THE CARDIAC GLYCOSIDES

The Cardiac Glycosides

A series of three lectures delivered in the College of The Pharmaceutical Society of Great Britain under the auspices of the University of London

By

PROFESSOR ARTHUR STOLL,
D.Sc., M.D. *(honoris causa)*

The Pharmaceutical Press
23 Bloomsbury Square
London, W.C.1

First Published [illegible] 1937

PRINTED AND BOUND IN GREAT BRITAIN
AT THE WORKS OF
W. HEFFER AND SONS LTD
CAMBRIDGE, ENGLAND

PREFACE

THIS book owes its publication to the series of lectures on the cardiac glycosides which I had the honour of delivering under the auspices of the Academic Council of the University of London in May of this year. These lectures provided me with an opportunity of surveying and discussing the previous investigations of the cardiac glycosides and, in particular, of presenting the results which have been obtained in my laboratory during the past few years. These latter investigations resulted in the isolation of the glycosides actually pre-existing in the fresh plant and thus made possible their individual study and the elucidation of their relationships to previously isolated glycosides now shown to be degradation products.

The work embraces different fields of chemical, pharmaceutical and medical research, and serves to illustrate the fact that chemical discoveries of theoretical interest may have important practical applications in pharmacy and medicine. While I have, in the main, been content to confine my consideration of the subject to the chemical discoveries made with regard to the cardiac glycosides, I have not hesitated to indicate briefly in what other ways their study may be of interest.

To the Academic Council of the University of London and to the members of the Board of Studies in Pharmacy I wish to express my gratitude not only for their kind invitation to discuss this branch of plant

chemistry, but also for the opportunity they have given me of reproducing the lectures in book form.

My sincere thanks are due also to my collaborators, Drs. E. Suter, W. Kreis, B. B. Bussemaker, A. Hofmann, A. Helfenstein and J. Peyer, whose whole-hearted co-operation and enthusiastic interest in the work has been a constant feature which I much appreciate. In particular, I wish to stress the large amount of actual experimental work performed by Dr. W. Kreis in the isolation of the genuine glycosides of squill and digitalis and in elucidating the relationships between the new digitalis glycosides and those previously known.

Finally, I wish to tender my grateful thanks to Professor C. B. van Niel for his kind assistance with the English manuscript and to Dr. W. H. Linnell for help in the preparation of the manuscript for publication.

A. S.

BASLE, *June*, 1936.

CONTENTS

ILLUSTRATIONS

THE CARDIAC GLYCOSIDES

LECTURE I

The Importance of Sugars

The chemistry of the sugars is one of the extensive fields of research where the accumulation of an enormous number of facts has resulted in placing our knowledge of the group on a firm, rational basis. Since the work of Armstrong, of Irvine, of Haworth, and their collaborators has been mainly responsible for the modern developments it is fortunately unnecessary to deal with this aspect of the subject in this country—the home of the sugar chemist.

It is necessary, however, to emphasise the great importance of the part played by sugars in the economy of nature. The very wide distribution of cellulose as a substance ensuring the rigidity of plant cells, and of starch and glycogen as reserve materials, is too well known to require more than a passing reference. Glucose, the common unit of these polymeric carbohydrates, is formed in the process of photosynthesis, the fundamental synthetic reaction in the household of living matter. The synthesis occurs in the leaves under the influence of chlorophyll whereby the energy of sunlight is transformed into the necessary chemical energy required to convert the water and carbon dioxide of the air into the sugar molecule. Glucose so formed, is both the building material and the primary source of chemical energy in all living beings. This dependence of the living world on the

sugars synthesised by the plant gives rise to an almost unlimited diversity of sugar conversions under the influence of living organisms. A large amount of information has accumulated concerning these conversions, and some of the processes have been elucidated to a considerable extent, as instanced by the investigations dealing with the fate of sugars during muscular contraction and in alcoholic fermentation. Again, the investigation of the enzymes which direct the formation of the simple and polymeric sugars and their natural derivatives, and the hydrolysis and the further decomposition of all these substances in the living cells, has opened up an extensive field of research.

Chemically, the sugars, or carbohydrates in a restricted sense, comprise the aliphatic polyhydroxyaldehydes and polyhydroxyketones, which correspond to the general formula of hydrates of carbon, $C_x(H_2O)_y$. This classification may be extended to include derivatives of these substances which can be obtained from them, such as the polyhydric alcohols (glycerol, arabitol, sorbitol, etc.) formed by reduction, and the sugar acids (gluconic acid, glucuronic acid, etc.) formed by oxidation. Some true sugars, such as rhamnose and digitoxose, do not correspond exactly to the formula $C_x(H_2O)_y$, but are hydrogenated derivatives, in which hydroxyl groups have been replaced by hydrogen. Rhamnose and digitoxose have been found in cardiac glycosides.

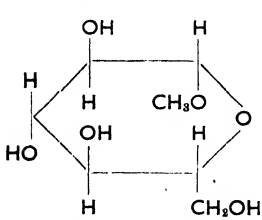
Apart from the occurrence of the sugars as such, or as so-called polysaccharides, there exists in nature an enormous diversity of natural substances which contain sugar in combination with substances

belonging to other classes of chemical compounds. All such sugar compounds are classified as *Glycosides*. One of the most important methods of investigation of such substances consists in their hydrolytic cleavage into the two major constituents of the glycoside, the carbohydrate and the organic compound with which this was combined, the latter being termed the *aglucone*.

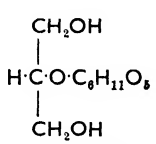
The Different Classes of Glycosides

A great number of glycosides have been discovered, especially in plants, and the functions which these substances perform in the vegetable organism are various; they may occur as reserve material, transport substances, excretory products, colouring matters, poisonous and protective substances. The last groups have often been used as medicines by mankind.

The following examples will serve to illustrate the great diversity of types of glycosides and will allow, at the same time, the cardiac glycosides to be classified among related compounds.



I. β-Methyl-*d*-glucoside



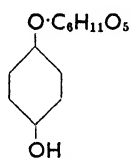
II. Floridoside

$$C_{18}H_{34}O_{16} + 2H_2O = C_6H_{14}O_6 + 2C_6H_{12}O_6$$

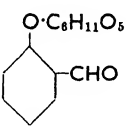
III. Clavicepsin Mannitol Glucose

The formula for β-methyl-*d*-glucoside (I), in which the chemical linkages are shown, demonstrates the structural characteristics of a sugar in glycosidal union. All other glycosides are similarly constituted,

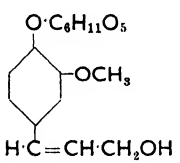
as far as the sugar fragment is concerned, but the methyl group is replaced by different organic radicals. β-Methyl-glucoside has been isolated from the leaves of Dipsacaceae.[1] Even the primitive representatives of the vegetable kingdom produce such glycosides; floridoside (II), which has been found in the alga *Rhodymenia palmata*, consists of galactose and glycerol,[2] and clavicepsin (III), which yields on hydrolysis one molecule of mannitol and two molecules of glucose, occurs in ergot.[3]



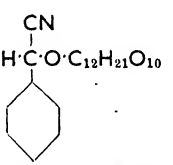
IV. Arbutin



V. Helicin



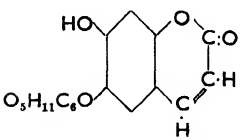
VI. Coniferin



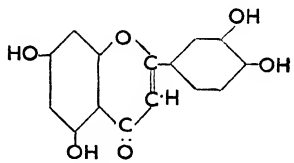
VII. Amygdalin

Particularly numerous are the glycosides derived from phenols and aromatic alcohols: reference may be made to arbutin (IV) (from the red bearberry, *Arbutus uva ursi*) consisting of hydroquinone and *d*-glucose,[4] helicin (V) (from the roots of some species of *Spiraea*),[5] the glucoside of salicylaldehyde, and coniferin (VI) (from the cambial sap of conifers), which consists of coniferyl alcohol and *d*-glucose.[6] The same group includes the cyanogenetic glycosides amongst which amygdalin (VII), the gentiobioside of mandelonitrile, is best known.[7]

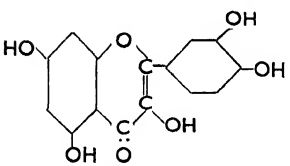
The addition of an oxide ring to phenols characterises the coumarin glycosides, such as aesculin (VIII), consisting of *d*-glucose and aesculetin (6:7-dihydroxycoumarin).[8]



