REVIEW ARTICLE

THE CARDIOACTIVE GLYCOSIDES

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INTRODUCTION

THE impressive description given by William Withering in 1785 of the action of digitalis leaves upon the failing heart resulted in such a fundamental advance in the treatment of cardiac diseases that, even to-day, therapeutic measures are still based upon the experiences he recorded. In the intervening period, however, it has been possible to adapt the methods of treatment better to the various diseases of the heart and circulation than was originally the case.

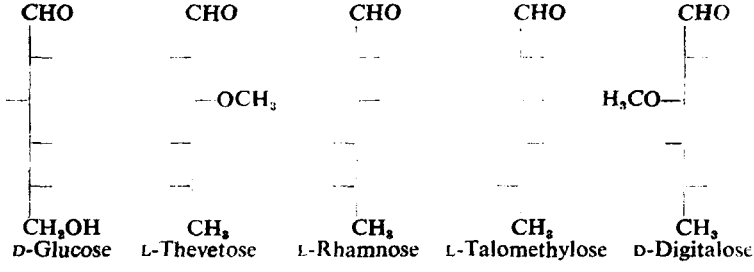
Since the time of Withering there has been introduced into therapy a whole series of cardioactive drugs fundamentally similar in effect to the leaves of *Digitalis purpurea,* but differing, for example, in their rapidity and duration of action.

The chemical investigation of the drugs from which the cardioactive glycosides are obtained also dates back to the middle of the 19th century. Particularly in the last two decades, however, it has made such rapid strides that to-day the constitution of practically every important cardiac glycoside is known, including the details of its steric configuration.

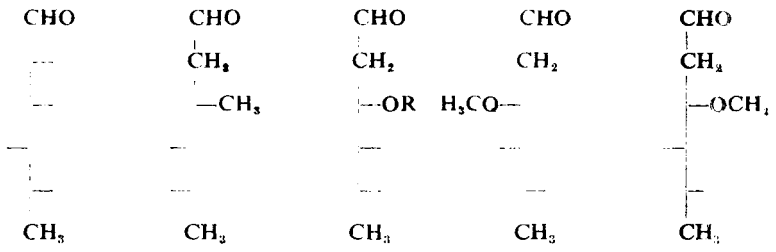
In this article, an attempt will be made to characterise the more important cardiac glycosides but without pursuing the long trail which led to their isolation and to the elucidation of their chemical constitutions. The main difference between modern processes of isolation and the earlier methods employed lies in the fact that account is now taken of the great sensitivity of the cardiac glycosides to high temperatures, to acids or alkalis, and to enzymatic cleavage, etc. Not only digitoxin but all the other cardiac glycosides known prior to 1930, with the exception of ouabain and scillaren A, are in fact, artificial products arising as the result of enzymatic cleavage from substances of a higher sugar content originally present in the plant. This reveals one reason for the difference in action repeatedly observed by physicians between the use of the older pure substances on the one hand and of the crude drugs on the other.

THE SUGARS OF THE CARDIOACTIVE GLYCOSIDES

One of the main characteristics of glycosides is the fact that they contain one or more sugar residues. In the cardiac glycosides, the following sugars have so far been discovered.



D-Glucose | L-Thevetose | L-Rhamnose | L-Talomethylose | D-Digitalose



D-Gulomethylose L-Oleandrose R = H: D-Digitoxose D-Diginose D-Sarmentose
R = CH_3: D-Cymarose

Apart from D-glucose, they are all desoxysugars, i.e., they contain 1 or 2 oxygen atoms fewer than the corresponding carbohydrate with 6 C atoms. While D-glucose and L-rhamnose are fairly widely distributed, the remaining sugars have so far been found only as components of cardiac glycosides.

For the sake of completion, we have also given the formulæ for L-oleandrose and D-diginose, although the glycosides derived from these sugars will not be discussed. The chemistry of the oleander glycosides has been known for a long time and has been described in a number of comprehensive publications. Diginose is the sugar obtained from the non-cardioactive diginin, a glycoside obtained from the leaves of *D. purpurea*, which would be out of place in this article. The constitutions and configurations of both these sugars have been confirmed by synthesis[1,2].

The sugar residue which, in the cardiac glycosides, may consist of a chain of up to 4 sugar molecules, is responsible for the water solubility of the glycoside on the one hand and, on the other, for its power of fixation to the heart muscle.

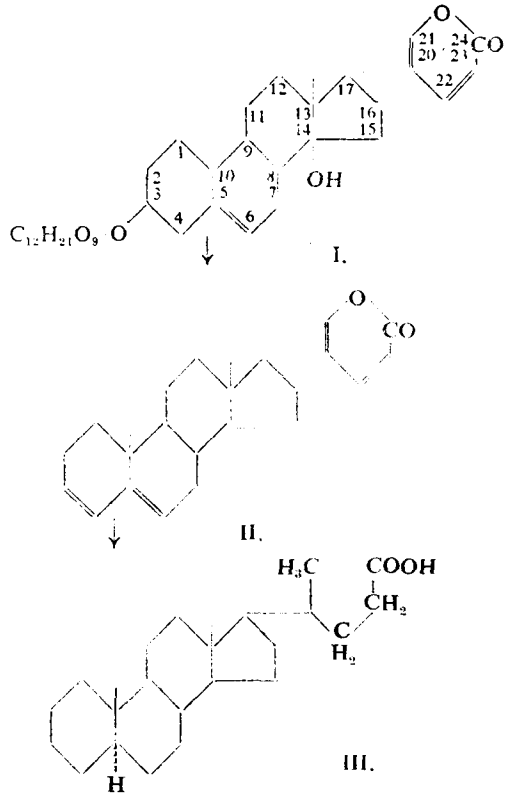
The Steroid Structure of the Aglycones

The aglycones of the cardiac glycosides possess much more complicated structures than the sugar components. These genins are the real carriers of the specific action even though, on account of their insolubility in water and their low power of fixation to the heart muscle, they are of no importance therapeutically. In structure they are very closely related to the steroids and they belong to this same large class of substances which also includes the sterols, the bile acids, the sex hormones, the hormones of the suprarenal cortex and vitamin D.

