone (70).

Complementing these observations, oxidative degradation of the side chain, which will be treated in detail later, definitely fixed the number of carbon atoms comprising it.

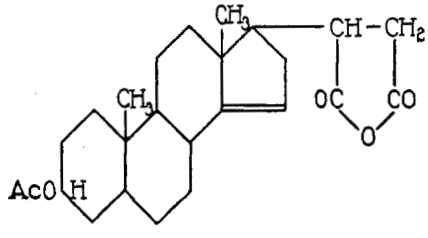
B. The ring system

Although the view has often been expressed by workers in the field that the cardiac aglycones are closely related to the sterols and bile acids, until very recently there has been no direct proof for such a statement, beyond

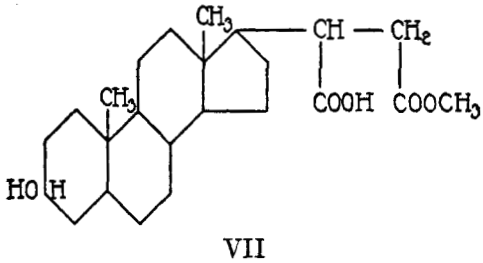
the fact that they are, like the sterols, reduced tetracyclic compounds. However, a direct relationship has now been demonstrated. The first evidence corroborating this view was forthcoming in the production of Diels' hydrocarbon (methylcyclopentenophenanthrene) upon dehydrogenation of strophanthidin (26) and of dianhydrouzarigenin (174) with



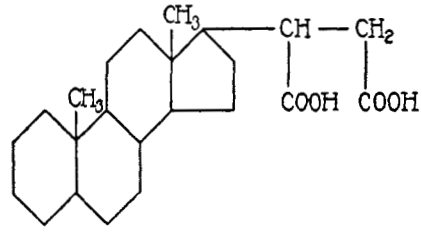
V
Isodigitoxigenic Acid



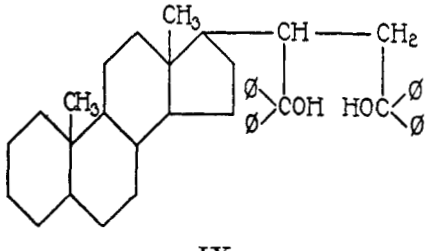
VI



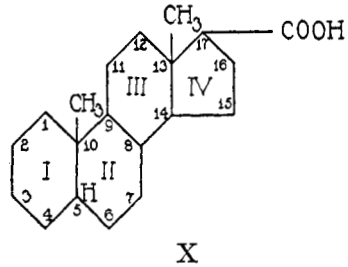
VII
Digitoxanoldiacid methyl ester



VIII
Digitoxandiacid



IX



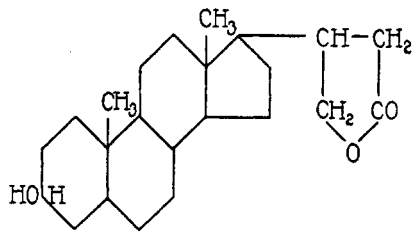
X
Etiocholanic acid

selenium. This compound has come to be regarded as a product characteristic of the sterols when they are subjected to the dehydrogenating action of selenium. Thus, it was indicated that, barring pyrolytic rearrangements during the course of the dehydrogenation, the two groups of substances did possess a common skeleton.

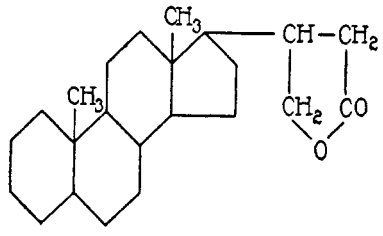
More recently a more direct proof has been provided—one which in

addition to furnishing decisive evidence on this point, also proved the point of attachment of the lactone side chain of the aglycone.

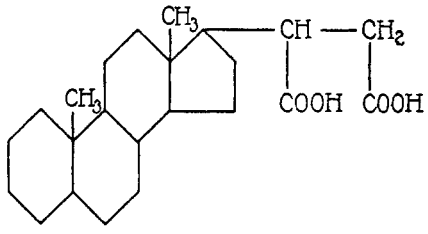
Jacobs and Elderfield (65) degraded a derivative of digitoxigenin to a saturated acid, $C_{20}H_{32}O_2$, which proved to be identical with etiocholanic acid as obtained by a similar degradation of the side chain of cholanic acid by Wieland, Schlichting, and Jacobi (184). The series of steps involved was as follows: Isodigitoxigenic acid (V) on treatment with acetic anhydride–acetyl chloride suffers cleavage of the lactone and removal of water, together with acetylation of the secondary hydroxyl group to yield the acetate of digitoxenoldiacid anhydride (VI) (77). This on saponification yielded digitoxenoldiacid, which as the half-ester was hydrogenated to



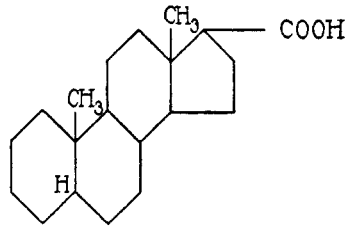
XI



XII



XIII



XIV

digitoxanoldiacid monomethyl ester (VII). After saponification of the ester group of the latter the remaining secondary hydroxyl group was then removed by oxidation to the ketone and reduction by Clemmensen's method giving digitoxandiacid (VIII) (65). The dimethyl ester of this acid was then degraded with loss of three carbon atoms of the side chain via the bis-diphenyl carbinol (IX), using the method of Wieland, Schlichting, and Jacobi (184), to yield a monobasic acid, $C_{20}H_{32}O_2$ (X), identical with etiocholanic acid.

A similar degradation of a fully hydrogenated derivative of dianhydrouzarigenin was simultaneously carried out independently by Tschesche (171). Starting with one of the isomeric saturated lactones (XI) obtained

by hydrogenation of dianhydrouzarigenin, the remaining secondary hydroxyl group was replaced by hydrogen to yield the deoxylactone (XII). This on vigorous oxidation with chromic acid gave a dibasic acid (XIII) which, on degradation by the method used in the case of digitoxandiacid, led to an acid $C_{20}H_{32}O_2$ (XIV). This acid, however, proved not to be etiocholanic acid but was identical with the spatially inverted isomer thereof, alloetiocholanic acid, which Tschesche prepared by degradation of the side chain of hyodeoxycholic acid (172).

Thus, independent proof was forthcoming from both laboratories not only of the conclusion that the carbon skeleton of the cardiac aglycones is identical with that of the sterols and bile acids, but also that the lactone side chain of the former is a fragment of the side chain of the latter and is attached to the skeleton at the same point, namely carbon atom 17.

Having established the ring system of the aglycones and the nature of the unsaturated lactone side chain, the individual characteristics of the various aglycones and their interrelationships will be discussed. In this connection, references will be given to communications in which the original observations were made, although it must be borne in mind that as the formulation of the aglycones underwent modification as additional evidence accumulated, such observations have of necessity undergone some reinterpretation (65).

C. Strophanthidin

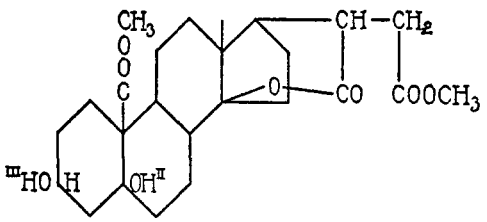
In addition to the lactone side chain, strophanthidin contains three hydroxyl groups and a carbonyl group. The carbonyl group has been shown to be in the form of an aldehyde, since oxidation with permanganate leads to the formation of an acid without degradation or ring cleavage but with disappearance of the carbonyl function (57). Of the hydroxyls, one (OH^{III}) has been shown to be secondary through the formation of a monoketone on oxidation with chromic acid (72). The remaining hydroxyl groups are tertiary.

One of the two tertiary hydroxyl groups (OH^{I}) must be involved in the strophanthidin–isostrophanthidin change, since appropriate derivatives of isostrophanthidin on oxidation still yield monoketones and hence retain the secondary hydroxyl group (70). This tertiary hydroxyl group must, in addition, be either γ or δ to the enolized aldehyde group concerned in the unsaturated lactone side chain in order to account for the formation of the lactals and lactones characteristic of the isostrophanthidin series. These conditions restrict the position occupied by this hydroxyl group to carbon atom 14.

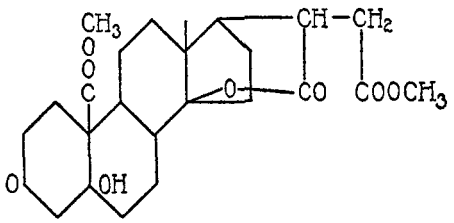
The remaining tertiary hydroxyl group (OH^{II}) must bear a 1:3 relationship to the secondary hydroxyl group (OH^{III}) for the following reasons.

α-Isostrophanthic dimethyl ester (XV) on oxidation with chromic acid gives a ketone, α-isostrophanthonic dimethyl ester (XVI), by oxidation of OH^{III} to carbonyl. In the latter compound OH^{II} has now become very labile and is easily removed as water (70), a property characteristic of β-hydroxyketones, to give anhydroisostrophanthonic ester (XVII). This demonstrates the relative positions of the two hydroxyl groups.

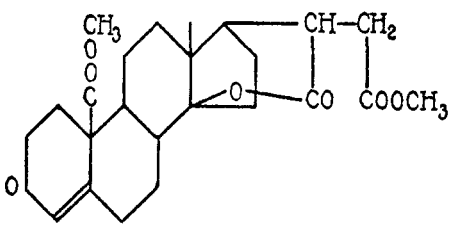
There remain to be proved the exact positions occupied by the aldehyde group, OH^{II}, and OH^{III}. The aldehyde group must be quaternary, for, as will be discussed later, (p. 217), it corresponds to one of the quaternary methyl groups of the sterols and other aglycones. This restricts its position to carbon atom 10 or 13. Carbon atom 13 is rendered improbable by



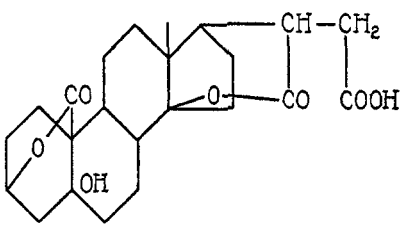
XV
α-Isostrophanthic dimethyl ester



XVI



XVII
Anhydroisostrophanthonic dimethyl ester



XVIII

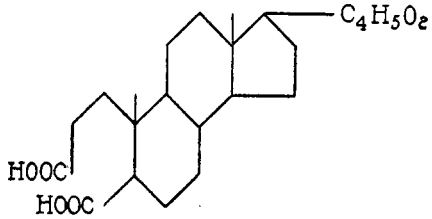
the observation, to be discussed at length later (p. 230), that in certain derivatives the aldehyde group, or carboxyl group derived from it on oxidation, is capable of undergoing lactal or lactone formation with OH^{I} (71) which has been placed definitely on carbon atom 14. Such lactals or lactones would then be β ones, a highly improbable arrangement. The alternative position, carbon atom 10, therefore remains as the point of attachment of the aldehyde group, which conclusion was substantiated by the following evidence.