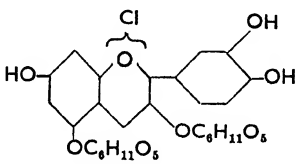
VIII. Aesculin



IX. Luteolin
+ Glucose = Galuteolin

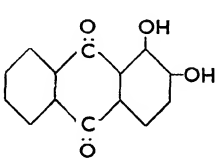


X. Quercetin
+ Rhamnose = Quercitrin



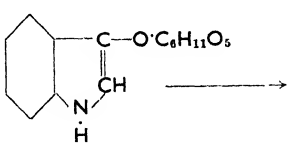
XI. Cyanin

A similar structure is encountered in the large group of yellow and red plant pigments, the flavones and flavonols, and the anthocyanins. The formulae given as examples are those of the flavone, luteolin (IX), which is combined with glucose in the glucoside, galuteolin;[9] the flavonol, quercetin (X), which with rhamnose comprises the glycoside quercitrin;[10] and the anthocyanin pigment cyanin (XI), which on hydrolysis yields cyanidin and two molecules of glucose.[11]

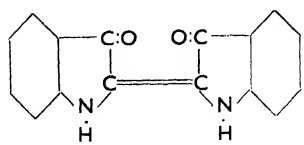


XII. Alizarin
+ Glucose + a Pentose = Ruberythric acid

Certain of the aromatic glycosides are dye-stuffs, and of these mention may be made of ruberythric acid[12] yielding on hydrolysis alizarin (XII), a pentose and glucose, and of indican (XIII), which consists of

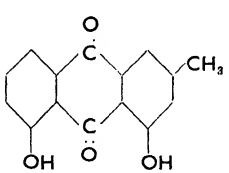


XIII. Indican

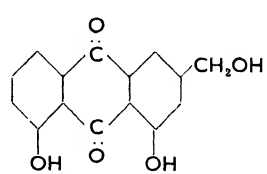


Indigotin

indoxyl and glucose.[13] Thus the three most important dyes, which for countless ages before the introduction of synthetic dyes served the old world inhabitants for the dyeing of textile fibres, are present in nature in the form of glycosides. Quercetin was used to obtain yellow and greenish shades, whilst alizarin furnished red, and indigo, blue colours.



XIV. Chrysophanic acid
Glycoside in Rheum officinale

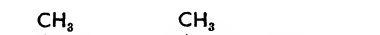


XV. Aloe-emodin
d-Arabinoside in Aloe and Senna

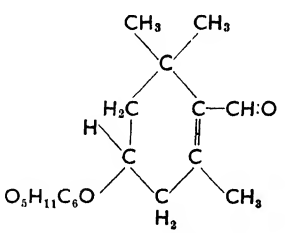
The importance of the glycosidal anthraquinone derivatives found in rhubarb, aloe and senna lies in their pharmaceutical applications. The above formulae show the aglucones of these glycosides, chrysophanic acid (XIV), isolated from rhubarb,[14] and aloe-emodin (XV), which can be obtained from aloe and from senna leaves.[15]

Furthermore, those isoprene derivatives known as the terpenes sometimes occur in nature in the

form of glycosides. Geranyl-glucoside (XVI), from *Pelargonium odoratum*,[16] consisting of geraniol and *d*-glucose, and picrocrocin (XVII), from saffron,[17] in which the constituents are saffranal and *d*-glucose, serve to illustrate this class of compounds. The saffron pigment, crocin (XVIII), is a near relative of these substances being the ester of gentiobiose and the dicarboxylic acid crocetin:[18] it is one of the few known representatives of organic carbohydrate esters occurring in nature.



XVI. Geranyl-glucoside



XVII. Picrocrocin

XVIII. Crocin

The more complicated terpenes lead to the sterols and to their glycosidal derivatives, the sterolines, such as sitosterol-*d*-glucoside, $C_{35}H_{60}O_6$, obtained from *Trifolium pratense*, from olives, and from the leaves of *Ginkgo biloba*.[19] The investigations of the last few years have shown that the cardiac glycosides, as well as the saponins, are very closely related to these sterol glycosides.

The Cardiac Glycosides

The chemical structure of the cardiac glycosides[20] will be discussed at length in the second lecture, and hence it may suffice at this juncture to say that all the cardiac glycosides that have been investigated in detail are hydroxylactones of sterol hydrocarbons in which one hydroxyl group is connected with a sugar molecule or a chain of several sugars.

The function performed by the cardiac glycosides in the plants is a problem that has not yet been solved, but several speculations have been advanced. It seems possible that these exceptionally poisonous substances serve as a guard against vermin and protect the plant against parasites, but objections may be raised to this teleological point of view and the cardiac glycosides considered as merely metabolic waste products. Furthermore, it is possible that the plant uses the water soluble glycosidal forms in order to transport the sparingly soluble sterol lactones from one part of the plant to another or the plant may store sugars or sterols in the form of such glycosides. As far as I am aware, a specific physiological function of the cardiac glycosides in the general metabolism of the plants has not been demonstrated.

The cardiac glycosides which have been carefully investigated occur in the families of the Scrophulariaceae, Apocynaceae, Ranunculaceae and Liliaceae, but it is probable that similar glycosides will be found also in representatives of other families. On the other hand, it is improbable that the distribution of the cardiac glycosides will be found to be as wide as that of the structurally similar saponins. The latter might

owe their general occurrence to the fact that they influence the surface tension of the plant saps, while the cardiac glycosides do not possess this physiologically important property.

The distribution of the cardiac glycosides in the plant varies from type to type and determines which part of the plant is used as the raw material for their isolation. In the case of *Digitalis*, the leaves and seeds are utilised; in *Strophanthus*, the seeds; in squill, the bulbs; in the lily of the valley, mainly the flowers; and in *Periploca* and ouabaio, the wood and the bark.

Ancient documents show that knowledge of the poisonous property of the cardiac glycosides is very old indeed: since olden times primitive tribes have used, and are using even to-day, the *Strophanthus* species to prepare arrow poisons. Again, the use of some of these drugs as remedies dates back into antiquity.

The Digitalis Glycosides

The credit for having introduced the cardiac glycosides into modern medicine must be ascribed to your fellow-countryman, William Withering. It was he who, as early as 1785, set forth the principles governing the dosage and the preparation of a drug containing cardiac glycosides in his monograph "An Account of the Foxglove and some of its Medical Uses: with practical remarks on dropsy, and other diseases." Even to-day this is still a highly instructive textbook on digitalis therapy.

The leaves of the red foxglove (*Digitalis purpurea* L.), used by Withering in his medicines, have since that time been the subject of careful pharmaceutical and chemical investigations. As a result of this research

work three glycosides, digitoxin, gitoxin and gitalin, had been isolated in a chemically pure state when our own investigations were commenced.

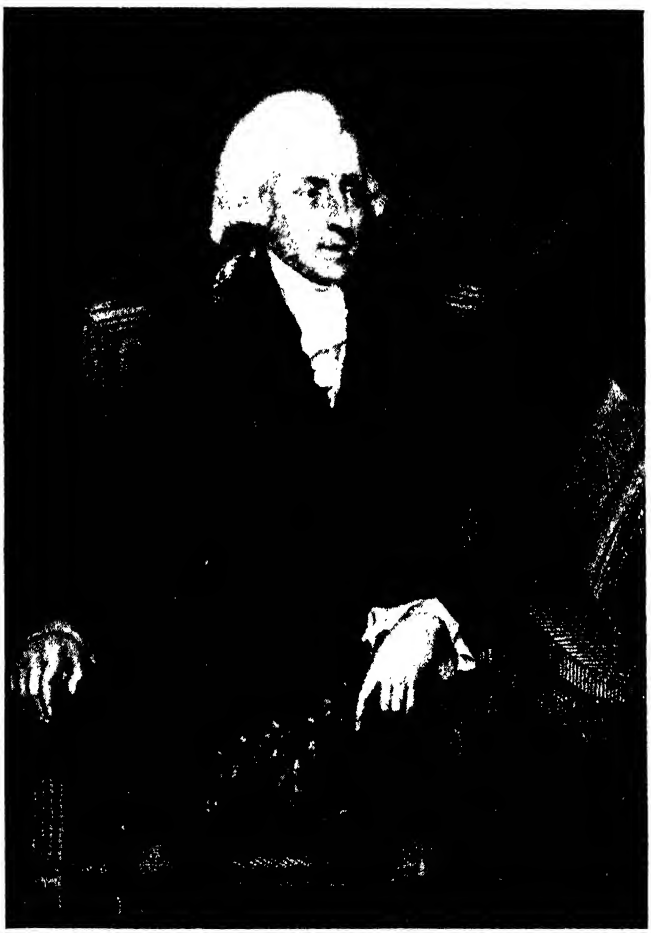
Digitoxin, which was probably already present in the digitaline preparations of Homolle and Quévenne, was first described as a pure crystalline substance "digitaline crystallisée" in 1869 by Nativelle in his famous publication,[21] but the careful chemical and pharmacological investigation of digitoxin by M. Cloetta,[22] was not published until 1920. Soon afterwards, Windaus[23] completed the analytical investigation of digitoxin by determining its empirical formula and by interpreting its behaviour on hydrolysis.