Our own investigations on the cardiac glycosides began more than 25 years ago with squill, a cardioactive drug from the Mediterranean countries which was used as a remedy for dropsy by the ancient Egyptians. Since we began our studies, we have isolated and thoroughly investigated the main glycoside of squill, scillaren A[3,4]. Our investigations are being continued on a number of other glycosides which accompany scillaren A in squill, but which are present only in very small quantities. They are also cardioactive and some of them are very beautifully crystalline.

In connection with scillaren A, a brief description will be given of the simplest chemical conversion of this cardioactive glycoside, or of its

aglycone, scillaridin A, into a product of animal origin, allocholanic acid[5].



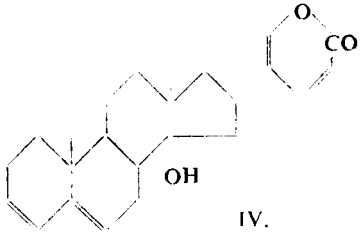
Schematic representation of the conversion of scillaren A into allocholanic acid.

Methyl alcoholic hydrochloric acid splits off the sugar residue and the tertiary hydroxyl group at C_{14} from scillaren A (I), resulting in the formation of two new double bonds, Δ^3 and Δ^{14}, and the production of anhydroscillaridin A (II). On catalytic hydrogenation, all the double bonds of this substance are reduced and, at the same time, the lactone ring is opened by reduction so that a saturated desoxycarboxylic acid is formed. This has been shown to be identical with allocholanic acid (III) obtained by the method of Windaus[6] and Wieland[7] from hyodeoxycholic acid. Hence in the light of the already known constitution of allocholanic acid, direct conclusions may be drawn regarding the constitution of the aglycone obtained from the squill glycoside, since the sequence of the reactions was such as to avoid any severe attack upon the molecule, particularly any alteration in the structure of the carbon skeleton. The close relationship between the two classes of compounds, the aglycones of the cardiac glycosides on the one hand and the bile acids on the other, is thus demonstrated in a very simple manner. That the unsaturated lactone ring in scillaren A must be 6-membered, is proved beyond doubt

by this degradation. Wieland[5] has demonstrated that the same lactone ring is also present in the toad poisons.

In the structural formula of scillaren A, deduced from the conversion of anhydroscillaridin A into allocholanic acid, the position of the sugar molecule and of one of the neighbouring double bonds remained uncertain. The determination of the position of the sugar residue was made more difficult by the fact that when the sugar is split off from scillaren A the hydroxyl group to which it is attached is lost at the same time and a new double bond is formed. The point of attachment of the sugar chain which is built up from rhamnose and glucose and is known as scillabiose, was at first assumed to be position 5. It was possible[9], however, to establish with certainty that the sugar is attached to the hydroxyl group at C_3. In principle, the method was the same as that which led to the conversion of anhydroscillaridin A into allocholanic acid, the only difference being that the sequence of the reactions was altered, the hydrolysis not being performed until after the catalytic hydrogenation so that the hydroxyl group at C_3 was retained. In this way, it was possible to show that the acid obtained from scillaren A is identical with epi-allo-lithocholic acid (3β-hydroxy-allocholanic acid), prepared by the method of Wieland[7] from hyodeoxycholic acid, and, at the same time, to obtain proof that the hydroxyl group at C_3 carrying the sugar molecule bears the cis-configuration with respect to the methyl group at C_{10}.

In order to explain the fact that, in contrast to other rhamnosides, the sugar in scillaren A splits off easily with the formation of a conjugated system of double bonds, there must be a double bond in the neighbourhood of the hydroxyl group carrying the sugar. Hence, it follows that, by analogy with other unsaturated sterols, this ethylene linkage must be situated at $\Delta^{5,6}$. The formulæ of scillaridin A and anhydroscillaridin A corresponding to these conclusions are as follows:



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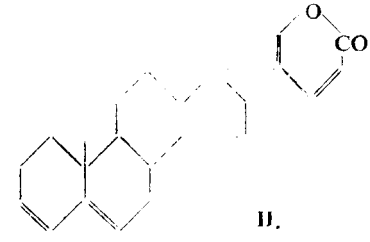
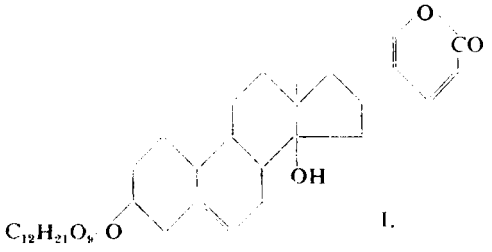


II.

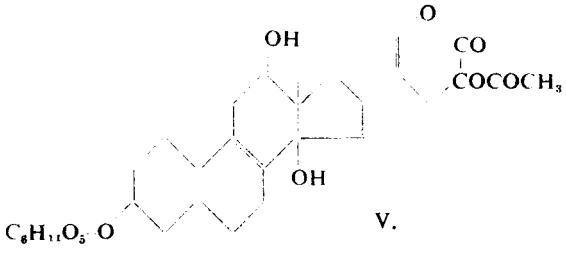
Although all the cardiac glycosides behave for the most part in a similar manner in their action on the heart, they exhibit certain differences which depend upon relatively small variations in the chemical structure. The following example illustrates how far the introduction of new groups results in changes in the type of action.

While rodents, particularly rats, are relatively speaking not very sensitive to glycosides of white squill and can tolerate comparatively large doses, they show an unusual sensitivity to one of the active principles of red squill. The red variety of squill contains a glycoside related to

scillaren A, scillirosid, which has only been prepared in a pure crystalline state and chemically identified in the last few years[10,11].



I.

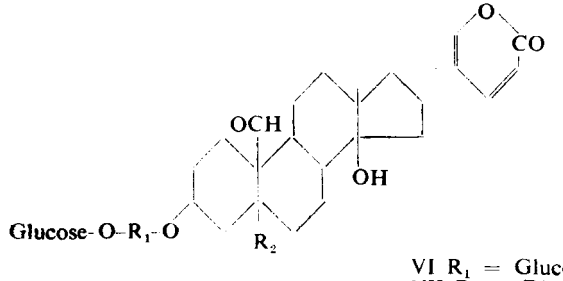


V.