The aldehyde group in β-isostrophanthidic acid, an isomer of the α substance of the same name which will be treated later (p. 216), is capable of existing as a lactal involving the secondary hydroxyl group, OH^{III} (71).

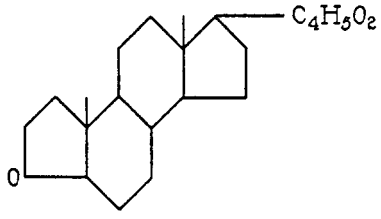
This lactal on oxidation leads to a lactone (XVIII). That OH^{III} is involved in this lactone is shown by its failure to give a ketone on oxidation. From this it follows that the aldehyde group must be in reactive proximity to OH^{III}, i.e., either γ or δ.

The final evidence needed definitely to place the aldehyde group, OH^{II}, and OH^{III} was supplied as follows. In a derivative of dihydrogitoxigenin (hexahydrodigitaligenin), wherein the hydroxyl groups have all been replaced by hydrogen save the secondary OH^{III} (which is common to all the aglycones), rupture of the ring bearing this hydroxyl group leads to a dibasic acid (XIX) (197). On pyrolysis of this acid a ketone (XX) was obtained which, in the light of the observations of Wieland and Dane (181) on the applicability of Blanc's rule to condensed ring systems, renders probable the fixing of OH^{III} on ring I.

This assumption was confirmed and OH^{III} definitely located on carbon atom 3 by the following series of reactions. Anhydroisostrophanthonic dimethyl ester (XVII) on ozonization suffers rupture of ring I with loss of a carbon atom in a manner similar to that obtaining in the oxidation of



XIX

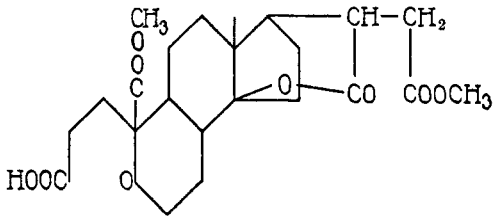


XX

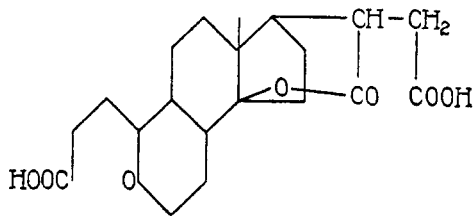
cholestenone, and a keto acid (undephanthontriacid) dimethyl ester (XXI) results (74). The latter, on being subjected to the action of weak alkali, undergoes the ketone decomposition of the β-ketonic ester group to give duodephanthondiacid (XXII). The latter on treatment with acetic anhydride–acetyl chloride is lactonized with the formation of the unsaturated lactone (XXIII) (79). Simultaneously, the isolactone on the other side of the molecule is cleaved, a change characteristic of the isoaglycone series (76). On catalytic hydrogenation, the unsaturated lactone is reduced to the saturated deoxyacid which, on saponification of the anhydride, gives dephanthanic acid (XXIV). This as the trimethyl ester, when subjected to the Wieland degradation over the trisdiphenylcarbinol (XXV), is degraded to dephanthic acid (XXVI) with the loss of four carbon atoms, three of which are accounted for by removal of the lactone side chain and the fourth by shortening of the side chain representing the fragment of ring I (64).

This series of reactions at once shows the presence of three consecutive secondary carbon atoms in the order $—CH_2—CHOH—CH_2—$, which

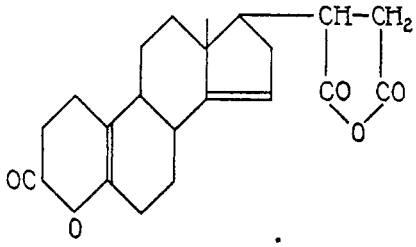
restricts the ring bearing OH^{III} to a terminal ring, ring I. It further follows that OH^{III} must be on carbon atom 3 and that OH^{II}, in order to be tertiary and in the β-position to OH^{III}, must be located on carbon atom



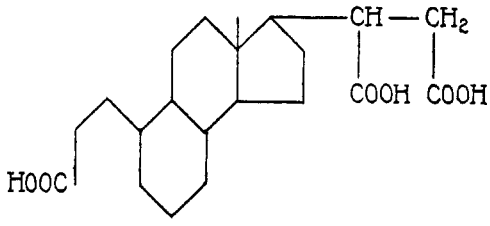
XXI
Undephanthontriacid dimethyl ester



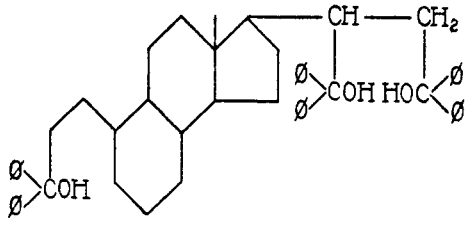
XXII
Duodephanthondiacid



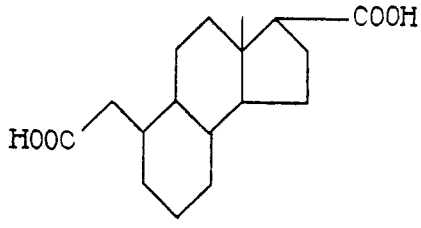
XXIII



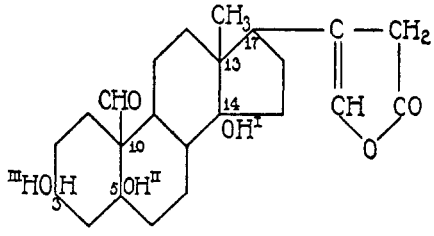
XXIV
Dephanthanic acid



XXV



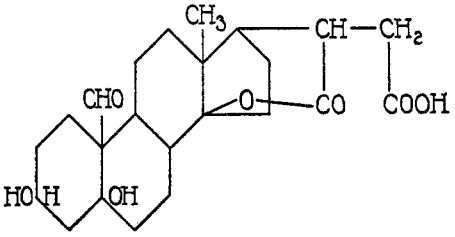
XXVI
Dephanthic acid



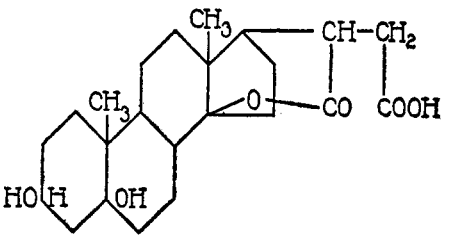
XXVII
Strophanthidin

5. These conclusions as to the relative positions of the aldehyde group and the hydroxyl groups have been confirmed by a study of the hydroxy acids formed by the use of the cyanohydrin reaction on the aldehyde group of dihydrostrophanthidin and its anhydro derivatives (67). In these acids the new carboxyl group no longer reacts with OH^{I} or OH^{III} but does lactonize on OH^{II} to which it is now γ. Strophanthidin therefore possesses the structure shown in formula XXVII.

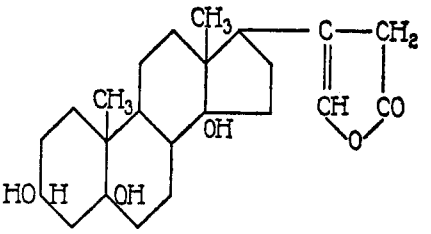
In discussing the positions occupied by the substituent groups in the other aglycones, it will be profitable to consider at the same time their interrelationships and transformations from one to another.



XXVIII
α-Isostrophanthidic acid



XXIX
Isoperiplogenic acid



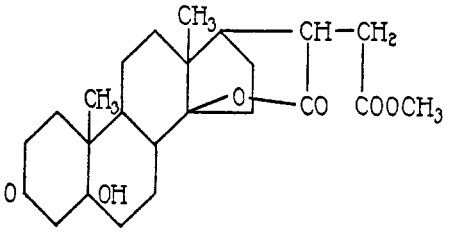
XXX
Periplogenin

D. Periplogenin

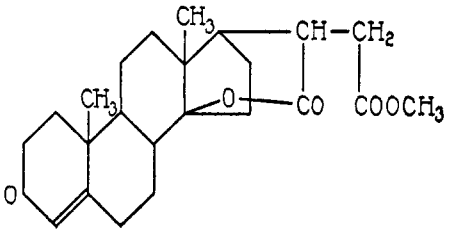
Jacobs and Elderfield (58) have correlated this aglycone with strophanthidin. α-Isostrophanthidic acid (XXVIII), on conversion into the semicarbazone and reduction of the latter by the Wolff–Kishner method, yields a substance wherein the aldehyde group has been replaced by methyl (XXIX). This substance is identical with isoperiplogenic acid, from which it at once follows that periplogenin differs from strophanthidin only in having a methyl group in place of the aldehyde group of the latter. The hydroxyl groups being in the same positions in both aglycones, periplogenin is represented by formula XXX.

E. Digitoxigenin

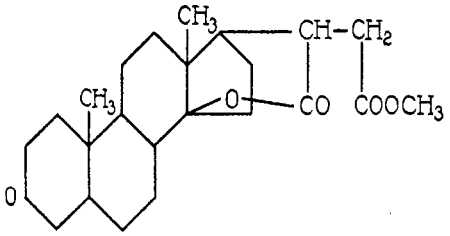
The same workers have also demonstrated the exact relationship of this aglycone to periplogenin and therefore to strophanthidin (59). Isoperiplogenic acid (XXIX), as the methyl ester, on oxidation with chromic acid yields isoperiplogonic methyl ester (XXXI). In the latter, as in the case of the analogous isostrophanthonic ester, OH^{II} has become labile and is easily split out, giving anhydroisoperiplogonic methyl ester (XXXII). This substance on catalytic hydrogenation gives a mixture of stereoisomers, one of which is identical with isodigitoxigonic methyl ester (XXXIII), the corresponding derivative of digitoxigenin. Digitoxigenin therefore is represented by formula XXXIV and differs from periplogenin in lacking OH^{II}.



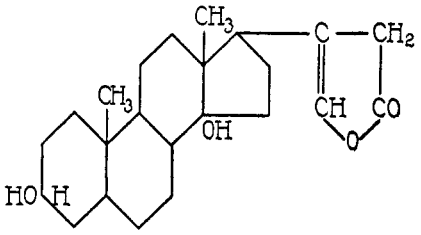
XXXI



XXXII



XXXIII
Isodigitoxigonic methyl ester



XXXIV
Digitoxigenin

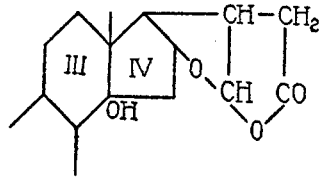
Inasmuch as digitoxigenin was converted into etiocholanic acid by degradation of the side chain (65), it follows from these interrelationships that the aldehyde group of strophanthidin occupies the position of one of the quaternary methyl groups of cholanic acid and specifically, as has been shown, this is carbon atom 10.