Digitoxin, which in pure form is sparingly soluble in water, has the formula $C_{41}H_{64}O_{13}$, and on hydrolysis with acids yields one molecule of digitoxigenin and three molecules of digitoxose, as shown by the following equation.

$$\underset{\text{Digitoxin}}{C_{41}H_{64}O_{13}} + 3H_2O = \underset{\text{Digitoxigenin}}{C_{23}H_{34}O_4} + 3\underset{\text{Digitoxose}}{C_6H_{12}O_4}$$

The combination of one molecule of aglucone with three molecules of digitoxose is also characteristic for the digitalis glycosides, gitoxin and digoxin, and hence glycosides with this composition will be designated as being of the digitoxin type. The structure of the aglucone will be treated in the second lecture, and the sugar digitoxose, specific for digitalis glycosides, will be discussed at the end of this lecture.

Gitoxin, the second representative of the glycosides from *Digitalis purpurea* leaves, was isolated as a chemically pure substance by Cloetta[24] and by Krafft.[25] Later, Windaus[26] established its formula and showed



Pl. 1 WILLIAM WITHERING

that its behaviour on hydrolysis could be represented as follows:—

$$\underset{\text{Gitoxin}}{C_{41}H_{64}O_{14}} + 3H_2O = \underset{\text{Gitoxigenin}}{C_{23}H_{34}O_5} + 3\underset{\text{Digitoxose}}{C_6H_{12}O_4}$$

Another glycoside, also isolated from the same material by Cloetta,[24] and investigated chemically by Windaus,[27] is gitalin, which on hydrolysis yields, in contrast with gitoxin, only two molecules of digitoxose

$$\underset{\text{Gitalin}}{C_{35}H_{56}O_{12}} + 2H_2O = \underset{\substack{\text{Gitoxigenin-}\\\text{hydrate}}}{C_{23}H_{36}O_6} + 2\underset{\text{Digitoxose}}{C_6H_{12}O_4}$$

The aglucone contained in gitalin can be converted into gitoxigenin by dehydration.

The glycosides which so far have been isolated from the seeds of *Digitalis purpurea* differ, quite unexpectedly, from those occurring in the leaves. Digitalinum verum, originally described by Schmiedeberg,[28] was studied later by Kiliani and Windaus,[29] the latter of whom determined its chemical composition as revealed by elementary analysis and hydrolysis. The results may be summarised as follows:—

$$\underset{\substack{\text{Digitalinum}\\\text{verum}}}{C_{36}H_{56}O_{14}} + 2H_2O = \underset{\substack{\text{primary aglucone}\\\text{(Gitoxigenin?)}}}{C_{23}H_{34}O_5} + \underset{\text{Digitalose}}{C_7H_{14}O_5} + \underset{\text{Glucose}}{C_6H_{12}O_6}$$

$$-2H_2O = \underset{\text{Digitaligenin (Dianhydrogitoxigenin)}}{C_{23}H_{30}O_3}$$

From this equation it appears that the aglucone originally contained in the glycoside is not isolated, but that under the influence of the rather severe treatment which is necessary in order to cause

hydrolysis, the primary aglucone, gitoxigenin, loses two molecules of water yielding dianhydrogitoxigenin. A similar behaviour is met with in the case of other glycosides, such as scillaren A, where hydrolysis proceeds with the loss of one molecule of water, and uzarin which yields an aglucone containing two molecules of water less than would be expected.

An explanation of the difference in the conditions under which hydrolysis of the various glycosides takes place has been offered by Jacobs.[30] A careful comparison of the behaviour of various glycosides towards acids led him to the assumption that only those glycosides in which the aglucone is combined with an α-deoxysugar can be readily hydrolysed. This condition is fulfilled in the case of digitoxin, gitoxin and gitalin, in which the carbohydrate constituent is digitoxose. In contrast, digitalinum verum, containing glucose and digitalose, which are not α-deoxysugars, can be hydrolysed only by drastic methods. An exception to this rule is observed in the above-mentioned case of scillaren A,[31] where hydrolysis occurs readily in spite of the fact that owing to the nature of the sugars present, rhamnose and glucose, a behaviour analogous to that of digitalinum verum might be expected. Yet the special structure of the aglucone may well account for the ready elimination of the sugar, which proceeds with the formation of an extra double bond. The specific nature of this hydrolysis is evidenced also by the elimination of the carbohydrate when the glycoside, scillaren A, is subjected to moderately high temperatures *in vacuo*, and further support of the assumption that the arrangement of the double bonds plays an important rôle in

the hydrolysis of this glycoside is found in the impossibility of hydrolysing it after hydrogenation.[32]

Having explained as far as possible the different results obtained by hydrolysis of glycosides a return may be made to the consideration of the individual digitalis glycosides isolated previous to the publication of our own investigations.

In addition to *Digitalis purpurea*, a related species of foxglove (*Digitalis lanata*) has been subjected to chemical and pharmacological investigations in recent years. In 1930, Sidney Smith[33] succeeded in isolating from the leaves of this plant not only the purpurea glycoside gitoxin, but, in addition, a glycoside which differed from those already known. This new glycoside, digoxin, is derived from a hitherto unknown aglucone, digoxigenin, whilst the carbohydrate constituent is identical with that of digitoxin and gitoxin.

$$\underset{\text{Digoxin}}{C_{41}H_{64}O_{14}} + 3H_2O = \underset{\text{Digoxigenin}}{C_{23}H_{34}O_5} + \underset{\text{Digitoxose}}{3C_6H_{12}O_4}$$

Perrot, Bourcet and Raymond-Hamet[34] obtained, from *Digitalis lanata*, a glycoside preparation, dilanine, which has not yet been investigated further.

To Mannich, Mohs and Mauss the same plant yielded a number of glycoside preparations which were designated as lanata-glycosides I–IV.[35] Some confusion has arisen concerning the nature of the various glycosides[38] owing to the fact that the original data and the interpretations suggested have been amended on a number of occasions.[36,37] Lanata glycoside III has been stated to be the acetyl derivative of digitalinum verum, and Mannich and Mohs have concluded finally that the lanata glycosides I and II are

mixtures of the digilanids. This group of substances will be discussed in detail in the last lecture dealing with the genuine digitalis glycosides.

The Strophanthins[20]

The cardiac glycosides previously discussed have all been obtained from two closely related plant species of the family Scrophulariaceae, and very little is known about the occurrence of cardiac glycosides in other members of this family. The largest number of plants, however, in which such substances have been found belongs to the Apocynaceae, of which the various *Strophanthus* species, particularly, are characterised by the relatively very high glycoside content. Botanically, the species of this genus can be distinguished from one another only with difficulty, and this, in turn, has handicapped any rapid progress in the chemical investigation of their glycosides. The work of Jacobs and his associates marked a great step forward by bringing about a certain order in the field of strophanthus glycosides by characterising the various members of the group and by establishing their relationships with other cardiac glycosides.

The glycosides which so far have been isolated from *Strophanthus* species are shown in Table I and will be discussed in the same sequence in which they occur there.

Cymarin was found by Jacobs[39] to be an essential constituent of the mixture of glycosides present in the seeds of *Strophanthus kombé*. Its composition has been determined by Windaus and Hermanns,[40] who obtained it from various species of *Apocynum* (*cannabinum*, *androsaemifolium* and *venetum*), and, on

hydrolysis, it yields the aglucone strophanthidin and the methylated deoxysugar cymarose.