Constitutional formulæ of scillaren A and scillirosid

Scillirosid, like scillaren A, has a sugar residue in position 3, although this consists only of glucose. On the other hand, it contains, in addition, a hydroxyl group, probably in position 12, as well as a double bond which is very difficult to hydrogenate and a characteristic acetyl group on the lactone ring. This acetyl group is very likely responsible for the specific toxic action in rodents. 0·1 to 0·2 mg. of scillirosid given with the food is sufficient to kill an adult rat, while the same animal could tolerate 200 times this amount of the closely related scillaren A without harm.

A glycoside with a 6-membered lactone ring has also been discovered in a species of the Ranunculaceæ. Karrer[12] isolated from the rhizome of *Helleborus niger*, the Christmas rose, a glycoside, hellebrin, with a powerful cardiac action. To this compound, he attributed the following formula VI with an aldehyde group at C_{10} and the δ-lactone ring with two double bonds characteristic of scillaridin[13].



VI R_1 = Glucose, R_2 = H
VII R_1 = Rhamnose, R_2 = OH

Reichstein and his co-workers[14,15], who demonstrated beyond doubt the presence of the aldehyde group, suggested formula VII. They found that hellebrin was decomposed by strophanthobiase to a monosaccharide, desglucohellebrin. On cleavage by the method of Mannich, this yields L-rhamnose and two isomeric genins, which have not yet been further investigated. In this case, too, it is possible that the sugar chain is present in the form of scillabiose.

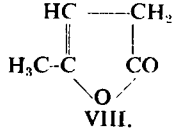
The aglycones of the cardioactive glycosides of the digitalis type and of strophanthus and certain other plants have a simple unsaturated 5-membered lactone ring in place of the 6-membered lactone ring with 2 double bonds present in scillaren A, scillirosid, hellebrin and the toad poisons. The remaining differences are connected with the peripheral structure of the molecule and depend upon the number and arrangement of the hydroxyl groups. Strophanthidin, the aglycone of k-strophanthin, like the aglycone of hellebrin, possesses the particular feature of an aldehyde group at C_{10}, whereas the other aglycones have a methyl group.

The reasoning which led to the above experimental proofs of the fine structure of the cardiac glycosides, was based upon the comprehensive knowledge which had been derived from the investigations into the steroids, and which, only about $1\frac{1}{2}$ decades ago, enabled the previously very incomplete conceptions of the chemical structure of the heart glycosides to be clarified.

Attempted Syntheses

That attempts to synthesise cardiac glycosides would soon follow and would lead to some interesting partial successes was to be expected. Thus Elderfield[16] and his co-workers, on the one hand, and Ruzicka[17] and co-workers on the other, starting from simple sterol derivatives and employing the Reformatsky reaction with bromacetic ester, succeeded in building up the 5-membered lactone ring characteristic of many aglycones. These experiments also showed that the previous assumption of a double bond in the β,γ-position to the carbonyl group of the lactone ring was incorrect and had to be replaced by a formulation in the α,β-position.

In all the structural formulæ given here, this alteration is already taken into account. The lactone ring was assumed by Jacobs and his co-workers to be β,γ-unsaturated, i.e., aldoenol lactone, since this position of the double bond appeared to agree particularly well with its reactions, and because, in particular, this structure was claimed to give the best



VIII.

explanation for the colour reaction with sodium nitroprusside, the so-called Legal test[18]. It had, however, already been observed at that time that, on catalytic hydrogenation, the aglycones were not reduced to saturated deoxycarboxylic acids like other enol lactones, e.g., α-angelica-

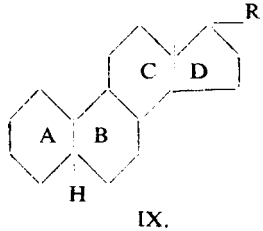
lactone[19] (VIII) or scillaren A[20], but that they took up only two atoms of hydrogen and that the double bond was reduced without opening of the lactone ring.

Moreover the aglycones with 5-membered lactone rings do not add on bromine on titration by Winkler's method[18]. That all aglycones so far investigated do, in fact, contain an α,β-unsaturated lactone ring, was shown by a thorough comparison with simple synthetic lactones and with lactones derived from steroids in which the position of the double bond had been established beyond question[16]. These comparisons concerned principally the behaviour of the lactone ring on cleavage with aqueous and with alcoholic alkali, the ultra-violet absorption and the colour reaction with potassium ferricyanide. The synthetic steroid lactones so far obtainable do not contain all the structural features of the natural cardiac aglycones. It was comparatively easy to prepare substances with a secondary hydroxyl group at C_3 and having the 5-membered lactone ring at C_{17} in the correct position. The introduction of the tertiary hydroxyl group at C_{14} has likewise been successfully achieved[21]. The synthesis of compounds which possess the configuration of the natural substances, both at C_{14} and at C_{17}, is being worked out.

The Configuration of the Aglycones

This brings us to a further problem, the solution of which has received particular attention in the last few years, namely the configuration of the individual linkages in the aglycones. By precise evaluation of X-ray photographs, Bernal and Crowfoot[22] have shown that the natural steroids must possess a flat, relatively elongated molecule.

This necessitates that rings B and C should be united in the trans-configuration, both in the cholanic (IX) and in the allocholanic (X) series, which differ from one another in the configuration of C_5.



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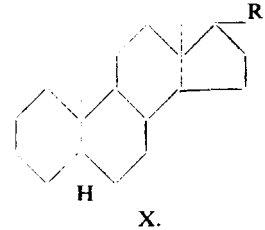
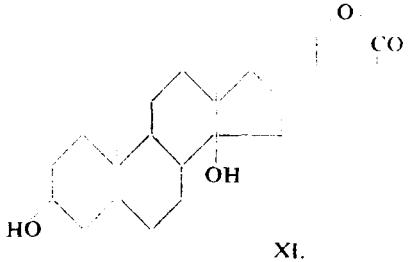


X.