F. Gitoxigenin

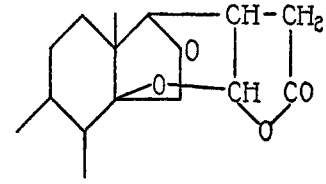
In some respects gitoxigenin shows certain divergences in its reactions from the three above-mentioned aglycones. These differences concern dihydrogitoxigenin and isogitoxigenin in particular.

Gitoxigenin is a trioxylactone, isomeric with periplogenin but differing from the latter in the position and character of one of the hydroxyl groups. This hydroxyl group is tertiary in periplogenin but secondary in gitoxigenin, as shown by the formation of a diketone, gitoxigenone, on oxidation. Both genins contain the secondary hydroxyl group (OH^{III}). The position to be assigned to the extra secondary hydroxyl group of gitoxigenin has been determined from a study of isogitoxigenin by Jacobs and Gustus (75) and of dihydrogitoxigenin by Jacobs and Elderfield (63).

When gitoxigenin is dissolved in alcoholic alkali and then reacidified, an iso compound is formed which differs in several respects from the usual isogenins, such as isostrophanthidin, isoperiplogenin, and isodigitoxigenin. Contrasting with these substances, it does not react with the usual aldehyde reagents after saponification of the lactone group. The lactal is unusually stable. After oxidation with hypobromite to isogitoxigenic acid, the lactone group of the latter substance is relatively resistant to hydrolysis in contrast to the behavior of the corresponding derivatives of the other



XXXV



XXXVI

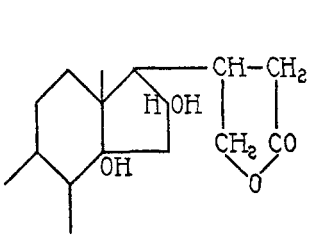
isogenins. The cause for this anomalous behavior lies in the fact that in the formation of isogitoxigenin the tertiary hydroxyl group which enters into the new oxidic ring in the other isoaglycones does not so function in this case. Instead, the extra secondary hydroxyl group of gitoxigenin is involved, as shown by the fact that isogitoxigenin gives only a monoketone on oxidation. This observation restricts the position of this secondary hydroxyl group to one of reactive proximity (γ or δ) to the enolized aldehyde group of the side chain.

However, when gitoxigenin is oxidized with chromic acid, the resulting diketone, gitoxigenone, no longer gives the Legal reaction. Oxidation experiments showed it to be a true isoaglycone wherein the tertiary hydroxyl group is involved in the new oxidic ring. This observation restricts the position of this hydroxyl group also to one of reactive proximity to the enolized aldehyde group of the side chain. These facts find expression in formula XXXV for isogitoxigenin and formula XXXVI for gitoxigenone.

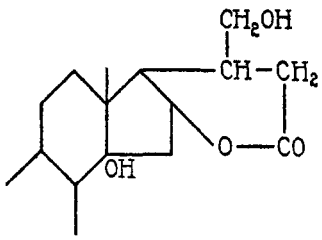
In view of the relationship of gitoxigenin to digitoxigenin, it appears better to designate gitoxigenone as oxoisodigitoxigenone. The rearrange-

ment involved in the formation of isogitoxigenin parallels that accompanying the formation of the other isoaglycones and apparently is due to the same factor, viz., a shift of the double bond of the side chain under the influence of alkali so that the side chain becomes *cis* to the hydroxyl group on carbon atom 16. In the case of oxoisodigitoxigenone, the presence of a carbonyl group on carbon atom 16 can also induce a similar shift of the double bond to a position α, β to the new carbonyl group with rearrangement as above to a *cis* configuration and consequent formation of the new oxidic linkage.

The results of a study of the hydrogenation of gitoxigenin confirm this interpretation. Cloetta (18) noted that gitoxigenin is hydrogenated to two substances, which Windaus, Westphal, and Stein (197) showed to be isomeric dihydrogitoxigenins. The nature of their isomerization was explained by Jacobs and Elderfield (63). Both dihydrogitoxigenins show mutarotation during which they are mutually interconvertible, a fact which suggested a rearrangement involving the lactone group. This view was



XXXVII



XXXVIII

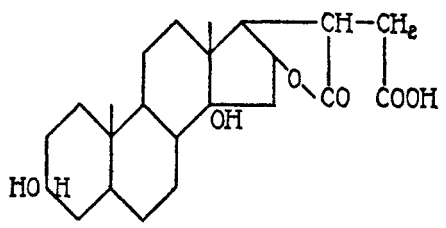
substantiated by the preparation of two isomeric dihydrogitoxigenin diacetates, neither of which exhibits mutarotation and which are not mutually interconvertible. It follows from this that in the isomerization of the normal α-dihydrogitoxigenin to the β form, the lactone bridge has shifted to the secondary hydroxyl group, as shown in formulas XXXVII and XXXVIII, respectively.

The placing of the tertiary hydroxyl group in a position 1:3 to the secondary one was confirmed by the behavior of the dihydrogitoxigenone derived from α-dihydrogitoxigenin. This substance exhibited the properties of a β-hydroxyketone, the tertiary hydroxyl group having become labile and easily removed as water.

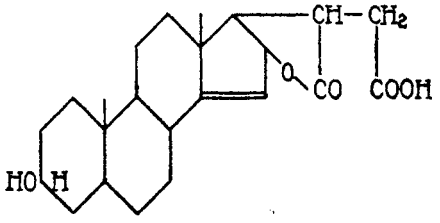
The correlation of gitoxigenin with digitoxigenin, while involving some difficulties, was accomplished by Jacobs and Gustus (77). Windaus and coworkers (191) had previously attempted to show the relationship between the two aglycones. Their procedure consisted in replacement of all of the hydroxyl groups, except OH^{III}, of both genins by hydrogen and

comparison of the fully saturated lactones thus obtained. However, the two lactones proved to be isomeric and not identical.

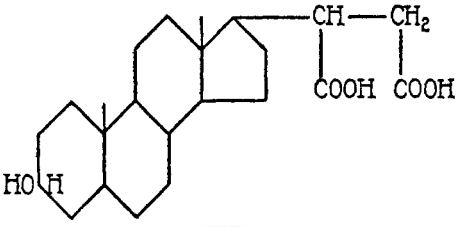
The former workers started with isogitoxigenic acid (XXXIX). On treatment with hydrochloric acid, the tertiary hydroxyl group of this substance was replaced by chlorine, which was then removed by alcoholic alkali to give an unsaturated hydroxylactone acid (XL). On catalytic hydrogenation of this substance, in addition to saturation of the double bond, the lactone group was opened to the saturated deoxyacid (XLI). The dimethyl ester of this acid proved to be identical with that obtained from the previously discussed digitoxanoldiacid (VII). Thus it was proved



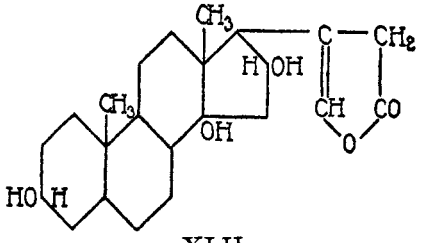
XXXIX
Isogitoxigenic acid



XL



XLI



XLII
Gitoxigenin

that gitoxigenin is hydroxydigitoxigenin, and further, that the secondary hydroxyl group on carbon atom 3 is common to both aglycones. Gitoxigenin is therefore represented by formula XLII.

G. Uzarigenin

As has already been noted, the substance obtained on hydrolysis of uzarin is a dianhydro derivative of the aglycone. Because of this fact, the positions occupied by the hydroxyl groups are not definitely known, with the exception of a secondary hydroxyl group on carbon atom 3. This hydroxyl group is the one retained in dianhydrouzarigenin, and its likely allocation on carbon atom 3 has been demonstrated by Tschesche (170). On oxidative cleavage of hexahydrodianhydrouzarigenin a ring at the

hydroxyl group in question is opened to give a dibasic acid. This on pyrolysis gives a pyroketone with loss of a carbon atom in a manner paralleling the reactions of hexahydrodigitaligenin.

Dianhydrouzarigenin has also been correlated with periplogenin by Tschesche (170). One of the isomeric hexahydrodianhydrouzarigenins on replacement of the remaining hydroxyl group by hydrogen gave the so-called fully saturated "α_2 lactone." This was identical with the corresponding fully saturated lactone, octahydrotrianhydroperiplogenin, prepared by Jacobs and Bigelow (52).

There is also evidence suggesting that uzarin contains an hydroxyl group on carbon atom 5. This is indicated by the production of etiocholanic acid from digitoxigenin, an aglycone which is unsubstituted at carbon atom 5, contrasting with the formation of alloetiocholanic acid by a similar degradation of hexahydrodianhydrouzarigenin. This may indicate that in uzarin one of the hydroxyl groups which is removed during the hydrolysis of the glycoside is on carbon atom 5. On hydrogenation of the double bond thus produced, addition to this carbon atom may occur in such a way as to lead to the spatially inverted isomer of etiocholanic acid. However, this type of evidence is far from conclusive since, as will be discussed later (p. 223), extensive wandering of double bonds occurs in the anhydroaglycones.

H. Ouabagenin

As previously noted, ouabain is a rhamnose glycoside and as such, in contrast to the glycosides of the deoxysugars, requires such severe hydrolytic treatment as to cause dehydration and decomposition of the aglycone. Consequently the aglycone has never been isolated as such. However, Jacobs and Bigelow (50) by use of a special technique have succeeded in isolating derivatives of the aglycone. Arnaud (2) described an anhydroheptaacetate of ouabain obtained by the action of acetic anhydride and zinc chloride. This substance contains two double bonds in addition to the side chain one, as shown by its hydrogenation to deoxydihydroouabain heptaacetate (50). The latter compound on acetolysis led to a crystalline cleavage product, $C_{24}H_{30}O_4$, which, on the basis of saponification and hydrogenation experiments, was shown to be the monoacetate of a trianhydrolactone. However, the acetyl-free substance was a C_{22} compound instead of the expected C_{23} derivative which would bring it into conformity with the other C_{23} genins. An explanation for this discrepancy was found when formaldehyde was isolated from the acetolysis mother liquors. It is quite probable that ouabain contains a primary alcoholic group, on carbon atom 10 or 13, corresponding to one of the quaternary methyl groups of the sterols, and that during the acetolysis this carbon atom is lost as formaldehyde.

In another study, Jacobs and Bigelow (51) have further shown the close similarity between ouabain and the other glycosides. Ouabain as the glycoside is isomerized to an iso compound under the influence of alkali with disappearance of the Legal reaction. Attempts to hydrolyze this compound again resulted in the decomposition of the aglycone. However, when acetylation of isoouabain with acetic anhydride in the presence of a trace of sulfuric acid was attempted, the glycosidic linkage was cleaved, a carbon atom was lost as formaldehyde as in the acetolysis of the above heptaacetate, three molecules of water were lost, and the monoacetate of a trianhydrohydroxylactone resulted. The acetyl group was easily removed on saponification to give a hydroxylactone, $C_{22}H_{26}O_4$, but the three double bonds were resistant to catalytic hydrogenation, thereby suggesting a benzenoid arrangement as in the case of trianhydrostrophanthidin (to be discussed later, p. 224).