TABLE I

STROPHANTHUS GLYCOSIDES AND SOME RELATED CARDIAC GLYCOSIDES

$C_{30}H_{44}O_9$ Cymarin	$+H_2O$	=	$C_{23}H_{32}O_6$ Strophanthidin	$+C_7H_{14}O_4$ Cymarose	
$C_{36}H_{54}O_{14}$ *k*-Strophanthin-*β*	$+2H_2O$	=	$C_{23}H_{32}O_6$ Strophanthidin	$+C_7H_{14}O_4$ Cymarose	$+C_6H_{12}O_6$ Glucose
$C_{30}H_{44}O_9 \cdot (C_6H_{10}O_5)x$ amorphous *k*-Strophanthin	$+(1+x)H_2O$	=	$C_{23}H_{32}O_6$ Strophanthidin	$+C_7H_{14}O_4$ Cymarose	$+xC_6H_{12}O_6$ Glucose
$C_{30}H_{44}O_9$ Allo-cymarin	$+H_2O$	=	$C_{23}H_{32}O_6$ Allo-strophanthidin	$+C_7H_{14}O_4$ Cymarose	
$C_{30}H_{46}O_8$ Sarmento-cymarin	$+H_2O$	=	$C_{23}H_{34}O_5$ Sarmentogenin	$+C_7H_{14}O_4$ Sarmentose	
$C_{30}H_{46}O_8$ Periplo-cymarin	$+H_2O$	=	$C_{23}H_{34}O_5$ Periplogenin	$+C_7H_{14}O_4$ Cymarose	
$C_{29}H_{44}O_{12}$ Ouabain	$+H_2O$	=	$C_{23}H_{34}O_8$ (Ouabagenin) (Decomposition, $-H_2O$)	$+C_6H_{12}O_5$ Rhamnose	
$C_{30}H_{46}O_9$ Oleandrin	$+H_2O$	=	$C_{23}H_{34}O_5$ (Gitoxigenin?) (Decomposition) $-2H_2O$ = Digitaligenin	$+C_7H_{14}O_5$ Digitalose?	
$C_{35}H_{54}O_{14}$ Uzarin	$+2H_2O$	=	$C_{23}H_{34}O_4$ (Uzarigenin) (Decomposition, $-H_2O$)	$+2C_6H_{12}O_6$ Glucose	
$C_{36}H_{52}O_{13}$ Scillaren A	$+H_2O$	=	$C_{24}H_{30}O_3$ Scillaridin A	$+C_6H_{12}O_5$ Rhamnose	$+C_6H_{12}O_6$ Glucose
$C_{30}H_{42}O_8$ Proscillaridin A		=	$C_{24}H_{30}O_3$ Scillaridin A	$+C_6H_{12}O_5$ Rhamnose	

The same species of *Strophanthus* contains also a series of glycosides which can be derived from cymarin by the addition of one or more molecules of glucose. The glycoside with one additional glucose

molecule, namely *k*-strophanthin-*β*, has been isolated in a crystalline state, whereas the more complex derivatives richer in glucose have been obtained, so far, only as an amorphous mixture. This amorphous mixture of glycosides finds therapeutic application under the name of strophanthin or kombé-strophanthin.

The relationships existing between all these glycosides have been elucidated by Jacobs.[41] This author found, in the seeds of *Strophanthus* species, an enzyme, strophanthobiase, which is characterised by its ability to remove all the glucose molecules from *k*-strophanthin-*β* and from the amorphous strophanthins richer in glucose. It was the study of the hydrolytic cleavage occurring under the influence of this enzyme which led Jacobs to the conclusion that the more complex glycosides differ from *k*-strophanthin-*β* only by their higher glucose content.

In the seeds of *Strophanthus kombé*, Jacobs[42] established the presence of still another enzyme which causes the conversion of cymarin into an isomeric compound, allo-cymarin, which proved to be without any detectable cardio-activity. This change must have involved the aglucone, strophanthidin, as, on hydrolysis, allo-cymarin yielded a different aglucone, allo-strophanthidin, in addition to unchanged cymarose.

According to Jacobs, strophanthidin is also the aglucone of the glycosides of *Strophanthus hispidus*,[43] for, on enzymatic hydrolysis, this mixture of glycosides furnished cymarin, but some differences were noticed in the behaviour of the hispidus and kombé glycosides as regards the rate of hydrolysis by acids.

From *Strophanthus sarmentosus*[44] the same investigator

obtained a complex preparation of glycosides which, on treatment with strophanthobiase, gave rise to a single glycoside, sarmentocymarin. The complex consists of a series of substances containing sarmentocymarin and additional glucose molecules. On hydrolysis, sarmentocymarin yields sarmentose and the aglucone, sarmentogenin, which has not yet been fully investigated.

The situation in the *Strophanthus* species so far discussed has been comparatively simple, as in each species the glycosides encountered were derived from only one aglucone. *Strophanthus Emini*, however,[45] has been proved to contain a mixture of glycosides derived from at least two aglucones, the previously mentioned strophanthidin, and periplogenin, so called on account of its occurrence in the glycosides of *Periploca graeca*. Because the hydrolytic decomposition of some of these glycosides requires conditions which lead to the formation of anhydro derivatives of the aglucones, our knowledge of this group is still limited.

Strophanthus gratus, widely used as an arrow poison, contains a glycoside, ouabain, which was also detected by Arnaud[46] in the roots and the bark of the ouabaio tree, a species of *Acocanthera*. In this case also hydrolysis does not proceed readily, and hence, in spite of the fact that the glycoside is composed of only one sugar molecule and an aglucone containing, like the others, twenty-three carbon atoms, the only guide to an understanding of its chemical composition has come from the isolation of anhydro derivatives.[47]

In the seeds of *Strophanthus gratus* the ouabain is accompanied by an amorphous mixture of glycosides

which has not yet been investigated. Various *Acocanthera* species also contain glycosides which have not yet been studied.

Not infrequently one finds glycosides with the same aglucone in representatives of plant families which are neither genetically nor biologically related to one another. Thus the previously mentioned periplogenin, which forms part of the molecule of the *Strophanthus Emini* glycosides, is also the aglucone common to a number of glycosides present in the stems of *Periploca graeca*,[48] a plant belonging to the family Asclepiadaceae. Jacobs was able to produce pure periplocymarin by the action of strophanthobiase on this mixture of glycosides, glucose being obtained as a by-product. The equation in Table I shows that the structure of periplocymarin resembles that of cymarin, and the assumption may be made that the other periploca glycosides are structurally similar to the corresponding complex glycosides of *Strophanthus kombé*.

It will be noticed that the investigation of a single crystalline identity was made possible only after the mixture of glycosides originally found in the *Strophanthus* species and *Periploca graeca* had been simplified by the elimination of glucose from the more complex glycosides through the agency of the enzyme strophanthobiase. In all these cases the enzymatic hydrolysis permitted the conversion of the complex glycoside mixture to simpler substances consisting only of the aglucone and one carbohydrate molecule.

A somewhat similar situation exists in the group of digitalis glycosides, but in this case the problem of the chemical constitution of the various members has been

solved much more completely, because the pre-existing glycosides, as well as the products formed at intermediate stages, have all been isolated in a chemically pure state. It is probable that the application of similar methods of attack will ultimately lead to a clarification of the situation in those cases where, at the present time, many difficulties and controversial findings are encountered. Although the results obtained so far are both fundamental and important they also reveal the many gaps in our knowledge of this field, and especially the amount of research which is still required to throw light upon the nature of the genuine glycosides of these plants.

However, mixtures of glycosides of the strophanthin type do not occur in all representatives of the family Apocynaceae. Thus, *Nerium oleander*, known since antiquity from the writings of Hippocrates, contains a glycoside, oleandrin, which is much more closely related to the glycosides of the Scrophulariaceae, the oleandrin aglucone being identical with one of the digitalis aglucones. As a result of studies by Windaus[49] on the hydrolysis of oleandrin, it seems probable that this glycoside is composed of gitoxigenin and digitalose, thus differing from digitalinum verum only by the loss of a glucose molecule. In both cases hydrolysis leads to the formation, not of gitoxigenin, but its dianhydro derivative, but the simple relation which oleandrin appears to bear to digitalinum verum cannot be considered as fully established until the conversion of digitalinum verum into oleandrin has been accomplished.

Of the many remaining members of the Apocynaceae in which the presence of cardiac glycosides has been

established or assumed, mention need be made only of *Thevetia neriifolia*, in which Chen[50] found a simple glycoside, thevetin, $C_{29}H_{46}O_{13}$, consisting of one carbohydrate and one aglucone molecule. Neither of the two products of hydrolysis has, however, been chemically defined.

The repeatedly mentioned difficulties encountered in the hydrolysis of glycosides which do not yield the primary aglucone are encountered also in the case of uzarin, a glycoside prepared from the roots of one of the Asclepiadaceae, *Gomphocarpus*. The aglucone has been obtained only in the form of derivatives formed by the loss of water. Notwithstanding this, Tschesche,[51] in an investigation of these anhydro derivatives, has succeeded in showing that the uzarin aglucone resembles those of the other cardiac glycosides in its constitution. The carbohydrate constituent of uzarin was shown to consist of two molecules of glucose.

Squill is the best known member of the family Liliaceae containing cardiac glycosides, and these will be discussed in detail in the second lecture.

W. Karrer[52] isolated from another representative of this family, *Convallaria majalis*, the lily of the valley, the glycoside convallatoxin, $C_{29}H_{42}O_{10}$, in a chemically pure state. This glycoside, which is distributed throughout the entire plant, but which seems to accumulate principally in the flowers, has been shown by Tschesche[53] to consist of rhamnose and an aglucone which loses water during hydrolysis.