The allocation of the aglycones of the cardiac glycosides to the cholanic series follows from the degradation of, for example, digitoxigenin to ætiocholanic acid. This degradation, as well as the numerous conversions which elucidated the connections between the individual aglycones, shows that the configuration of the asymmetric C-atoms 8, 9, 10, 13 and 17 in all these substances is the same as that present in the bile acids. We shall, therefore, discuss the steric relationships for those positions where particular isomerisms can occur. The two possible positions for substituents on asymmetric carbon atoms in the steroid skeleton will be denoted by the Greek letters α or β and indicated in the usual manner by dotted or by continuous valency lines. As point of reference, the C-atom in

position 10 will be selected and substituents which bear a *cis*-configuration with respect to the methyl group at this point will be defined as β-orientated.

The proof that epimerism occurs at C_3 based on the fact that cholesterol, for example, is precipitated by digitonin while certain derivatives are not, has been known for a long time. It has recently been shown, however, that not all 3β-hydroxysteroids are precipitated by digitonin, but that the precipitation can be prevented by certain substituents at other positions in the molecule. Thus, the aglycones of the cardioactive glycosides are themselves not precipitated by digitonin, and hence the configuration at C_3 had to be proved by chemical degradation in the manner shown above for scillaren A[9]. Hunziker and Reichstein[23] obtained epi-ætiolithocholic acid (3β-hydroxyætiocholanic acid, XII) from digitoxigenin (XI). The β-configuration of the 3-hydroxyl group



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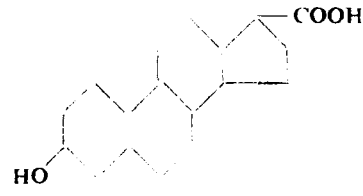

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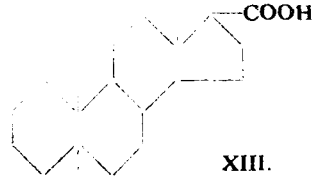


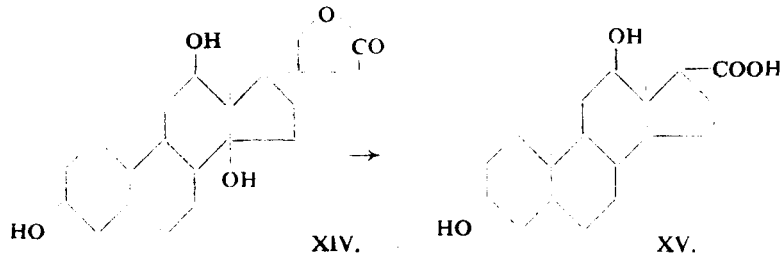
XIII.

is also found in sarmentogenin[24] and in gitoxigenin, which has been shown, by a series of reactions which do not affect the configuration at C_3 to be related to digitoxigenin[25]. Uzarigenin[26] likewise possesses a β-orientated 3-hydroxyl group, since its anhydro derivative gives a precipitate with digitonin. The opposite configuration at C_3 is found in digoxigenin[27] as has been shown by degradation to the corresponding 12-epi-ætiodeoxycholic acid.

Strophanthidin and periplogenin must possess the same configuration at all asymmetric centres, since they can be transformed into identical derivatives by methods which do not affect the steric structure[28]. The hydroxyl groups at C_3 and C_5 must bear a *cis*-configuration to one another, because they can be esterified by thionyl chloride with the formation of a neutral sulphite[29]. The β-configuration of the two hydroxyl groups follows from the ring closure between the aldehyde group at C_{10} and the 3-hydroxyl group in the anhydro-strophanthidin derivatives[30], and from the fact that, if a carboxyl is added to the aldehyde group by cyanhydrin synthesis, lactone formation takes place with the 5-hydroxyl group[31]. The substituent at C_5 therefore has the same configuration as

the hydrogen atom in the other aglycones, and hence strophanthidin and periplogenin also belong to the cholanic series. Only uzarigenin possesses the allocholanic configuration, since it is degraded to ætio-allocholanic acid (XIII)[32].

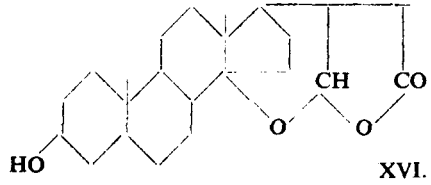
The above-mentioned degradation of digoxigenin (XIV) to 12-epi-ætiodeoxycholic acid (XV) proves that the 12-hydroxyl group has the β-configuration.



XIV. XV.

In scillirosid, the configuration at C_{12} must be the same as in digoxigenin since, in certain derivatives of *iso*-scillirosid, the presence of an oxide ring between C_{21} and C_{12} can be demonstrated, which would necessitate that the side-chain and the 12-hydroxyl group should possess the *cis*-configuration[33].

The 14-hydroxyl group which is common to all the aglycones of the cardiac glycosides must be formulated as β-orientated, since the oxide ring between C_{14} and C_{21} in the *iso*compounds of the aglycones can only be formed when the hydroxyl groups and the lactone side-chains possess the *cis*-configuration.

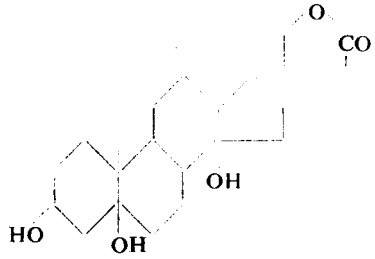


XVI.

That the isomerisation of the aglycones by alkalis takes place without change of configuration at C_{17}, is proved by the degradation of digitoxigenin via *iso*-digitoxigenin (XVI) to ætiocholanic acid[34] and by degradation of anhydro-uzarigenin to allo-ætiocholanic acid[32]. Both acids possess β-orientated carboxyl groups. It may be mentioned here that Reichstein and his co-workers have demonstrated the β-configuration of the 14-hydroxyl groups in periplogenin[35] and in digitoxigenin[36] by another method, namely, by oxidative degradation to a 20-keto-21→14-lactone. Hence, in the cardiac aglycones, rings C and D are joined in the *cis*-position, in contrast to the bile acids and the steroids. For the *cis*-union of rings C and D Reichstein[37] has proposed the term 14-*iso*, Ruzicka the term 14-allo[38].