On saponification of the trianhydrohydroxylactone an acid was isolated as the methyl ester, $C_{23}H_{30}O_5$. The presence of a lactal as well as a secondary hydroxyl group in this substance was shown by its oxidation with chromic acid to a ketolactone ester. This paralleled the experience with the other isoaglycones. The hydroxyl group which emerged from the acetolysis reaction was also shown to be secondary and presumably identical with OH^{III} of strophanthidin and related aglycones.

Unfortunately, a more exact correlation of the aglycone of ouabain with other members of the group has not been accomplished, owing to its degradation to a C_{22} derivative during removal of the sugar component.

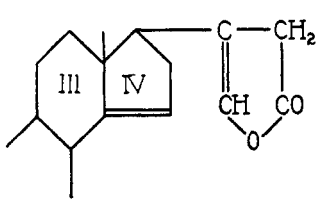
I. Anhydro derivatives of the cardiac aglycones

When strophanthidin is allowed to stand in alcoholic hydrogen chloride solution, a sparingly soluble dehydration product separates. This was shown by Jacobs and Collins (53) to be the ethyl half-acetal of monoanhydrostrophanthidin. Under the influence of the reagent the aldehyde group of strophanthidin forms an internal cyclic acetal with OH^{III} in a manner analogous to the formation of simple glycosides of the sugars. Hydrolysis with dilute acid removes the ethylal group, giving the hydroxyaldehyde which exists in either the free aldehydic or lactal form. The identity of the hydroxyl group (OH^{I}), which is removed, was shown by the failure of monoanhydrostrophanthidin to yield an iso compound of the usual type under the influence of alkali.

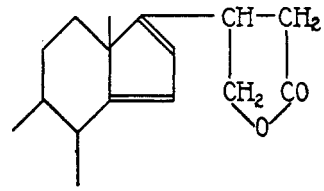
However, the ethylal of monoanhydrostrophanthidin when saponified with alkali leads to an aldehydo acid, the nature of which was shown by the formation of an oxime (56). On reacidification, the ethylal is hydrolyzed, and, in addition, lactonization occurs, not, however, with the reformation of monoanhydrostrophanthidin, but with the formation of an

isomeric substance which no longer gives the Legal reaction (66). The nature of this isomerization is explained by a shift of the $\Delta^{\beta,\gamma}$-lactone double bond from its original position in monoanhydrostrophanthidin (XLIII) to a position, conjugate with the new double bond arising from loss of OH^{I}, which would then permit relactonization to occur in the formation of isomonoanhydrostrophanthidin (XLIV or XLV).

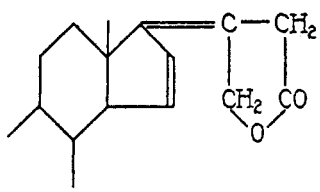
When monoanhydrostrophanthidin or strophanthidin itself is boiled with alcoholic hydrogen chloride, the ethylal of dianhydrostrophanthidin results. This compound arises from loss of both OH^{I} and OH^{II}. In its reactions it resembles very closely the monoanhydro derivative, except in its behavior after saponification of the lactone group. The aldehydo acid



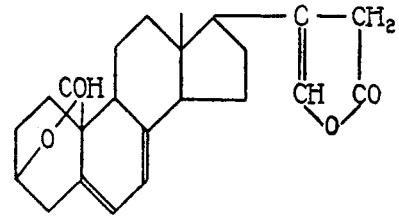
XLIII



XLIV



XLV



XLVI

thus obtained shows no tendency to relactonize. The explanation for this behavior apparently lies in the fact that the double bond formed by loss of OH^{I} in this case shifts to ring II in conjugation with that arising from loss of OH^{II} (formula XLVI). Influences tending to cause a shift of the side chain double bond are thus removed, and nothing is present which would facilitate relactonization of the lactone group when once opened.

The double bonds of both monoanhydro- and dianhydro-strophanthidin can be catalytically hydrogenated, that of the side chain being the last to be reduced (55). On oxidation of either of the anhydrostrophanthidins or of their reduced products, the lactal between the aldehyde group and OH^{III} is oxidized to a lactone, with the formation of dilactones. The unsaturated dilactones can be hydrogenated to the saturated ones, but the predominating stereoisomer obtained by this method is different from the

one which is obtained by reduction of the double bonds and subsequent oxidation of the lactal to the lactone (68). On exhaustive hydrogenation dianhydrostrophanthidin suffers reduction of the lactal to an alkylene oxide in addition to saturation of the double bonds. The same substance (octahydrotrianhydrostrophanthidin) is also obtained by Clemmensen reduction of hexahydrodianhydrostrophanthidin.

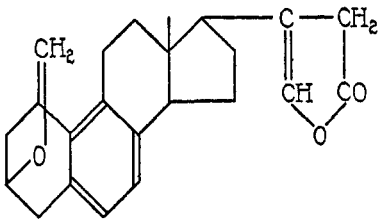
Upon treatment with concentrated hydrochloric acid, dianhydrostrophanthidin loses a third molecule of water and trianhydrostrophanthidin results (55). The formation of this substance is accompanied by loss of both the aldehydic and hydroxyl functions remaining in the dianhydrostrophanthidin molecule. This third molecule of water thus is apparently lost by dehydration of the lactal form of dianhydrostrophanthidin, with the formation of an alkylene oxide. At the same time, trianhydrostrophanthidin manifests several properties in striking contrast to those of its precursors. Whereas both monoanhydro- and dianhydro-strophanthidin can be completely catalytically reduced, the double bonds of trianhydrostrophanthidin (with the exception of the original $\Delta^{\beta,\gamma}$-lactone double bond) have resisted all attempts at catalytic hydrogenation. Trianhydrostrophanthidin does not add bromine to its double bonds as do its precursors, and on oxidation with permanganate in acetone, in contrast to dianhydrostrophanthidin, it suffers degradation of the lactone side chain with loss of three carbon atoms to a monobasic acid (69). On oxidation with chromic acid, a ring is opened with the formation, without degradation, of a dilactone acid. All of these reactions strongly indicated the presence of a benzenoid ring in trianhydrostrophanthidin, a fact which was confirmed by its ultra-violet absorption spectrum (25) and more recently by the production of benzenetetracarboxylic acid from it on oxidation with nitric acid (175).

The characteristic reactions of trianhydrostrophanthidin, which were studied rather early in the work on the aglycones, were largely the cause of misconceptions regarding their structure. For the logical deduction that oxidation of a side chain in an aromatic compound to a carboxyl group indicated attachment of such a side chain to an aromatic ring led to the placing of the lactone side chain of strophanthidin on a six-membered ring. Furthermore, a glance at the sterol skeleton with its two quaternary carbon atoms shows that the formation of a benzenoid ring in such a skeleton without rearrangement is impossible, and there had been no evidence of a rearrangement noted in the formation of trianhydrostrophanthidin. However, the direct correlation of digitoxigenin with cholic acid has made necessary a revision of the earlier ideas regarding trianhydrostrophanthidin. But one explanation is satisfactory—namely, that during its formation a sort of retropinacone rearrangement has occurred wherein the quaternary

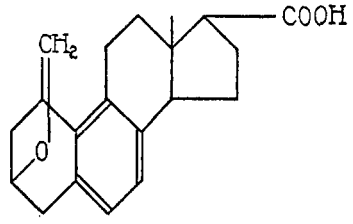
aldehyde group attached to carbon atom 10 wanders to carbon atom 1 (66, 175). There is some evidence suggesting that this rearrangement has already occurred during the formation of monoanhydro- or dianhydrostrophanthidin.

The structure of trianhydrostrophanthidin, therefore, is represented by formula XLVII; and the products obtained by oxidation of it with permanganate and chromic acid, by formulas XLVIII and XLIX, respectively. Other degradation products of trianhydrostrophanthidin have been described by Jacobs and Elderfield (66).

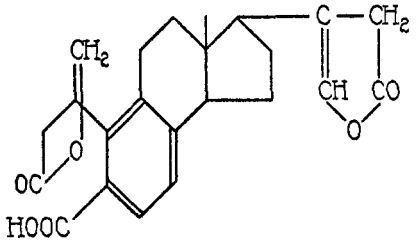
Dihydrostrophanthidin differs from strophanthidin in the lability of OH^{I}, the formation of a monoanhydro derivative of the former proceeding



XLVII
Trianhydrostrophanthidin



XLVIII



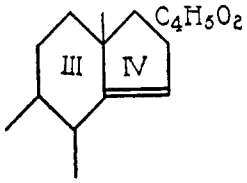
XLIX

with greater ease than in the case of the latter (85). A study of monoanhydrodihydrostrophanthidin (L) has been made by Jacobs and Elderfield (61). Alkaline permanganate oxidizes the aldehyde group to carboxyl, and simultaneously two hydroxyl groups are added to the double bond to give hydroxydihydrostrophanthidinic acid (LI). This substance as the methyl ester, on oxidation with chromic acid, suffers rupture of a ring with formation of a keto acid (LII), OH^{III} simultaneously being oxidized to a ketone. On catalytic reduction both ketone groups are reduced, and the hydroxy acid thus formed spontaneously lactonizes as the reduction proceeds (LIII). This easy lactonization, which presumably must involve either a γ- or δ-lactone, at once showed the position occupied

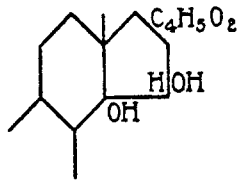
by the double bond in monoanhydrodihydrostrophanthidin under these conditions, inasmuch as ring IV is the only ring which can be opened to give a keto acid leading to a δ-lactone on reduction.

At the same time, it might be said that by the formation of a carboxyl group on carbon atom 15 in the above keto acid, independent proof was furnished of the presence of a CH_2 group at that point of the sterol skeleton, and also of the point of attachment of the side chain.