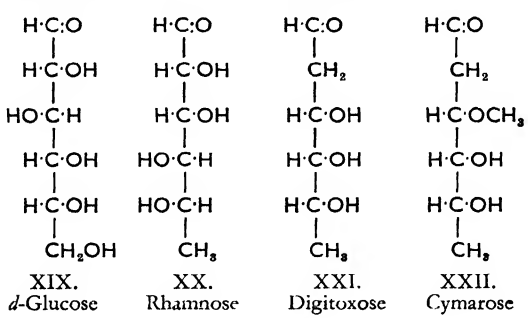
The occurrence of cardiac glycosides in the family Ranunculaceae has been known since olden times, and a great deal of research on the glycosides of *Adonis*

vernalis and of *Helleborus* species has been carried out, but our knowledge of the chemical composition of these glycosides is still very limited.

The Sugars of the Cardiac Glycosides

The discussion of the various types of cardiac glycosides is intended to demonstrate that the great diversity of its members is primarily the result of the occurrence of a large variety of aglucones. These are mutually closely related, possess the same nucleus, but differ in detail, especially concerning the position of the hydroxyl groups. These questions will be discussed in the second lecture.

The investigations of the carbohydrate constituents of the cardiac glycosides have shown that only a few types are present—a result which was to be expected because in all natural glycosides the carbohydrate constituents show but little variation.



XIX. *d*-Glucose — XX. Rhamnose — XXI. Digitoxose — XXII. Cymarose

The formulae given in XIX, XX, XXI, XXII, written in open chain formation, represent the simple cardiac glycoside sugars, the steric configuration of which has been investigated.

In addition to the ubiquitous glucose and the widely distributed rhamnose there are the two α-deoxysugars,

digitoxose and cymarose, characterised by the lack of a hydroxyl group at the carbon atom adjoining the aldehyde group. Cymarose[54] and digitoxose[55] are similar in their steric configuration and differ only in the former being the methyl ether of the latter.

Digitoxose is responsible for the Keller–Kiliani test in which a blue colour is developed when concentrated sulphuric acid is cautiously added to a solution of the glycoside in acetic acid containing ferric chloride.

Isomeric with cymarose is sarmentose,[56] also an α-deoxysugar, but the configuration and the position of the methyl group have not been determined. The same holds for digitalose,[57] which may be a methyl ether of rhamnose.

The four sugars, digitoxose, cymarose, sarmentose and digitalose have been found, so far, to occur naturally exclusively in the cardiac glycosides. It is noteworthy that the only other naturally occurring deoxysugar is deoxyribose, found in yeast nucleic acids. The peculiar structure of these sugars has considerably handicapped the chemical investigation of the cardiac glycosides. This difficulty, added to the previously mentioned complications arising during the hydrolysis, and to the complex nature of some of the mixtures of glycosides, accounts for the fact that the interesting class of cardiac glycosides has only recently begun to yield its secrets to investigators, and that many problems in this field still remain unsolved.

LECTURE II

The Squill Glycosides and the Structure of the Aglucones

The first lecture consisted of a general survey of the chemical nature of glycosides, especially of those possessing cardioactive properties, and a summary of the results of investigations of other authors on cardiac glycosides.

The starting-point of our own work on the cardiac glycosides was squill (*Scilla maritima*). The isolation and investigation of the then unknown cardiac glycosides of squill necessitated the working out of new methods, which were afterwards successfully applied to the digitalis glycosides.

The fact that the aglucones of squill had never been investigated led us to submit one of them, scillaridin A, to a careful research into its chemical structure. These results will be considered later and will be followed by a review of the results obtained by other workers on the structure of the aglucones of cardiac glycosides.

The isolation, in pure form, of the active substances of a drug which is used therapeutically, besides enlarging purely scientific knowledge also serves several practical purposes. For instance, it permits exact dosage to be used in therapeutic applications and the physician is no longer handicapped by decomposition or by fluctuations in activity which may occur in many drugs, including squill. J. Markwalder[58] has shown that the activity of galenical preparations of squill may vary to the extent of several hundred per cent., and

C. Focke[59] has proved that fresh squills of different origins show considerable variations in activity.

Squill is one of the oldest drugs used in medicine. Its history was reviewed in 1910 by Gordon Sharp,[60] and more recently in detail by E. Hirschfeld.[61] In ancient times the Egyptians, the Greeks and the Romans used squill in the treatment of many illnesses, and they worshipped the plant as a general protector against evil. The earliest mention of squill is found in a medical prescription contained in the Papyrus Ebers (about 1500 B.C.)* A sixth century manuscript of Dioscurides contains a characteristic drawing of the squill plant. These two documents are reproduced in plates 2 and 3.

Later, in the middle of the eighteenth century, G. L. B. van Swieten[62] emphasised the importance of squill as a remedy against dropsy and used, with clear insight, fresh squill because he found it to be especially active. But when Withering,[63] soon afterwards, introduced the foxglove as a cardiac remedy, squill became neglected, even though F. Home[64] discovered, almost at the same time, that squill also was cardioactive. In spite of the neglect in practical application, squill and its preparations were always mentioned in pharmaceutical textbooks. Yet it was not until 1918 that a German physician, F. Mendel,[65] at Essen, advocated anew the use of squill for many cardiac diseases.

* According to Joachim's translation, the corresponding passage reads as follows:—

"A different one for curing diseases of the heart:

Flour of dates	.. ..	1/4
Bulbs (squills?)	.. ..	1/32
Amamu plant	.. ..	1/3
Sweet beer	.. ..	1/3 dena
Tehebu tree	.. ..	1/2

Boil, sift and take for four days."

Pl. 2 Medical prescription contained in the Papyrus Ebers (about 1500 B.C.)

Pl. 3

Squill Plant
(From a Sixth Century Manuscript of Dioscurides)

Mendel's procedure was, however, decidedly inferior to that of van Swieten because the former used commercial squill powder in which a large proportion of the active substances had undergone decomposition, instead of the very active fresh drug. It is outside the scope of this lecture to mention all the workers who have carried out investigations on squill from a pharmaceutical and chemical point of view, and reference should be made to our original paper[31] in which the matter is dealt with exhaustively. Efforts to isolate the pure cardioactive substance had met with no success until the beginning of our investigations about 15 years ago, probably because previous workers had, like Mendel, used raw materials in which the active substances were more or less decomposed.

Thus the scillipicrin, scillitoxin and scillin of Merck[66] represented purified extracts rather than pure substances. Similarly the scillain of Jarmersted,[67] and of Kurtz,[68] and the apparently crystalline substances of Waliszewski,[69] scillipicrin, scillenin and scillimarin, have not been characterised as pure compounds, whilst definite statements concerning their physiological activity are lacking. The same must be said of the substances scillitin and scillidiuretin, prepared more recently by Kopaczewski,[70] and which are stated to be toxic. They have been wrongly considered to be the cardioactive principles of squill.

The preparations obtained by A. J. Ewins[71] (1911), before the beginning of our own investigations, proved to be very much more cardioactive than those just mentioned, and approached two-thirds of the activity of the pure substance which we isolated subsequently. Ewins started with a concentrated Tinctura

Scillae of the British Pharmacopoeia, and it is probable, therefore, that his raw material had been prepared from dried squill: alterations in the cardioactive substances originally present in the plant had almost certainly occurred, and hence the preparation of the genuine active principle in crystalline form was practically impossible.

The method generally used in our laboratory for the preparation of sensitive natural substances is based, firstly, on the use of raw material which is as fresh as possible, and which thus offers the best chance of containing all the active substances in unchanged form and, secondly, on mild treatment during the grinding, extracting and purifying operations. It is essential that enzymatic action be avoided—a fact not appreciated until recently—and that degradation under the influence of chemical agents such as acids and bases, the destructive influence of light and of oxygen, and, especially, thermal decomposition, be prevented. Consequently, these operations are carried out at low temperature, if possible *in vacuo*, and with the exclusion of oxygen.

Similar methods were elaborated in Willstätter's laboratory in connection with the investigations on chlorophyll and on enzymes. In our laboratory they were applied from the beginning to pharmaceutical problems, the first examples of their application being furnished by the isolation of ergotamine[72] from ergot of rye and *l*-hyoscyamine instead of atropine from *Atropa Belladonna*.[73] Atropine, which is *dl*-hyoscyamine, is not contained, as such, in belladonna leaves, but is formed by racemisation during extraction.

Although the close resemblance in the physiological

action of the foxglove and the squill had led to the assumption that squill also contained cardiac glycosides, at the beginning of our investigations, about 15 years ago, no one had a clear concept of the nature of the active principle of squill. The beautiful and characteristic colour reactions of digitalis glycosides proved to be negative in the case of squill extracts, and, at first, the only available test for following the purification process was the determination of the toxicity of the various fractions for frogs. This state of affairs lasted until a hitherto unknown colour reaction for squill glycosides was discovered. It was found that the toxicity ran parallel to the intensity of the so-called Liebermann's cholesterol reaction, in which cholesterol gives rise to deeply coloured solutions with acetic anhydride and concentrated sulphuric acid: active and partially purified extracts of squill first show a strong red colour which changes rapidly to blue and then to a bluish-green.