Under the influence of one of the enzymes found in strophanthus seeds, the glycoside cymarin undergoes a peculiar rearrangement[39], resulting in the formation of an isomeric physiologically inactive glycoside which

Jacobs and his co-workers have named allocymarin. On hydrolysis with acids, this yields allo-strophanthidin which is not isomerised by alkalis, but possesses the same functional groups as strophanthidin and must, therefore, be stereoisomeric with it. The systematic removal of the asymmetry at the oxygen-substituted asymmetric centres does not lead to identical derivatives from strophanthidin and allo-strophanthidin[40]. It must, therefore, be assumed that a change of configuration at C_{17} occurs on allomerisation. Thus, the side-chain takes up a *trans*-configuration with respect to the 14-hydroxyl group and isomerisation with the formation of an oxide ring can no longer take place. Periplocymarin (periplogenin-cymarosid) can also be allomerised by means of enzymes[41]. Reichstein[35] has shown that alloperiplogenin (XVIII) differs from periplogenin (XVII) only in the configuration at C_{17}, i.e., the allo-linkage carries an α-orientated side-chain. This may also be assumed to be the case for allo-strophanthidin.



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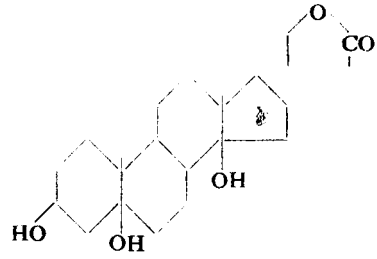


XVIII.

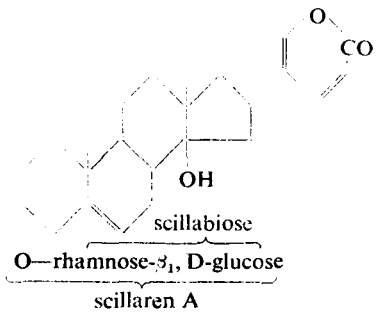
In the last few years, the introduction of simple sugars into natural aglycones and into synthetically prepared steroid lactones has been successfully accomplished, so that a whole series of partially synthetic glycosides are now available[42,43,44,45]. In addition, it has been shown that, in pharmacological experiments, synthetic substances obtained from natural aglycones by introduction of sugar residues often exhibit a high activity which, in certain cases, exceeds that of the natural glycosides. Up till now, however, no clinical results are available relating to the therapeutic activity and utility of these compounds. In the case of substances with synthetic lactone rings, the activity, although detectable, was only very slight. They lack certain structural features necessary for a high activity, such as, above all, certain steric relationships. This would almost seem to justify the old contention that Nature, in many cases, provides the physician with the remedy in its optimum form. In this connection, the extremely favourable properties of penicillin may be recalled.

The Sugar Components of the Genuine Glycosides

The close relationship which the various cardiac glycosides bear to one another is also manifest with regard to the position and composition of the sugar residue. In all the cardiac glycosides so far examined, this occupies the 3-position, where the aglycone is usually united with a

desoxy-sugar. This, in some cases, may in turn be combined with further desoxy-sugars and finally with 1 to 2 molecules of glucose. The glucose is readily split off by enzyme action and, for this reason, as already mentioned, the previously isolated glycosides such as digitoxin and gitoxin, or even digoxin from *Digitalis lanata,* lacked the terminal glucose, whereas, in the genuine glycosides obtained by excluding enzyme action, the glucose is present. The simplest example of a genuine cardiac glycoside is once more provided by scillaren A the composition of which is depicted schematically in the following diagram:

SCILLAREN A



XIX.

The sugar residue is situated at C_3 and is composed of rhamnose and glucose. Acids break the linkage with the aglycone and split off scillabiose[3,4], while scillarenase, an enzyme accompanying the glycoside in squill, breaks the linkage between rhamnose and glucose[46]. Thus, by means of this enzyme, and only in this way, a step-wise degradation may be carried out and the intermediate product proscillaridin A, a beautifully crystalline glycoside, obtained. Proscillaridin A has too low a solubility for therapeutic purposes, the only sugar present being rhamnose.

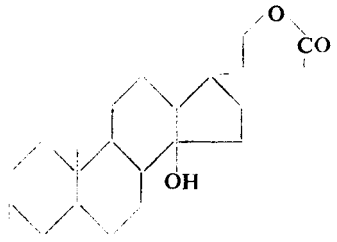
In the case of the genuine cardiac glycosides of the digitalis type, the conditions are somewhat more complicated. We had already learnt in our experiments with squill to prevent the action of the enzyme responsible for splitting off glucose. When we applied this process to the extracts of the glycosides of *Digitalis purpurea,* we obtained, instead of the already well-known crystalline glycosides digitoxin and gitoxin, amorphous but likewise very active products. The failure to crystallise was a great obstacle to the complete purification and identification of the genuine glycosides of *D. purpurea.* Only after we had first obtained experience with the glycosides of *D. lanata*[47] were we able to complete the investigations of the purpurea glycosides[48]. From *D. lanata,* by a process excluding enzymatic action, it was possible to obtain a crystalline glycosidal preparation which soon proved to be a mixture of 3 very different isomorphous glycosides which we designated digilanid A, digilanid B and digilanid C.

The marked differences in the distribution of the individual components between chloroform and aqueous methyl alcohol enabled the total

digilanid preparation to be resolved into the homogeneous components by repeated systematic partition between the two solvents. Separation of the components by fractional crystallisation is rendered very difficult by the isomorphous nature of the crystals.

The space at our disposal is not sufficient for a detailed description of the three digilanids A, B and C, and the products of their step-wise degradation. We shall, therefore, confine ourselves to digilanid A. Analogous conditions exist for digilanids B and C. The sugar chain is identical in all three digilanids.

SCHEME FOR DIGILANID A



O-digitoxose-digitoxose-acetyldigitoxose-β, D-glucose

XX.