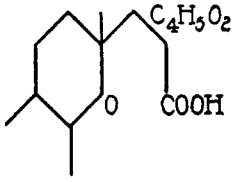
In another investigation, evidence has been obtained that in neutral or acid solution the double bond of monoanhydrodihydrostrophanthidin may also occupy the position between carbon atoms 8 and 14 (67). Oxidation of monoanhydrodihydrostrophanthidin in chloroform solution with per-



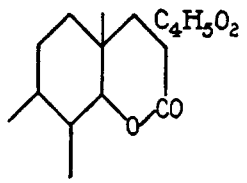
L



LI



LII

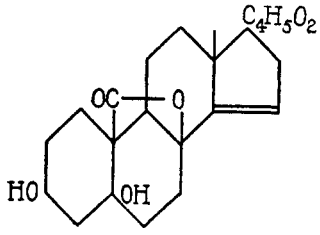


LIII

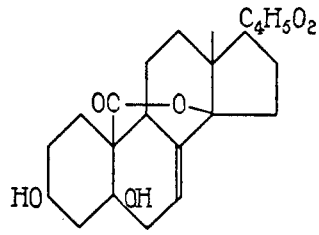
benzoic acid or with permanganate in acetic acid solution results in the formation of an ethylene oxide on the double bond. On hydration of this oxide, the resulting glycol promptly loses water in a variety of ways, depending on the form in which the aldehyde carbon atom happens to be. If the latter has been oxidized to a carboxyl group before opening the oxide, treatment with very weak acid (50 per cent acetic acid) gives an anhydrolactone (either LIV or LV), wherein the carboxyl group lactonizes on one of the new hydroxyl groups and the other new hydroxyl group is removed with formation of a double bond. But if the aldehyde group be reduced to CH_2OH, then opening of the ethylene oxide leads to the formation of a new oxidic linkage (LVI or LVII), involving one of the new hydroxyl groups and that derived from reduction of the aldehyde group. These reactions are in strong contrast to those displayed by

hydroxydihydrostrophanthidinic acid (LI), which is unusually stable towards dehydrating or lactonizing agents.

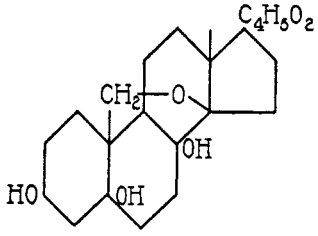
Other anhydro derivatives of strophanthidin have been studied. Of these, one formed by a reaction characteristic of the iso series has already been mentioned. β-Isostrophanthic lactone acid (LVIII) (to be discussed later, p. 229) on treatment with acetic anhydride–acetyl chloride



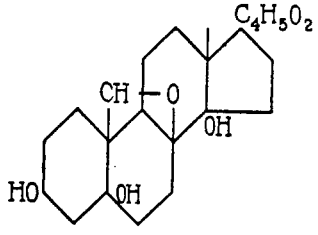
LIV



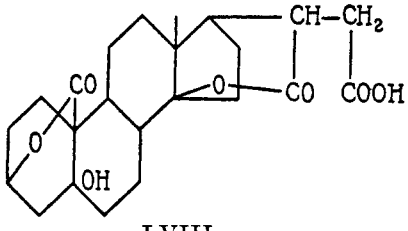
LV



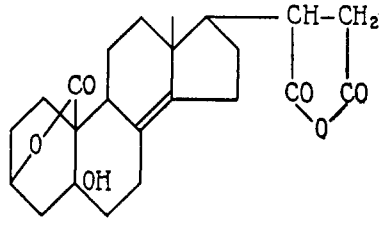
LVI



LVII



LVIII



LIX

undergoes cleavage of one of the lactone rings with the formation of an unsaturated anhydride (LIX) (76). The double bond in this substance apparently occupies the same position as that occurring in monoanhydrodihydrostrophanthidin, at least in acid solution, since an ethylene oxide was obtained which exhibited the same behavior, after hydration. The double bond in the anhydroanhydride can be catalytically hydrogenated.

By the action of methyl alcoholic hydrogen chloride on β-isostrophanthic lactone acid, the isolactone is similarly cleaved, and an unsaturated di-

methyl lactone ester is obtained corresponding to the above unsaturated anhydride. It, however, is not identical with the similar unsaturated dimethyl ester prepared from the acetic anhydride–acetyl chloride product. Besides differing sterically from the former substances, the double bond apparently lies in another position, since it cannot be catalytically hydrogenated.

Dihydrodigitoxigenin readily forms an anhydro compound which, on oxidation with alkaline permanganate, behaves in a manner analogous to the corresponding dihydrostrophanthidin derivative (62). With perbenzoic acid or permanganate in acetic acid, an ethylene oxide is formed, but all attempts at opening the latter have led to non-crystalline products.

Mention has already been made of the dianhydro derivative of gitoxigenin, digitaligenin, which results from the hydrolysis of digitalinum verum, or from the action of acid on gitoxigenin itself. In this substance only the hydroxyl group on carbon atom 3 remains. The tendency for gitoxigenin or dihydrogitoxigenin to go to a dianhydro derivative is so strong that special procedures are necessary for the formation of monoanhydro derivatives. Two isomeric anhydro derivatives of dihydrogitoxigenin corresponding to the two dihydrogitoxigenins have been prepared (63) by subjecting the appropriate dihydrogitoxigenin to the action of zinc chloride in acetic anhydride. In this way, monoanhydrodiacetates were obtained, wherein the tertiary hydroxyl group was removed and the other two hydroxyl groups were acetylated. The double bond in both derivatives resists catalytic hydrogenation.

Periplogenin easily forms a trianhydro derivative by loss of its three hydroxyl groups under the influence of acid (52). In this substance all of the double bonds can be catalytically reduced, thus indicating that they are not in a benzenoid arrangement as in trianhydrostrophanthidin. Absorption spectra data substantiated this conclusion (25).

Other anhydro derivatives of various genins have already been discussed in connection with other phases of the work, such as anhydroisostrophanthonic dimethyl ester and the anhydro derivatives of ouabagenin and uzarigenin.

J. Steric considerations and rearrangements

While the degradation of digitoxigenin to etiocholanic acid at first glance might seem to indicate that the aglycones belong to the cholic acid series, such a conclusion is not warranted. During the series of reactions leading to etiocholanic acid the hydroxyl group in digitoxigenin on carbon atom 14 was removed and the resulting double bond then hydrogenated. Undoubtedly, this hydrogenation reëstablished asymmetry on this carbon

atom, hence any conclusion of the original configuration of the aglycone at this point is extremely uncertain. At the same time, as has been mentioned, deductions from the degradation of dianhydrouzarigenin to etioallocholanic acid are uncertain for similar reasons.

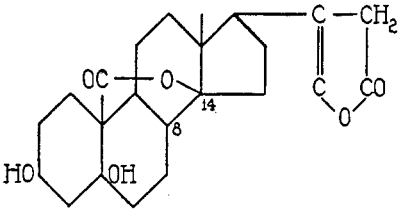
It is possible, however, to make certain limited deductions regarding the manner of linkage of the rings in some of the aglycone derivatives. In digitoxigenin, and therefore in gitoxigenin, rings I and II are probably linked in a *cis* configuration as in the cholanic acid series, for there is no apparent reason for expecting disruption of the asymmetry on carbon atom 5 on the way to etiocholanic acid. However, in the case of strophanthidin and periplogenin such a statement is rendered uncertain, since asymmetry on this carbon atom was destroyed and then reëstablished in the correlation of these aglycones with digitoxigenin. There is no evidence beyond this for placing the aglycones definitely in either the cholanic or allocholanic acid series.

Certain reactions of strophanthidin and its derivatives involve rearrangements which must affect the centers of asymmetry of the molecule. The aldehyde group of normal or α-isostrophanthidin or α-isostrophanthidic acid exhibits no tendency to exist in the form of a lactal with OH^{III}. Similarly, α-isostrophanthic acid, in which the aldehyde group has been oxidized to carboxyl, shows no tendency to lactonize on this hydroxyl group. However, if α-isostrophanthidic acid be boiled with alkali, an isomerization occurs, and the isomerized acid, β-isostrophanthidic acid, can be isolated through its sparingly soluble ammonium salt (71). The aldehyde group of this substance exists either as a lactal involving OH^{III}, as shown by its oxidation with hypobromite to β-isostrophanthic lactone acid (LVIII), or as the free aldehyde, as shown by its oxidation with permanganate to β-isostrophanthic acid which readily lactonizes, in contrast to the α-isomer. This change is not undergone by α-isostrophanthic acid, where the aldehyde group has been oxidized to carboxyl, a fact which at first led to its explanation on the usual basis of a change of the aldehyde group from a position *trans* to OH^{III} to a *cis* position by enolization on an adjacent CH group under the influence of alkali.

However, the placing of the aldehyde group on a quaternary carbon atom, 10, necessitated a different explanation. The most logical one appears to be based on the assumption that in the α series, rings I and II are linked in a *trans* arrangement as in allocholanic acid. Under the influence of alkali a rearrangement at carbon atom 5 occurs, so that in the β series the two rings are *cis* to each other as in cholanic acid, an arrangement which permits interaction of the aldehyde or carboxyl group on carbon atom 10 with OH^{III} (78a). Such an explanation is supported by a study of the atomic model and by the persistence of the differences between the

two series through the α- and β-isostrophanthonic esters. However, both isostrophanthonic esters yield the same anhydroisostrophanthonic esters, owing to loss of asymmetry on carbon atom 5 (79). Whether during the reduction of α-isostrophanthidic acid to isoperiplogenic acid by the method of Kishner and Wolff a similar rearrangement occurs under the influence of the sodium ethylate remains to be determined.

Another type of isomerization noted in certain derivatives appears to involve the centers of asymmetry located on carbon atoms 8 and 14. When strophanthidin is dissolved in strong acid, pseudostrophanthidin is formed, which has been shown to contain a lactal group between the aldehyde group and OH^{I}. Similarly, from strophanthidinic acid a dilactone, strophanthidinic lactone, results (LX) (57). A *trans* configuration between rings II and III, as assumed in many sterols, would prevent the formation of an oxidic bridge between the aldehyde group and OH^{I} in strophanthidin. After isomerization to pseudostrophanthidin, rings II and III may be in a *cis* configuration, an arrangement which a study of the



LX

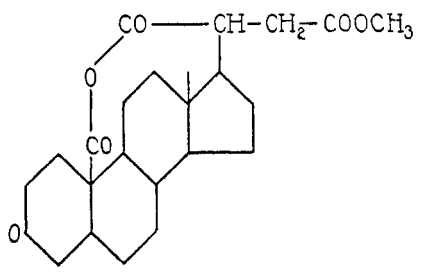
model shows to permit formation of such a bridge readily. Another explanation for this isomerization may be postulated on a preliminary removal of OH^{I} with the formation of a double bond between carbon atoms 8 and 14, followed by subsequent addition to this double bond with the formation of an oxidic linkage on carbon atom 8.

When β-isostrophanthic acid is dissolved in strong hydrochloric acid, in addition to lactonization of the carboxyl group on carbon atom 10 with OH^{III} a further isomerization occurs (71). The substance differs from β-isostrophanthic lactone acid in its physical properties. It has been called δ-isostrophanthic lactone acid. Similarly, α-isostrophanthic acid under the same conditions is isomerized to γ-isostrophanthic acid, except that in this case no lactone formation occurs. The same center of asymmetry is involved in both cases. Whether this type of isomerization is the same as that which occurs in the case of the formation of pseudostrophanthidin remains a subject for future investigation. A similar type of isomerization has also been noticed in the case of digitoxigenin derivatives (77).