It appears to be outside the scope of this lecture to discuss in detail the rather extensive investigations[31] which have been necessary in order to achieve the isolation of the active principle in pure, crystalline form; it will suffice to indicate only the more important stages of the final procedure, and the reasons which led to the adoption of the individual operations.

After having found in Liebermann's colour reaction a simple guide to control the purification process, highly active preparations, containing crystalline fractions free from inactive admixtures were obtained comparatively easily. Nevertheless, it was necessary to standardise these preparations physiologically by toxicity tests on frogs or cats owing to the fluctuations

in activity resulting from their complex composition. They consisted of at least two glycosides, the beautifully crystalline scillaren A and an amorphous fraction called scillaren B, consisting probably of at least two glycosides. Scillaren A, however, predominates and accounts for about two-thirds of the entire quantity. On attempting to isolate chemically pure scillaren A from batches obtained by various methods, the yields were inconsistent, and it was not until years later that it was discovered that a beautifully crystalline scillaren A fraction could be obtained in very high yields, if the ground, fresh squill skins were allowed to stand for a few days covered with ethyl acetate. A closer examination revealed, however, that this glycoside was not identical with genuine scillaren A; it occurred in crystals of a different shape and was much less soluble in aqueous media. Chemical investigation proved that the preparation contained more aglucone and less sugar than scillaren A: the scillaren A, originally present in squill, had, during the prolonged extraction, lost one molecule of glucose through the action of an enzyme, scillarenase, also present in squill. In other words, scillaren A had been converted into proscillaridin A—a degradation that will be discussed in more detail later.

Having recognised the occurrence of enzymatic cleavage, it was necessary to devise methods to prevent it taking place if a high yield of the genuine glycoside, scillaren A, was desired. The observations made with scillaren A recalled previous experiences encountered during our research work on chlorophyll, where it was discovered that when chlorophyll extracts were left in contact with the leaf meal for

relatively long periods the aliphatic alcohol phytol was split off from the chlorophyll molecule. The crystalline chlorophyllids prepared from such extracts were recognisable as alteration products only after investigation of this reaction.

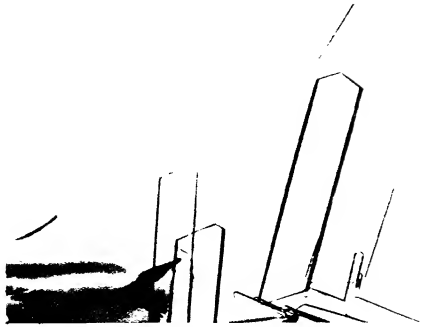
The method finally adopted for the isolation of the squill glycosides, in which the possibility of enzymatic cleavage is eliminated, may be outlined as follows: only fresh, living squill contains the initial glycosides, but the glycoside-splitting enzyme is also present and performs its destructive action as soon as the squill is ground preparatory to extraction. This enzyme action can best be prevented by the addition of salts, such as ammonium sulphate. The addition of these salts brings about, at the same time, the coagulation of mucilaginous substances which facilitates the extraction, during which the glycosides are taken up by ethyl acetate in the form of "*tannoids*," the tannoid substances then being separated from the glycosides by suitable treatment with lead salts. In this very simple way a so-called "pure glycoside preparation" free from non-cardioactive impurities is obtained. By simple solution of this mixture of glycosides in a little methyl or ethyl alcohol and by adding water or ether, a large proportion of the preparation can be crystallised out. Repeated recrystallisation yields the homogeneous and very beautifully crystalline scillaren A of about 1200 frog units toxicity per mg. A similar separation of the components can also be achieved by starting with the "glycoside tannoid complex" obtained from ethyl acetate as the differences in solubility of the tannoids are even greater than those of the glycosides.

The mother liquor contains scillaren B which, so far, has not been obtained in crystalline form. Its chemical investigation has created many difficulties because acid hydrolysis does not give clear results. Crystalline aglucone preparations have been obtained in low yield from scillaren B, and quantitative determination of the sugars resulting from its decomposition seems to indicate that they are not present in a simple ratio. Scillaren B, possessing a toxicity of about 2000 frog units per mg., is also much more readily water-soluble.

The beautifully crystalline scillaren A has been subjected to an intensive investigation. On acid hydrolysis it yields, quantitatively, the well crystallised scillaridin A and a disaccharide, scillabiose, which on continued hydrolysis is converted into rhamnose and glucose. The enzymatic cleavage mentioned above, caused by the enzyme contained in squill, scillarenase, differs from the acid hydrolysis in that the enzyme does not attack the linkage of scillaridin with the disaccharide, but yields proscillaridin A and glucose. The two modes of hydrolysis of scillaren A are presented in the diagram on page 31.

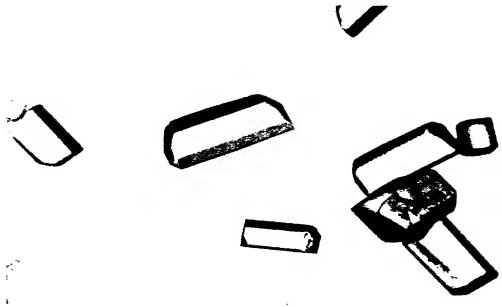
Photographs of crystals of scillaren A and proscillaridin A are reproduced in plates 4 and 5 respectively.

Scillarenase, the enzymatic nature of which has been established by a special investigation,[74] has not been obtained in cell-free state and must be considered, according to Willstätter, to be a true desmoenzyme. The enzyme is specific for scillaren A and does not attack even free scillabiose. It is also inactive towards the digitalis glycosides, which also contain a terminal glucose molecule like scillaren A.

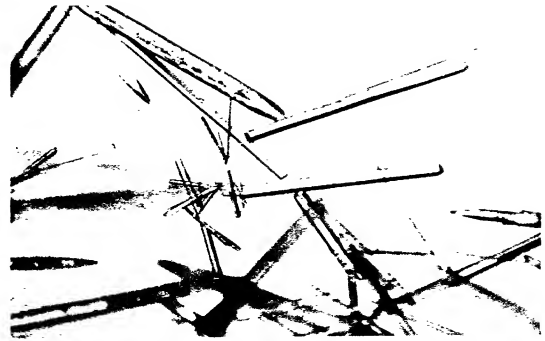


Pl. 4. Scillaren A (from 50 per cent. methanol)

Pl. 5. Proscillaridin A (from methanol)



Pl. 6. Scillaridin A (from absolute ethyl alcohol)

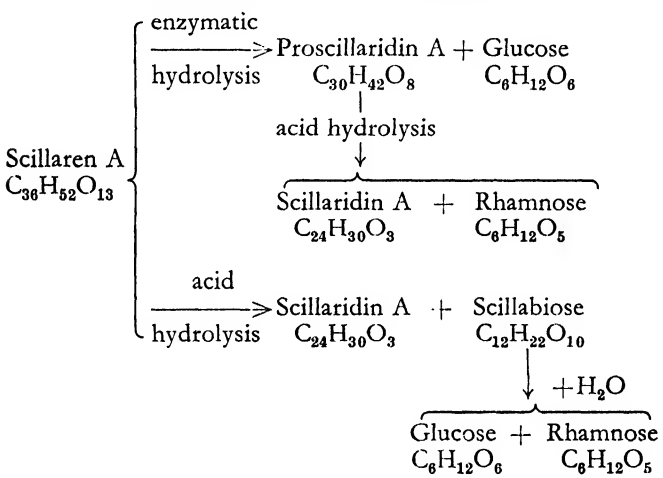


Pl. 7. Anhydroscillaridin A (from absolute ethyl alcohol)

The enzymatic transformation of scillaren A to proscillaridin A proceeds similarly to the cleavages of the strophanthus glycosides, which were described in the first lecture (e.g., the cleavage of *k*-strophanthin-*β* investigated by Jacobs).[41] The experience gained of this enzymatic decomposition afterwards became of special importance in the isolation of genuine digitalis glycosides and in their partial degradation.

TABLE II

THE HYDROLYSIS OF SCILLAREN A



The pure crystalline scillaren A, which accounts for two-thirds of the total quantity of the cardiac glycosides of squill, is not well suited for therapeutic use as it is only sparingly soluble in aqueous solvents, and these solutions gradually undergo a partial hydrolysis which yields the less active and even less soluble scillaridin A and scillabiose. The total glycosidal preparation containing also the fraction of scillaren B is more easily soluble in aqueous solvents and, at room

temperature, remains constant for many years. Standardised methods of manufacture permit the production of preparations which, as is invariably proved by physiological tests, show only insignificant fluctuations in composition.