The formula shows the unsaturated lactone ring in position 17 and 2 hydroxyl groups in positions 3 and 14, the one in position 3 carrying the sugar chain. The latter consists of 3 molecules of digitoxose and a terminal glucose. The third molecule of digitoxose also carries an acetyl group which is characteristic for the digilanids and is responsible for the isomorphism. If it is removed, the A and B components lose their power of crystallisation[49]. They yield amorphous substances which have proved to be identical with the likewise amorphous genuine glycoside of *D. purpurea*.[48] These, in turn, by enzymatic removal of the terminal glucose, are converted into digitoxin or gitoxin. Thus the relationship between the glycosides of *D. lanata* and *D. purpurea* is clearly shown. The difference between the genuine glycosides of the two plants consists therefore in the presence of an acetyl group in the lanata glycosides.

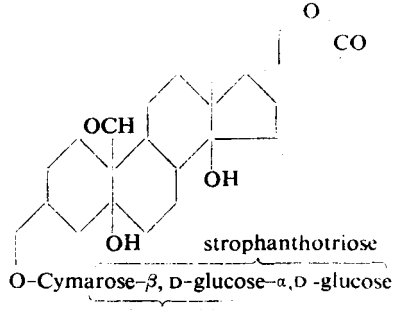
For digilanid C, which has a composition very similar to that of digilanid A, there is no corresponding glycoside in *D. purpurea*. The digilanid C-structure has so far been found only in *D. lanata* and, as Mannich[50] has shown, in *D. orientalis*, a variety very similar to lanata which is found in Asia Minor.

The best example of the step-wise degradation of a genuine glycoside is provided by strophanthin or k-strophanthosid as we designate the crystalline active principle of the seeds of *Strophanthus kombé*. Jacobs[51] in New York had already isolated in small quantities from this drug two crystalline glycosides: cymarin, consisting of the aglycone strophanthidin and cymarose, and k-strophanthin-β, which contains one glucose molecule more than cymarin. The main part of the glycosides

isolated from this drug remained amorphous but, in the form of strophanthin, a greatly purified preparation, they have been widely used for many years. In this connection, the pioneer work of Fraenkel[52] deserves especial mention.

By employing a special process utilising the peracetyl derivative (heptacetyl) we have succeeded in converting the main portion of the total glycosidal preparation from strophanthus seeds into a crystalline and pure form and have introduced it into therapy as k-strophanthosid, known commercially as "Strophosid"[53]. The following scheme shows clearly the composition and the step-wise degradation by which all the cleavage products could be isolated and characterised.

K-STROPHANTHOSID



XXI.

The aglycone, strophanthidin, possesses hydroxyl groups at C_3, C_5 and C_{14}. At C_{17} the 5-membered unsaturated lactone ring is attached and at C_{10} the aldehyde group characteristic of strophanthidin. As in the case of the other cardioactive glycosides of the digitalis group, the oxygen atom at C_3 forms the bridge to the sugar chain, which, in k-strophanthosid, consists of cymarose and two molecules of glucose. If the terminal glucose is split off by enzymatic hydrolysis, k-strophanthin-β is produced. By splitting off a further molecule of glucose with the specific enzyme strophanthobiase, k-strophanthin-β is converted into cymarin which consists only of the aglycone strophanthidin and cymarose. The linkage between the aglycone and the sugar chain is broken by acid. From k-strophanthosid, a sugar, strophanthotriose, is obtained which consists of one molecule of cymarose, and two molecules of glucose. Cleavage of k-strophanthin-β leads to strophanthobiose, composed of cymarose and glucose. Acid cleavage of cymarin yields the aglycone and cymarose.

The example of k-strophanthosid enables an exceptionally good insight to be obtained into the fine structure of the glycosidal linkages and, hence, into the nature of the enzymes which are responsible for sugar cleavage and are specific for the cardioactive glycosides. As the nature of these enzymes was originally unknown, nothing could be said regarding the configuration of the linkages between the sugars. It was found that a well-known enzyme, the α-glucosidase of yeast, was capable of splitting the linkage between the two glucose residues of k-strophanthosid, so that,

by means of an enzyme obtained from an entirely different source, the genuine glycoside could be converted into k-strophanthin-β. Thus, the α-glucosidal nature of the linkage with the terminal sugar residue was established. So long as the sugar residue remains attached to the aglycone, however, the linkage between glucose and cymarose can only be broken with the specific enzyme strophanthobiase.

Once it is separated from the aglycone with acid, the disaccharide strophanthobiose can be split by means of the well-known β-glucosidase emulsin[54]. The linkage between cymarose and glucose is therefore of a β-glucosidal nature and strophanthobiase, as far as its specific power of hydrolysing glycosides is concerned, must be classed with the β-glucosidases.

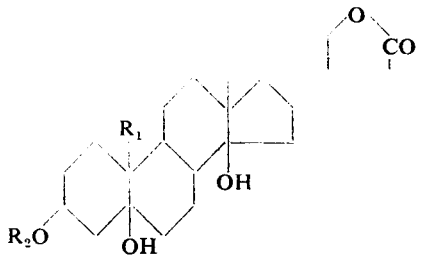
Thus, in k-strophanthosid, the inner glucose molecule has the β-form, while the outer has the α-form. k-Strophanthosid is the first heterosaccharide to be found in nature in which an α-glucosidal configuration has been established with certainty and it is therefore also the first natural heterosaccharide containing both an α- and a β-glucose residue.

In an exactly similar manner to that used in the case of k-strophanthosid, it has been possible to show that, in the other cardiac glycosides, the linkage between the terminal glucose and the desoxysugar is also of a β-glucosidal nature and hence that the specific enzymes digilanidase, digipurpidase and scillarenase belong to the β-glucosidases[54].

Other Glycosides of Strophanthus Species

The cardiac glycosides so far discussed in no way exhaust the multiplicity of this class of substances. In addition to the digitalis and strophanthus types and to *Scilla maritima*, many other plants are known which contain cardiac glycosides. Some of these possess aglycones or sugars which were not previously known. Some of them, however, are only variations of already known cardiac glycosides in the sense that both the aglycone and the sugar have already been found in the glycosides of digitalis or strophanthus though here they are differently coupled. Moreover, most varieties of strophanthus contain, in addition to the strophanthidin glycosides a number of other cardioactive glycosides, the preparation of which in a pure, intact form has, however, not yet been accomplished. On the other hand, after enzymatic hydrolysis, it has been possible to prepare the glucose-free glycosides which correspond in composition to cymarin (XXII). The variations may be seen from the following formula.