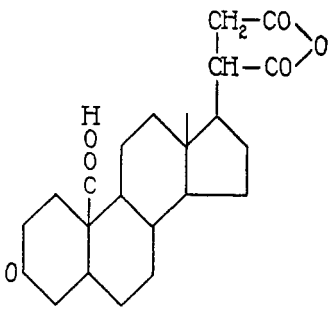
Finally, a derivative of strophanthidin which owes its origin to a change

in the centers of asymmetry of the molecule is the anhydride ester (LXI) (60). By the use of reactions already discussed, OH^{I} and OH^{II} were removed and the double bonds hydrogenated to give the keto acid anhydride (LXII). This on treatment with methyl alcoholic hydrogen chloride gave the anhydride (LXI), the formation of which appears to involve a rather long stretch. However, from a study of the model, it is apparent that this anhydride formation is quite possible, if during the hydrogenation of the double bond arising from loss of OH^{I} asymmetry is reëstablished on carbon atom 8 in such a manner that the original *trans* configuration of rings II and III becomes *cis*.

The oxidation product of gitoxigenin, α-oxoisodigitoxigenone, undergoes isomerization to a β-compound in acid or alkaline solution (78). This appears to be readily explained on the basis of the well-known epimeriza-



LXI



LXII

tion of a CH group (carbon atom 17) adjacent to a carbonyl group (carbon atom 16).

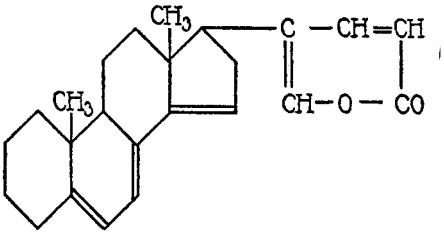
K. The squill aglycones

Stoll and coworkers have made some progress on the problem presented by scillaridin A. This aglycone, in contrast to the aglycones previously discussed, does not give the Legal test. However, the presence of a lactone group has been clearly demonstrated, which, however, apparently is not a $\Delta^{\beta,\gamma}$-lactone of the type common to the previously considered aglycones. Stoll, Hoffmann, and Helfenstein (156) by the action of methyl alcoholic potassium hydroxide have succeeded in opening the lactone group and at the same time esterifying the carboxyl group liberated to give scillaridinic methyl ester. The hydroxyl group liberated then readily loses water with a second hydroxyl group in reactive proximity with the formation of an oxidic bridge to give isoscillaridinic-A-acid methyl ester. (The term "iso" is used in this connection only to denote the presence of an oxidic ring,

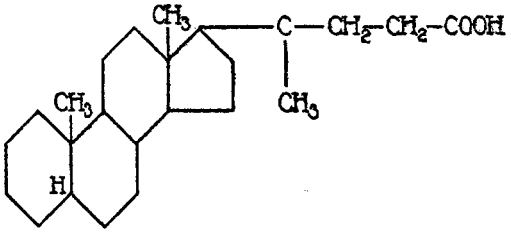
and is not synonymous with the term as used in connection with the isomerization of the other aglycones.) However, by rapid manipulation it was possible to methylate the hydroxyl group originally bound in lactone formation, with the formation of methyl scillaridinic-A-methyl ester. This, together with the formation of alkali salts, was taken to indicate the phenolic nature of the lactone hydroxyl group in scillaridin A.

Scillaridin A loses another molecule of water on treatment with acid. This anhydroscillaridin A on treatment with alkali no longer gives an iso compound, showing that the hydroxyl group of scillaridin A which functions in the formation of the oxidic bridge during the isomerization is no longer present.

The hydrogenation of scillaridin A, anhydroscillaridin A, and their derivatives has been described (157 b) and leads to the conclusion that the former contains four double bonds and the latter five. Scillaren A con-



LXIII



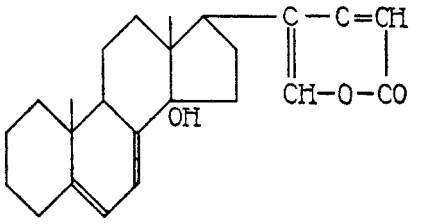
LXIV
Allocholanic acid

tains but three double bonds, one hydroxyl group being removed from the aglycone during hydrolysis. In all cases, partial reduction of the lactone occurred, giving a mixture of the saturated lactone and deoxyacid.

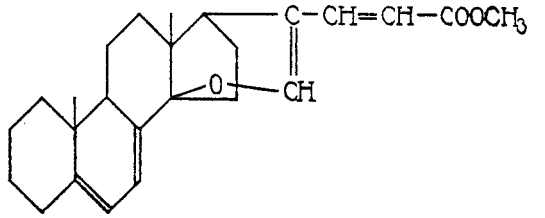
From the mixture of isomers obtained by complete reduction of anhydroscillaridin A to the saturated acid, scillanic acid, one component has very recently been obtained pure by Stoll, Hoffmann, and Helfenstein (155). On further examination this acid was found to be identical with allocholanic acid (LXIV). This finding furnished confirmation of the suspected close relationship between scillaridin A and the bile acids. In addition, however, it forced a revision of the previously adopted formulations for scillaridin A and therefore for scillaren A and proscillaridin A. Stoll and coworkers had previously accepted $C_{25}H_{32}O_3$ as representing scillaridin A. Inasmuch as the formulation for allocholanic acid has been definitely established to be $C_{24}H_{40}O_2$, it follows that scillaridin A must be revised to $C_{24}H_{30}O_3$.

In the light of these latest findings, the structure of anhydroscillaridin A is represented by Stoll, Hoffmann, and Helfenstein by formula LXIII;

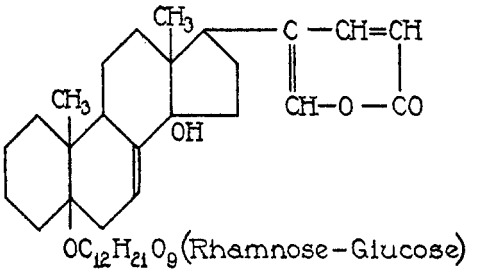
on hydrogenation this yields allocholanic acid (LXIV). Scillaridin A is represented by formula LXV. From this it is obvious that the previously advanced idea that the hydroxyl group of the lactone group is of phenolic character must be revised, since the hydroxyl group in question unquestionably is located on one of the carbon atoms of the side chain. The presence of the unsaturated δ-lactone in contrast to the γ-lactones of the members of the digitalis–strophanthin group accounts for the failure of scillaridin A to give the Legal test and also for the reduction to the saturated deoxyacid.



LXV
Scillaridin A



LXVI



LXVII
Scillaren A

The remaining hydroxyl group of scillaridin A is placed on carbon atom 14, in order to bring it into reactive proximity to the lactone hydroxyl group of the side chain to account for the formation of isoscillaridinic-A-acid methyl ester (LXVI). The evidence for the positions assigned to the double bonds rests on a less secure basis,—namely, certain analogies to ergosterol exhibited by scillaridin A, in particular the Rosenheim trichloroacetic acid color test.

Finally, a complete formula (LXVII) for scillaren A has been advanced. The evidence for placing the hydroxyl group which bears the sugar residue on carbon atom 5 consists in the resistance to hydrolytic cleavage exhibited by the glycoside. From this it is argued that the hydroxyl group in ques-

tion is probably tertiary. However, whether the difficulty of hydrolysis of the glycosidic linkage is due to this factor or whether it may be conditioned by the presence of the normal deoxyhexose, rhamnose, as in the case of the hydrolysis of ouabain, remains to be determined.

Nothing is known concerning scillaridin B.

IV. NITROGENOUS CARDIAC PRINCIPLES

A. The toad poisons

Any account of the digitaloid substances would be incomplete without mention of another group of very interesting compounds which are, however, of animal origin—the so-called toad venoms. These substances, which exhibit a potent digitalis action, are secreted by certain (parotid) glands on the surface of the animals. Abel and Macht (1) were the first to report the isolation of a non-nitrogenous, crystalline principle, bufagin, from such a secretion of the tropical toad, *Bufo agua* (*marinus*). In addition, a relatively large amount of adrenalin was isolated.

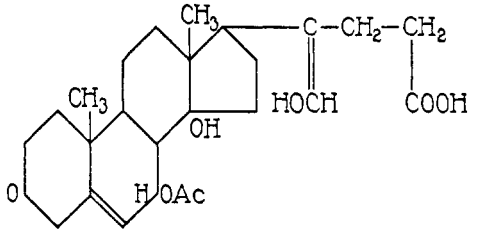
The chemistry of these materials has recently been developed to a considerable extent. As in the case of the plant glycosides, confusion has been occasioned by uncertainty as to the source of the material under examination. This has particularly been the case in the study of Ch'an Su or Senso, the dried venom of the Chinese toad, where conflicting reports have come from several workers.

Wieland and coworkers have made an extensive study of the principle from *Bufo vulgaris*. A substance, bufotalin (186), $C_{26}H_{36}O_6$, was first obtained which corresponded to, but was not identical with, bufagin. Subsequently, Wieland and Alles (180) obtained a non-glycosidal, neutral, nitrogenous substance, bufotoxin, $C_{40}H_{62}O_{11}N_4$, which was shown to be a conjugation of bufotalin with arginine and suberic acid. Thus it was concluded that bufotalin is a sort of "genin" of the toad poison in question, bufotoxin, in which the suberylarginine parallels somewhat the position of the sugar in the non-nitrogenous glycosides. On hydrolysis it was shown that bufotoxin, which contains one double bond, yields not bufotalin but a still more unsaturated substance, bufotalein, $C_{24}H_{30}O_3$, along with suberylarginine and acetic acid.

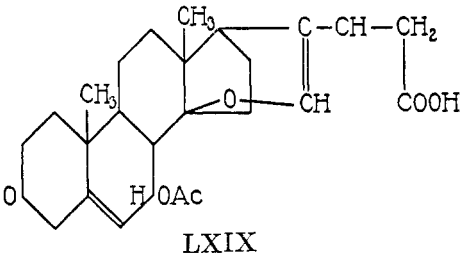
Oxidation of bufotalin with chromic acid leads to a monoketone, bufotalone; hence it contains one secondary hydroxyl group. Two more of the oxygens are contained in an acetoxyl group which can be removed on saponification. A lactone accounts for two more oxygens and the sixth is in the form of a tertiary hydroxyl group. Bufotalin, therefore, is the acetate of a doubly unsaturated trihydroxylactone, $C_{24}H_{34}O_5$. However, since the toad poisons, as far as they have been investigated, do not give the Legal nitroprusside reaction, it must be concluded that neither of the double

bonds of bufotalin is in the lactone ring, or else that the lactone is not a γ but rather a δ one.

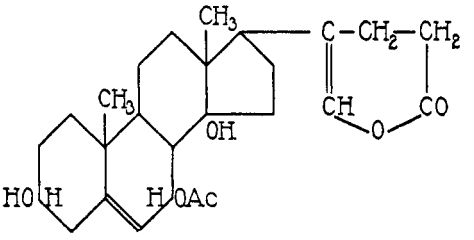
Concentrated hydrochloric acid removes the acetoxyl and tertiary hydroxyl groups from bufotalin to give bufotalein. After acetylation of the remaining hydroxyl group, catalytic hydrogenation of acetylbufotalein leads to a mixture of acetylbufotalans, the fully saturated acetoxylactone, and the acetate of a monobasic acid arising from reduction of the lactone (183). After removal of the acetyl group of this acid, the remaining hydroxyl group can be replaced by hydrogen by distillation *in vacuo* and catalytic hydrogenation of the resulting unsaturated acid. The final



LXVIII



LXIX



LXX

Bufotalin

product is isobufocholanic acid, $C_{24}H_{40}O_2$, which, however, is not identical but isomeric with any of the known cholanic acids. The isomerization may be due to the formation of isomerides on hydrogenation.