The single therapeutic dose for the adult is 0·8 mg., or 1200 frog units; the daily dose corresponds to three to four times this quantity. The intravenous dose is 0·5 mg.; in the form of suppositories 1 mg. is administered twice daily.

The indications for scillaren fall into two groups; it fulfils all the requirements of a cardiotonic of the digitalis type, and it has been shown by physiological and clinical tests to produce copious diuresis. One of the characteristics of scillaren is its activity in cases where digitalis or strophanthus fail to act, or act insufficiently, or where intolerance to digitalis exists. But scillaren has a value of its own and does not simply live on the contra-indications or failures of other heart remedies. On account of its high therapeutic index and rapid elimination it maintains compensation in those cases where prolonged treatment is necessary.

Its diuretic activity is due to its direct action on the renal epithelium and, indirectly, to its action on the heart, whereby renal circulation is improved. Scillaren is particularly valuable as an azoturic diuretic, since it has been shown that it increases the elimination of urea, especially in cases of uraemia.

The investigation of the chemical structure of scillaren A, a substance of complicated composition, has advanced by several stages and has proceeded parallel with the progress of similar researches into other cardiac glycosides and the sterols in general.

The earliest phase of our investigation showed that acid hydrolysis of scillaren A led to the aglucone scillaridin A and the carbohydrate scillabiose,[31] which on further hydrolysis yielded one molecule of glucose and one molecule of rhamnose.

Analysis of the aglucone part, the well-crystallised scillaridin A, yielded the molecular formula $C_{24}H_{30}O_3$, showing that the aglucone contains one molecule of water less than might be expected on the basis of the equations for the hydrolysis of scillaren A and proscillaridin A. This loss of water proceeds with the formation of a double bond, and scillaridin A must be considered as the anhydro-derivative of the aglucone originally present in the glycosides.

Under certain conditions, such as sublimation in high vacuum, or by dissolving the substance in dehydrating solvents, scillaridin A loses another molecule of water with formation of a further double bond and the equally beautifully crystalline anhydroscillaridin A is obtained. Plates 6 and 7 show crystalline scillaridin A and anhydroscillaridin A.

For the further elucidation of the structure of scillaren A it was logical to consider its relationships with two other groups of substances. On the one hand, the similar physiological activity of scillaren A and the other cardioactive glycosides would indicate that similarities in chemical configuration exist. On the other hand, the intensity of the Liebermann reaction showed a much closer resemblance to certain sterols, such as ergosterol, than to the other aglucones.

The relationships between our substances, the sterols and the other aglucones thus determined the working hypothesis. The connections with these

two groups had to be established and, in doing so, it was possible to adapt the methods worked out by other authors for our purposes, although new methods had also to be devised. The chemical reactions were concerned, on the one hand, with the carbon nucleus of scillaridin A and, on the other hand, with the groups attached to the skeleton, in this case the groups containing oxygen.

Investigation of the oxygen atoms in scillaridin A[75] proved to be a simple matter; two of the three oxygen atoms belong to a lactone ring which could be established by a suitable titration. In this respect scillaridin A resembles the other aglucones of the cardiac glycosides, where the presence of a lactone ring could also be ascertained. The third oxygen atom is probably present as a tertiary hydroxyl group. When analysed by Zerewitinoff's method scillaridin A shows one active hydrogen atom, which is removed during its transformation to anhydroscillaridin A. The supposition of the tertiary character of the hydroxyl group is further supported by the fact that it does not yield a carboxyl- or keto-group on oxidation, and that the hydroxyl group is easily eliminated in the form of water by sublimation or by treatment with acids.

Indications as to the nature of the carbon nucleus of scillaridin A were obtainable firstly from the results of complete catalytic hydrogenation, which permits the determination of the number of unsaturated linkages present. In this way it was proved that scillaridin A contains four carbon double bonds,[76] and anhydroscillaridin A, five. Secondly, analytical determination of the composition of the initial material and of the

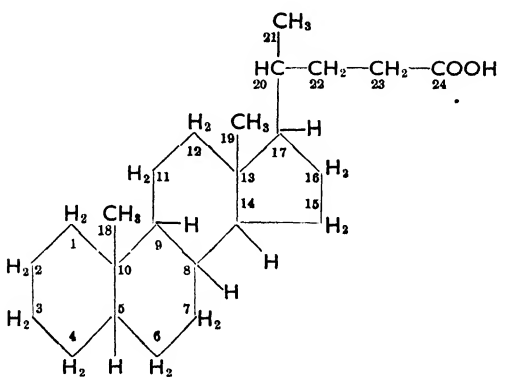
hydrogenation products showed the presence of four carbon rings in both substances.

This result was in agreement with the findings for other well investigated aglucones of cardiac glycosides. Furthermore, the presence of four carbon rings suggested that scillaridin A, like other cardiac aglucones, might belong to the domain of the sterols and bile acids, both of which exhibit such a cyclic system.

The catalytic hydrogenation of scillaridin A and anhydroscillaridin A also permitted the detection of a characteristic difference between these substances and the aglucones of other cardiac glycosides. In the case of the squill aglucones it appeared that, besides the hydrogenation of the double bonds, a second reaction proceeds which is not known to occur with other aglucones, namely, the cleavage of the lactone ring by reduction resulting in a considerable yield of saturated carboxylic acids. Thus the exhaustive hydrogenation of anhydroscillaridin A gave, in uniformly crystalline form, the saturated analogue of the carboxylic acid from which the squill aglucones are derived. This contains no other functional groups besides the two oxygen atoms of the carboxyl group and was therefore called scillanic acid.[76]

It might be expected that the reduction of double bonds contained in a complicated and probably branched system of carbon rings would yield mixtures of optical isomers which, in consequence of similar properties, would be difficult to separate. Fortunately, by fractional crystallisation of the methyl ester of scillanic acid a separation of the mixture was achieved and a homogeneous acid was isolated, which was called α-scillanic acid. By a careful comparison

of the melting points, the forms of the crystals and the optical rotations of the acid and of its methyl and propyl esters, it was shown that this acid is different from cholanic acid, the well-known key substance of the bile acids, but that it is identical with its isomer, allocholanic acid.[77] By this transformation of the squill aglucone into a substance well known in the chemistry of the bile acids the principal features of the structure of scillaridin A were elucidated and the differences between scillaridin A and the other aglucones also become obvious.

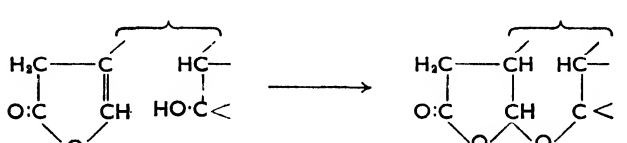


XXIII. Cholanic (Allocholanic) Acid

The formulae of cholanic and allocholanic acids, which differ only by steric isomerism of carbon atom 5, have been proposed on the basis of the results of many careful researches by Wieland, Windaus, Rosenheim and King and others, and, although there may be some doubt regarding details in consequence of the fact that as yet no derivative of the bile acids containing the original skeleton has been synthesised, the formula in all probability is correct. The derivation of a

formula for scillaridin A and its derivatives in accordance with the accepted structure of allocholanic acid is facilitated by the fact that the readily comprehensible formation of allocholanic acid from scillaridin A fixes the position of the lactone ring, the carboxyl group of the acid being necessarily derived from the carbonyl group of the lactone.

The nature and size of the lactone rings and the position of the free hydroxyl group in scillaridin A could be ascertained by a study of the action of alkali on the squill substances, a procedure which proved valuable in the investigations of W. A. Jacobs[78] on other aglucones. A. Windaus[40] had found previously that the action of alcoholic anhydrous alkali is complicated by the occurrence, in addition to the expected saponification of the lactone ring, of another reaction involving the lactone group and a neighbouring hydroxyl. The use of aqueous alkali is less suitable, because it necessitates longer exposure to alkali which, in turn, causes the resinification of the sensitive substances. The formulation of this reaction, according to W. A. Jacobs (XXIV and XXV), shows the formation of an oxide ring, which cannot be opened without further decomposition.[78]

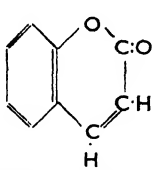


XXIV. XXV.

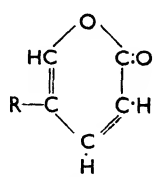
The original lactone ring is reformed on acidification, so that one obtains, by the reaction with alkali, products isomeric with the initial material, which are

called *iso*-strophanthidin, *iso*-digitoxigenin, etc. According to the above formulae the *iso*-substances are characterised by the loss of the typical double bond in the lactone ring.