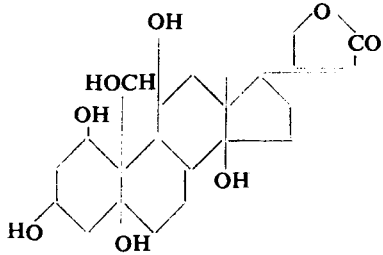
They differ from cymarin principally in the stage of oxidation of the C-atom 19 in the genin. The glycosides of periplogenin, periplocymarin (XXIII) (19-desoxo-strophanthidin) and emicymarin (XXIV), have no oxygen atom in this position. The former contains the sugar cymarose, the latter the sugar digitalose first found in digitalinum verum, the seed glycoside of *Digitalis purpurea*. Cymarol (XXV) possesses an alcoholic hydroxyl group at C_{19}. Its aglycone, k-strophanthidol, can be obtained from strophanthidin by reduction of the aldehyde group[55]. In cymarol, the hydroxyl group at C_3 is united to cymarose by a glycosidal linkage.



XXII R_1=CHO,R_2=cymarose
XXIII R_1=CH_3,R_2=cymarose
XXIV R_1=CH_3,R_2=digitalose
XXV R_1=CH_2OH,R_2=cymarose

Cymarol can be shown to be present in all strophanthus seeds which contain the glycosides of periplogenin and strophanthidin. These glycosides are found in the seeds of *Strophanthus kombé*[56], *S. eminii*[57], *S. nicholsoni*[57] and *S. hispidus*[58]. However, the botanical differentiation of strophanthus seeds is not always easy and the results of examinations of hispidus seeds in particular are for this reason rather uncertain.

The glycosides of the seeds of *S. gratus* and *S. sarmentosus* differ fundamentally from the glycosides described above. Strophanthidin, k-strophanthidol and periplogenin glycosides cannot be detected in the seeds of either of these varieties. In gratus seeds, only one glycoside, g-strophanthin, has been found and this has been shown to be identical with ouabain isolated from the bark and wood of *Acocanthera ouabaio,* an African plant of the Family Apocynaceae[59]. The sugar component of ouabain, the name by which g-strophanthin is more usually known, consists of a single molecule of rhamnose. Even under conditions which avoid enzymatic cleavage, only ouabain is obtained from gratus seeds[53]. k-Strophanthin-β remains intact in contact with gratus seeds, from which it may be concluded that these seeds do not contain a glucosidase and probably no glucose-containing glycoside. Being a rhamnosid, ouabain is very difficult to split. The hydrolytic cleavage was first accomplished



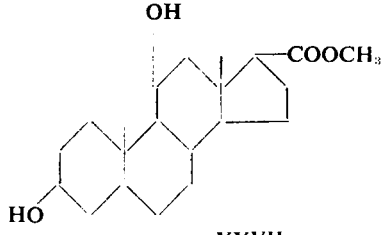
XXVI.

by Mannich and Siewert[60], who proposed for ouabagenin a complete structural formula (XXVI) with a hydroxyl group at C_5.

Since, however, under certain conditions ouabain yields a heptacetate, its genin must contain four acylable hydroxyl groups in addition to

those involved in the glycosidal linkages. This argues against a tertiary hydroxyl group at C_5, unless it is assumed that, in ouabain, in contrast to other cardiac glycosides, the sugar is attached at this point. By a number of ring closures, Mannich has provided good evidence for the positions of the other hydroxyl groups. As regards the configuration of the asymmetric C-atoms to which the oxygens are attached, it can only be said that all the hydroxyl groups are *cis*-orientated to one another.

The seeds of *Strophanthus sarmentosus* contain a mixture of glycosides none of which has yet been prepared in its native state, although three glycosides, sarmentocymarin[61] and sarmentosids A and B[62] have so far been obtained after enzymatic removal of glucose. Sarmentocymarin, an already well-known glycoside, yields, on acid hydrolysis, sarmentogenin and the methyl ether of a 2-desoxy-methylpentose, sarmentose. Although the configuration of this sugar has not yet been established with absolute certainty, on page 850 we have assigned to it the configuration of 2-desoxy-D-idomethylose (2-desoxy-D-gulomethylose). On the assumption that sarmentose possesses a straight C-chain, this is the only possible configuration, since, from each of three of the four pairs of isomers theoretically possible, at least one partner is known and sarmentose is neither identical nor enantiomorphous with any of these. The structure of sarmentogenin has been established by degradation to 3β-11α-diacetoxy-ætiocholanic acid methyl ester[24] as that depicted in formula XXVII.



XXVII.

In the case of sarmentosids A and B only the sugar components are known. Sarmentosid A contains L-talomethylose[63], a sugar which had not previously been found in a natural product. Sarmentosid B contains the sugars D-glucose and digitalose[62]. A striking feature here is that the glucosidal linkage is resistant to the attack of the specific enzyme.

Cardiac Glycosides of Unofficial Drugs

Of the remaining drugs which contain cardiac glycosides, only those whose active principles have been chemically investigated during the last 10 years will be discussed. First of all, mention should be made of *Convallaria majalis*. Convallatoxin, the glycoside contained in its leaves and flowers, was isolated and described a long time ago by W. Karrer[64]. Reichstein and Katz[65] were, however, the first to elucidate its constitution, using the method of Mannich which hydrolysed it to strophanthidin and L-rhamnose. The seeds of *C. majalis* also contain cardiac glycosides. One of these was isolated in a pure state by Schmutz

and Reichstein[66] and given the name convallosid. It is split by strophanthobiase to yield convallatoxin and D-glucose. Thus, its sugar structure corresponds to that of scillaren A. Nevertheless, it has not yet been possible to split off the sugar chain as a whole, but this would presumably be in the form of scillabiose. Convallosid is very probably the genuine glycoside of the seeds of *C. majalis*.