Very recently, Wieland and Hesse (182) have reported the dehydrogenation of bufotalin with selenium. They obtained a hydrocarbon believed to be chrysene, which has also been obtained from the sterols. In addition, they have succeeded in opening the lactone ring of bufotalone. The free acid obtained, however, was still missing a water molecule. It was concluded that the enolized aldehyde group had participated in the formation of a new oxidic linkage with one of the remaining hydroxyl groups. This transformation is represented by formulas LXVIII and LXIX, from which Wieland's tentative structure for bufotalin is at once obvious (LXX).

The venom of the Japanese toad, *Bufo japonicus* (or *Bufo vulgaris for-*

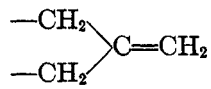
mosus), has been studied by Kotake (115) and Wieland and Vocke (185). The former isolated a substance, gamabufotalin (gama being the Japanese word for toad), to which he gave the formula $C_{27}H_{38}O_6$. This substance, on saponification, in addition to opening the lactone was reported to lose C_3H_5O to give a non-lactonizing gamabufotalinic acid. On catalytic hydrogenation, gamabufotalin loses C_3H_5O and absorbs six atoms of hydrogen to give a substance of the formula $C_{24}H_{38}O_5 \cdot C_2H_5OH$. In addition, gamabufotalin contains two acylatable hydroxyl groups.

The observations of Wieland and Vocke are somewhat at variance with Kotake's. They isolated gamabufotoxin, $C_{38}H_{60}O_{10}N_4$, which contains a lactone and one double bond. It is a suberylarginine derivative, but the "genin" is different from the bufotalin isolated from *B. vulgaris*. Direct hydrolysis of gamabufotoxin leads to the formation of an anhydrogamabufagenin, $C_{24}H_{32}O_4$. In addition to gamabufotoxin, gamabufogenin, $C_{24}H_{34}O_5$, was isolated in small amounts as such. On treatment with concentrated hydrochloric acid gamabufagenin loses one molecule of water to give an anhydrogamabufagenin, which is not identical with that obtained by direct hydrolysis of gamabufotoxin. However, the latter can be isomerized to the former by the action of strong acid. In their behavior towards acid these two substances differ from bufotalin, which loses one acetyl group and one molecule of water under the influence of acid. Gamabufagenin is a doubly unsaturated trioxylactone in which two of the hydroxyl groups are acylatable and hence presumably secondary. Since its anhydro derivative still absorbs only two molecules of hydrogen, the new double bond in the latter is inert. Gamabufogenin is very similar to, but not identical with, Kotake's gamabufotalin. However, the hydrogenation products of the two seem to be identical. This may be taken to indicate that Kotake's material is an acetyl derivative of a C_{24} genin, in which case his C_3H_5O, which is lost, would become C_2H_2O.

More recently, Chen, Jensen, and Chen (14) have reported the isolation of a gamabufagin from identified *B. formosus*, presumably the same species investigated by the earlier workers, which gave analytical figures corresponding to Kotake's gamabufotalin. The gamabufotoxin obtained by them, however, more closely resembled Wieland's material.

Jensen and Chen (90) have examined the bufagin of Abel and Macht. They succeeded in isolating from the secretion of *B. marinus* a crystalline bufotoxin, marinobufotoxin, for which the formula $C_{42}H_{64}O_{11}N_4$ was given. At the same time the formula for bufagin was revised to $C_{28}H_{36}O_6$ and a monoacetyl derivative of the substance was noted. Subsequently, Jensen (91) noted the formation of formic acid from bufagin under the action of alkali and considered it to be a formoyl derivative of a triply unsaturated (hydrogenation), monohydroxy, C_{23} lactone, thereby bringing it into con-

formity with the plant aglycones. A still further revision of the formula for marinobufotoxin by Jensen and Evans (92) to $C_{38}H_{58}O_{10}N_4$ has been made more recently. Observations were also made which indicated that the formic acid produced by alkali does not have its origin in a formoyl group in the bufagin, but rather in a



grouping which breaks at the double bond to give formaldehyde. The latter then undergoes a Cannizzaro reaction with the production of formic acid.

The bufagins and bufotoxins of a number of other species of toads have been isolated by Chen and Chen (16). Inasmuch as the active principles gave the impression of being different substances, these workers have proposed using the name of the species from which the substances are obtained as a prefix. Bufagin and bufotoxin thus become generic terms.

The Chinese have long made use of a remedy known as Ch'an Su (in Japan, Senso) which purports to consist of the dried venom of a Chinese toad. Chen and Chen (15) are of the opinion that *Bufo bufo gargarizans* is the source of Ch'an Su. However, owing to the conflicting results obtained by various investigators, this surmise would seem to be open to question. Shimizu (152), who was the first to attempt its investigation, isolated a crystalline substance from Senso, either identical or isomeric with Wieland's bufagin, as well as an amorphous bufotoxin. Subsequently, Kodama (111) isolated a bufagin and gave it the formula $C_{27}H_{34}O_7$. He also obtained an amorphous bufotoxin.

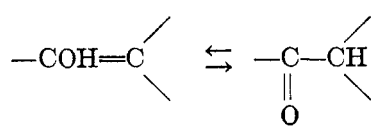
Kotake (116), the first to attempt an exhaustive study of the chemistry of the constituents of Senso, isolated a cinobufagin (here prefixed by "cino-" in line with Chen's suggestion for nomenclature) to which he assigned the formula $C_{29}H_{38}O_7$. It contains an acetyl group which is removed by alkaline saponification, which simultaneously opens the lactone. Cinobufagin gives a diacetate, $C_{33}H_{42}O_9$, but on oxidation with chromic acid only a monoketone is formed. On catalytic hydrogenation two isomeric tetrahydrocinobufagins, $C_{29}H_{42}O_7$, are formed. Kotake's cinobufagin therefore contains a lactone, an acetoxyl group, one secondary hydroxyl group, and two double bonds, leaving one oxygen atom to be accounted for. From the mother liquors from the cinobufagin, after treatment with hydrogen chloride gas, he was able to isolate a chloro derivative, apparently corresponding to Kodama's bufotoxin (111), to which the formula $C_{27}H_{35}O_5Cl$ was assigned. It is considered to be derived

from cinobufagin by loss of the acetoxyl group and replacement of one hydroxyl group by chlorine.

Jensen and Chen (89) have reported the isolation from Ch'an Su of a cinobufotoxin, $C_{43}H_{64}O_{12}N_4$, and a cinobufagin, $C_{29}H_{38}O_7$, agreeing with Kotake's substance. The former, as expected, was found to be a conjugation of a genin, which was not isolated, with suberylarginine. Subsequently, Jensen (91, 92) revised these formulas to $C_{39}H_{58}O_{11}N_4$ and $C_{25}H_{32}O_6$, respectively. The usual methods accounted for all the oxygens as a lactone, an acetoxyl group, one secondary hydroxyl group, and one tertiary hydroxyl group. Cinobufagin therefore appears to be an acetyl derivative of a C_{23} genin.

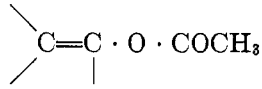
Kondo and Ikawa in a series of studies have elucidated the chemistry of a bufotalin isolated from Senso which, although first giving the impression of being identical with Wieland's substance, appeared on later study to be quite different from the ones previously reported (112). This they have called pseudobufotalin. From the Senso with which they worked, they failed to isolate bufotoxin and concluded that perhaps it may have been decomposed during drying of the toad venoms. ψ-Bufotalin possesses the formula $C_{26}H_{36}O_6$ and is an acetyl derivative of a substance $C_{24}H_{34}O_5$,—deacetyl-ψ-bufotalin. ψ-Bufotalin contains one acylatable hydroxyl group, but differs from Wieland's bufotalin in the behavior of the deacetyl compound. Once the original acetyl group is removed, deacetyl-ψ-bufotalin gives only a monoacetate, the hydroxyl group originally acetylated being resistant to reacetylation. Contrasting with bufotalin, ψ-bufotalin when treated with strong acid retains the acetyl group, giving only a monoanhydro-ψ-bufotalin, compared with bufotalein from bufotalin. ψ-Bufotalin is also different from Kotake's bufagin.

ψ-Bufatalin on oxidation with chromic acid gives a monoketone, ψ-bufotalone, which in turn gives a monoöxime (113). This on saponification loses the acetyl group, and simultaneously the lactone is opened to give deacetylbufotalonic acid, $C_{24}H_{34}O_6$. From the methyl ester of the latter compound a monoacetate was obtained, indicating that the hydroxyl group originally bound in lactone formation is primary or secondary. But deacetyl-ψ-bufotalonic ester gives a dioxime. This is explained by a keto–enol tautomerism involving one of the hydroxyl groups, and indicates that the arrangement



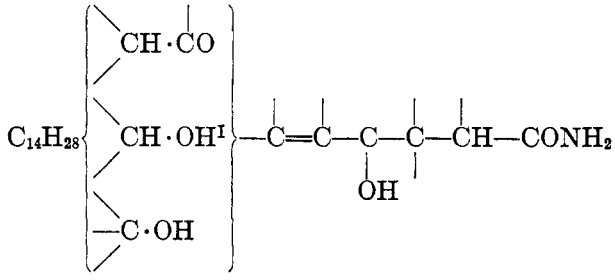
is present. That the hydroxyl group involved in this change is the one originally acetylated in ψ-bufotalin is shown by the production of a mono-

oxime from deacetyl-ψ-bufotalin itself. This interpretation is strengthened by the observation that ψ-bufotalin on catalytic hydrogenation absorbs two molecules of hydrogen to give two isomeric tetrahydro-ψ-bufotalins, which on saponification give deacetyltetrahydro-ψ-bufatalins. However, in these substances the originally acylated hydroxyl group has become active, since diacetates were obtained. The retention of the tertiary hydroxyl group was indicated by the formation of a monochloro compound. Thus, the group

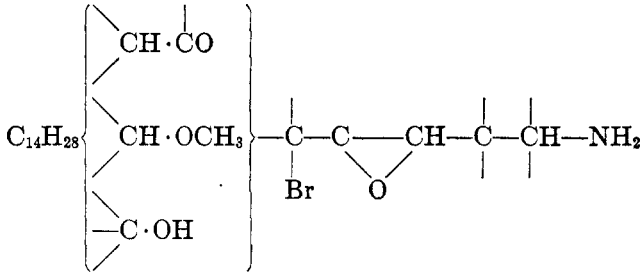


is present in ψ-bufotalin.

In a third paper the same workers have placed the second double bond of ψ-bufotalin (114). Deacetyl-ψ-bufatalin on treatment with ammonia yields an amide (LXXI) by opening of the lactone. Bromine and sodium



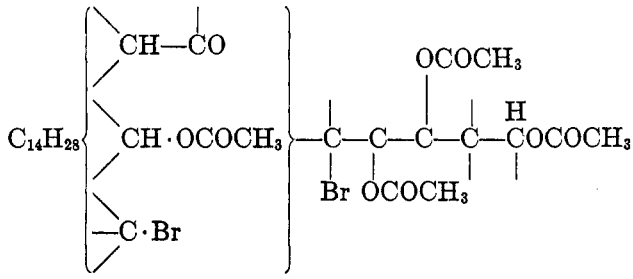
LXXI



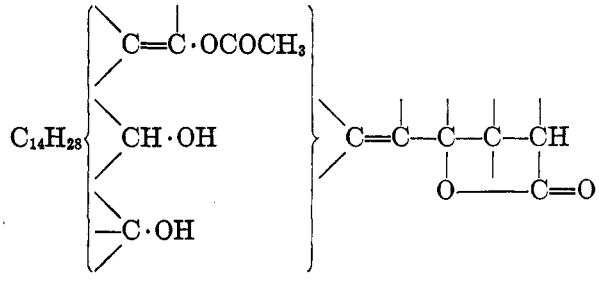
LXXII

methylate yielded the urethan, with simultaneous methylation of the secondary hydroxyl group. At the same time, hypobromous acid added to the double bond with the formation of a bromohydrin from which water was spontaneously lost, resulting in the formation of an ethylene oxide. Hydrolysis with alcoholic alkali gave the methoxyamine (LXXII),

which was demethylated by heating with hydrogen bromide in acetic acid. The tertiary hydroxyl group was retained, as shown by conversion to a dibromide. Finally, acetic anhydride opened the ethylene oxide to yield a dibromotetraacetate (LXXIII). Thus, the second double bond in ψ-bufotalin was shown to be α,β to the lactone, and ψ-bufotalin may be represented by formula LXXIV.



LXXIII



LXXIV

B. Erythrophloeum guineense

The bark of this tree, often called "sassy-bark," has long been the source of an ordeal poison of the natives of East Africa. A graphic description of its use is given by Proctor (146). The active principle of the bark, while displaying a powerful digitalis action, contains nitrogen and falls in the alkaloid class. Gallois and Hardy (34) first investigated it and reported the isolation of a crystalline alkaloid, to which they gave the name "erythrophleine" and which gave a crystalline hydrochloride and platinichloride. Subsequently Harnack and Zabrocki (38) took up its study, but obtained erythrophlein only as an unstable oil. On boiling with acid or alkali it is decomposed, yielding a partly crystalline, nitrogen-free erythrophleic acid. They surmised that erythrophlein probably consisted of a nitrogenous side chain joined to a nitrogen-free complex to which the physiological action is due. In a later paper Harnack (39) succeeded in

obtaining solid platinichloride and potassium bismuth iodide salts from analyses of which he deduced formulas of either $C_{28}H_{43}NO_7$ or $C_{28}H_{45}NO_7$ for erythrophlein. Its decomposition with acid gave methylamine and erythrophleic acid, $C_{27}H_{38}O_7$ or $C_{27}H_{40}O_7$. More recently, Power and Salway (144) obtained erythrophlein as an oil and checked Harnack's formulation. Maplethorpe also failed to obtain it crystalline (127). Petrie (140) reported *Erythrophloeum laboacherii*, a native of Australia, to yield the same erythrophlein as that previously obtained. Kamerman (93) reported the same substance from *E. lasianthum*, and developed a method for isolating it as the phosphotungstate.

V. THE SUGAR COMPONENTS OF THE CARDIAC GLYCOSIDES

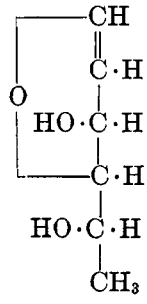
In the preceding discussion mention has been made of the sugars which have been isolated after hydrolysis of the cardiac glycosides. Inasmuch as some of them have been shown to possess unusual structural features, it will be profitable to treat them in more detail. Digitoxose, cymarose, and sarmentose are 2-deoxysugars, and with the exception of deoxyribose from thymus nucleic acid (122) are the only members of this group of sugars which have been encountered in nature. They are characterized by their lability towards acid as compared with the usual hexoses, and by the ease with which their glycosides undergo hydrolysis. The Keller–Kiliani test (the production of a blue color when an acetic acid solution of the sugar containing iron is allowed to come in contact with a layer of sulfuric acid) is characteristic of the group.

A. Digitoxose

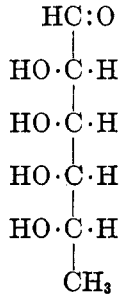
This sugar was first isolated by Kiliani (102) from digitoxin. He assigned to it the formula $C_6H_{12}O_4$, noted that in its reaction with phenylhydrazine only a phenylhydrazone rather than an osazone was formed (103), and obtained acetic acid upon oxidation of it with silver oxide (104). Later he showed it to be an aldose by the formation of digitoxonic acid upon oxidation with bromine water, and obtained α,β-dihydroxyglutaric acid and mesotartaric acid on oxidation with nitric acid (105). These facts were sufficient for the derivation of the structure $CH_3 \cdot (CHOH)_3 \cdot CH_2 \cdot CHO$ or that of a 2-deoxyhexomethylose. In a later paper, Kiliani (106) concluded that the hydroxyl groups on C_3 and C_4 are *cis* to one another. This was supported by the application of Hudson's rule to digitoxonic lactone and to the lactone of digitoxoseheptonic acid derived from the sugar by the cyanohydrin reaction. In addition, these hydroxyl groups were assigned positions to the left of the chain.

Windaus and Schwarte (195), starting with a substance obtained by Cloetta (17) by vacuum sublimation of digitoxin, indicated that the hy-

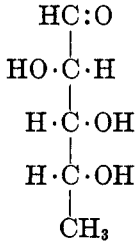
droxyl group on C_5 was also *cis* to those on C_3 and C_4. Cloetta's substance was shown to be an anhydro derivative of digitoxose of the glucal type, to which the formula LXXV was assigned. By oxidation of this glucal with perbenzoic acid, an aldohexomethylose was obtained to which was assigned



LXXV

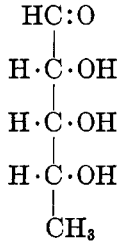


LXXVI

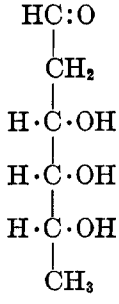


LXXVII

or



LXXVIII



LXXIX

the configuration LXXVI, on the basis of physical constants and the properties of the phenylosazone, taken together with Kiliani's work.

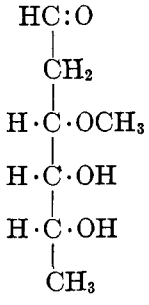
Micheel (130), however, has shown that Hudson's rule apparently does not hold in this case. The above glucal (LXXV) on ozonization leads to a pentomethylose (LXXVII or LXXVIII). This was shown to possess the

same configuration on C_4 and C_5 of the digitoxose chain as *d*-arabomethylose, by the preparation of identical phenyl- and *p*-bromophenyl-osazones from both sources. Hence, since the hydroxyl groups on C_3 and C_4 are *cis* to one another, it follows that all three hydroxyl groups are *cis* and that digitoxose belongs to the *d*-series. Digitoxose therefore is represented by formula LXXIX and is 2-deoxyallomethylose.

B. Cymarose

Cymarose was first obtained by Windaus and Hermanns (193) from cymarin. They showed it to be a methyl ether of a 2-deoxyhexomethylose and suggested that it might be a methyl ether of digitoxose. Recently Elderfield has shown the position occupied by the methoxyl group to be C_3, and has demonstrated the configurational identity with digitoxose. When oxidized with 50 per cent nitric acid, cymarose yielded the lactone of α-hydroxy-β-methoxyglutaric acid (27), thus rendering probable the allocation of the methoxyl group on C_3. This was substantiated by the preparation of two different fully methylated lactones from cymarose, one of which possesses a γ-lactone ring and the other a δ-lactone ring. Thus, both of the hydroxyl groups on C_4 and C_5 are unsubstituted.

The relationship of cymarose to digitoxose was shown by the preparation of identical fully methylated γ-lactones from both sugars (27). Cymarose therefore is represented by formula LXXX.



LXXX

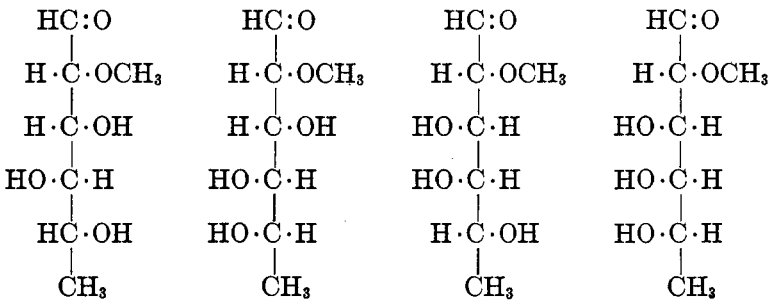
C. Sarmentose

This sugar was isolated from sarmentocymarin by Jacobs and Bigelow (49). It is a methyl ether of a 2-deoxyhexomethylose isomeric with cymarose. The position occupied by the methoxyl group and the configuration are unknown at present.

D. Digitalose

Digitalose differs from the above sugars in that it is not a 2-deoxysugar. However, it does contain a methoxyl group. It was obtained as a syrup by

Kiliani (107) from digitalinum verum. On oxidation with bromine water, crystalline digitalonic lactone was obtained, which was shown to have the formula $C_7H_{12}O_6$. From this he concluded that digitalose was $C_7H_{14}O_6$. He also obtained acetic acid by oxidation of the lactone with silver oxide, thus indicating the presence of a terminal methyl group. Further, digitalonic lactone gave on oxidation with nitric acid an α,β-dihydroxy-α'-methoxyglutaric acid (108). Schmidt and Zeiser (149) have recently shown that this oxidation product after complete methylation is identical with *l*-arabotrimethoxyglutaric acid. From this it follows that digitalose is represented by one of the formulas LXXXI to LXXXIV.



E. Linkage of the sugars with the aglycones

Jacobs and Hoffmann (84) have shown that in cymarin the sugar is presumably joined in glycosidic union with the secondary hydroxyl group (OH^{III}) of strophanthidin. Cymarin on acetylation with acetic anhydride in pyridine solution yields a monoacetyl derivative. If it be accepted that acetylation has occurred on the free hydroxyl group of the cymarose unit, then the strophanthidin has remained unacetylated. Since strophanthidin itself under such conditions forms only a monoacetyl derivative, the conclusion appears warranted that the ordinarily acetylatable OH^{III} is the point of glycosidic union. While no direct evidence is at hand regarding this point in respect to the other glycosides, they probably will be found to contain a similar glycosidic linkage.

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