The roots of *Adenium somalense*, a plant of the Family Apocynaceae, together with varieties of strophanthus and acocanthera, are used by certain native African tribes, particularly in Kenya, for the preparation of arrow poisons. It has been found that they contain a crystalline glycoside to which Hartmann and Schlittler[67] have given the name somalin. On acid hydrolysis, somalin is decomposed into digitoxigenin and cymarose and is therefore so simple in structure that, apart from the configuration of the glycosidal linkage, all the structural details can be deduced merely by hydrolytic cleavage.

The aglycone of the glycosides present in the nuts of *Thevetia neriifolia* has been identified as digitoxigenin[68]. At the same time, it was shown that thevetin is decomposed both by the drug enzymes[69,70] and by strophanthobiase[70] and the digestive enzymes of snails[71], yielding the glucose-free neriifolin. The isolation of acetylneriifolin suggests that the genuine glycoside is present as an acetyl derivative of thevetin.

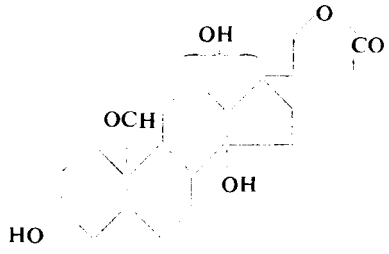
Similar glycosides have been found in the nuts of *Cerbera odollam*. Of these cerberosid[72], which is assumed to be the genuine glycoside, is converted by enzymatic degradation [73,74] into neriifolin or monoacetyl neriifolin (cerberin)[75,76].

Thevetose is also the characteristic desoxysugar of the glycosides contained in the nuts of *Tanghinia venenifera*, another member of the Apocynaceae[77]. Frèrejacque and Hasenfratz were able, after isolating several glucose-free glycosides from the amorphous, acetyl-containing tanghinosid, to prepare gentiobiose, so that here, too, the nature of the sugar residue has been to a large extent cleared up[78].

The leaves of *Adonis vernalis* (Ranunculaceae) contain at least two glycosides, cymarin and adonitoxin[79], which, together, may be considered to be mainly responsible for the pharmacodynamic action of this drug. Adonitoxin has been further investigated by Katz and Reichstein. It contains the sugar component L-rhamnose united with an aglycone which is isomeric, but certainly not identical with strophanthidin. This aglycone, likewise, possesses an aldehyde group and the simply unsaturated γ-lactone ring characteristic of the genins of digitalis and strophanthus glycosides, but, in contrast to strophanthidin, it has only one tertiary and two acylable hydroxyl groups.

From the seeds of *Cheiranthus cheiri* (Goldlack), a cardioactive glycoside cheirotoxin can be isolated[80]. On cleavage by the method of Mannich, this yields strophanthidin and a sugar syrup from which phenyl glucosazone can be obtained. In addition to glucose, however, cheirotoxin contains a pentose, the D-lyxose[81] which is the first pentose found as a component of cardiac glycosides.

The sap of *Antiaris toxicaria* provides two glycosides α- and β-antiarin which, according to Kiliani[82], differ only in their sugar components. β-antiarin contains L-rhamnose, α-antiarin the sugar of D-gulomethylose[83], which is found here for the first time as a constituent of a natural product. Reichstein and co-workers were the first to prepare intact antiarigenin, to which they assigned the formula XXVIII with a secondary non-acylable hydroxyl group, the position of which has not yet been determined.



XXVIII.

Evonosid, the glycoside from the seeds of *Euonymus europæus*, contains a sugar chain consisting of two molecules of glucose and one of rhamnose. On treatment with strophanthobiase, step-wise degradation takes place as a result of which the intermediate product evobiosid and the end-product evomonosid are formed[84].

The seeds of practically all the known species of *Coronilla* belonging to the section Eucoronilla contain cardioactive glycosides. We have investigated the seeds of *Coronilla glauca* more exactly[85]. Cautious extraction yielded an amorphous mixture which it was impossible to separate. On acid hydrolysis, the genin portion completely resinified while, in the sugar portion, only glucose was found. From the yield of the latter it was concluded that the genuine glycosides were built up from the genin and, on the average, two molecules of sugar. Enzymatic degradation of the glucosidal mixture with the specific enzyme of Coronilla seeds led direct to the aglycones, a result which had previously not been obtained with any other hydrolytic enzyme specific for cardiac glycosides.

From the mixture of aglycones, in addition to a considerable quantity of a furocumarin, it was possible to isolate in a crystalline form four different compounds with the carbon skeleton of the genins of the cardiac glycosides. These are:

Allo-glaucotoxigenin	$C_{23}H_{32}O_6$
Corotoxigenin	$C_{23}H_{32}O_5$
Coroglaucigenin	$C_{23}H_{34}O_5$
Glaucorigenin	$C_{23}H_{32}O_6$

Allo-glaucotoxigenin, corotoxigenin and glaucorigenin contain an aldehyde group at C_{10} of the steroid skeleton; coroglaucigenin has a methyl group in this position. Allo-glaucotoxigenin is inactive; it represents the

allo form, which probably arises through the action of an allomerising enzyme present in the seeds.

The above review shows that the investigations of the composition, structure and configuration of the individual cardio-active glycosides and on their synthesis have reached impressive proportions. Moreover, it emphasises the important part played by enzymatic studies in this field of research.

The chemical investigations on the cardiac glycosides which have so far been made have provided a fairly clear and complete picture, and have laid the foundations for the pharmacological investigations and for the clinical application and differentiation of the various glycosides. Nevertheless, it will be well worth while, particularly in view of the rapid increase in diseases of the heart and circulation, to expand still further our knowledge of the completed and improved therapeutic armamentarium, to which the glycosides known to-day have certainly contributed. Only on the basis of long experience will it be possible to establish rules which will make it easy, in each individual case, to select the best from the good preparations.

If data on the pharmacological action and the therapeutic application of cardiac glycosides have not been given in this article, it is because it would be out of place here to discuss the treatment of heart diseases. It should, however, be stressed that in this field therapy cannot be carried out according to any pre-arranged scheme, but that it still requires, more now in fact than ever, the whole art of the experienced physician.

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