The formation of the *iso*-compounds of scillaridin A and the digitalis and strophanthus aglucones is explained by this series of reactions, but in the case of scillaridin A the analytical figures showed that, contrary to the behaviour of other aglucones, a methyl group had entered the molecule. This occurrence of methylation of a lactone by alcoholic alkali recalls similar observations on the coumarins, and by analogy a similar formulation of the lactone ring in scillaridin A and in coumarins appears to be justified. Since, however, the skeleton of scillaridin A had been shown to agree with that of allocholanic acid, it was impossible to assume a lactone ring condensed with benzene as in coumarin, yet it proved possible to formulate this lactone ring (XXVII) in accordance with that of coumarin (XXVI), as shown by the following formulae:



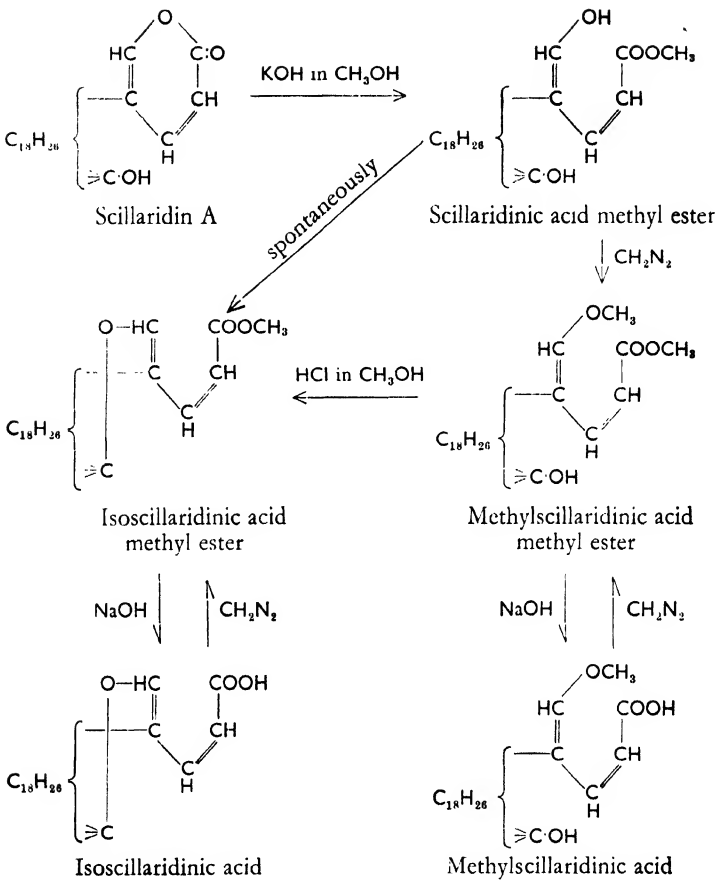
XXVI. Coumarin



XXVII. Lactone group of scillaridin A

The conversion of scillaridin A to *iso*-compounds can be interpreted through intermediate products,[75] the existence of which was proved by the possibility of methylation with diazomethane. Thus the following diagram (Table III) depicts the reactions carried out with these substances:

TABLE III



Scillaridin A

Scillaridinic acid methyl ester

Isoscillaridinic acid methyl ester

Methylscillaridinic acid methyl ester

Isoscillaridinic acid

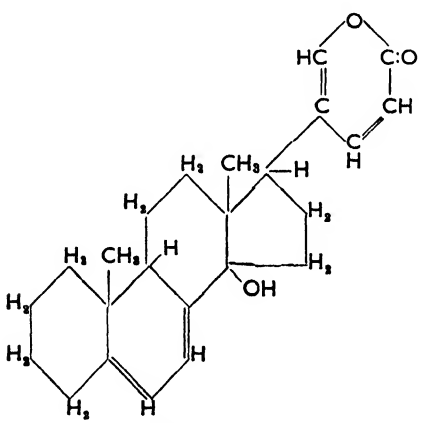
Methylscillaridinic acid

Afterwards, the same reactions were also shown to occur with the glycoside scillaren A. Of the reaction products which have been tested physiologically only one, formed in the first stage, proved to be cardioactive, which seems to show that the physiological activity does not depend exclusively upon the presence

of the lactone ring, although the latter increases the activity considerably.

Returning to the aglucone scillaridin A, a position must be assigned to the hydroxyl group which will account for the formation of the oxide ring of the *iso*-compounds. Such a position is found at carbon atom 14, and this is applicable also to other aglucones which exhibit the phenomenon of the formation of the oxide ring during isomerisation.

The formula of scillaridin A (XXVIII), which follows from the structure of allocholanic acid and the reactions just mentioned concerning the lactone ring and the hydroxyl group, is given below:

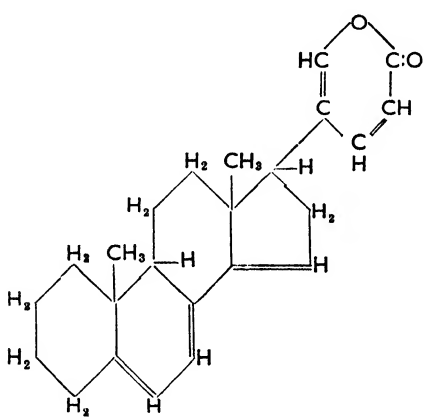


XXVIII. Scillaridin A

Of the four double bonds, two are shown in the lactone ring according to the corresponding positions in coumarin. The other two have been assumed to be in positions analogous to those of ergosterol, this assumption being based upon the exactly similar response given by scillaridin A and ergosterol to Rosenheim's colour reaction with trichloracetic acid.

However, this structural detail has not yet been proved and also, with respect to the formulation of the lactone ring, it must be said that while the formula given explains all the reactions observed without difficulty, other formulations are not excluded.

It follows from the above facts that anhydroscillaridin A may be represented by the formula given below (XXIX), and that the scheme given in Table IV illustrates the mechanism of the formation of the derivatives produced by the action of methyl alcoholic potassium hydroxide.

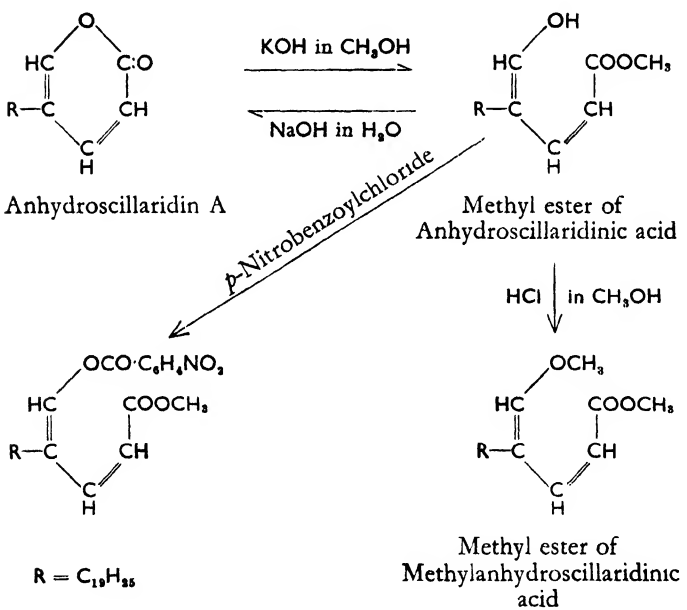


XXIX. Anhydroscillaridin A

In the case of anhydroscillaridin A, the proof that the alcoholic alkali esterifies the carboxyl group during the saponification of the lactone ring, arises from the fact that the substance formed by hydrogenation of the enolic reaction product is neutral. If the alkyl group had entered as a result of ether formation at the alcoholic hydroxyl, the hydrogenation product would have been a saturated acid.

W. A. Jacobs and his collaborators have subjected the other aglucones of cardiac glycosides, particularly strophanthidin, to an extensive series of investigations,[20] with the result that many structural details of several members, and the characteristic differences and mutual relationships of the individual aglucones, have been elucidated, whilst finally, the structure of the carbon nucleus has been established.

TABLE IV



The following table (Table V) presents the molecular formulae of the aglucones of cardiac glycosides of vegetable origin concerning which the analytical data are well established:

TABLE V

Strophanthidin	$C_{23}H_{32}O_6$
Digitoxigenin	$C_{23}H_{34}O_4$
Gitoxigenin	$C_{23}H_{34}O_5$
Digoxigenin	$C_{23}H_{34}O_5$
Periplogenin	$C_{23}H_{34}O_5$
Sarmentogenin	$C_{23}H_{34}O_5$
Uzarigenin	$C_{23}H_{34}O_4$
Ouabagenin	$C_{23}H_{34}O_8$
Scillaridin A	$C_{24}H_{30}O_3$

The occurrence of 23 carbon atoms in all these aglucones, with the exception of scillaridin A, gave rise to the assumption that the substances would possess a similar structure. Proof of this assumption was obtained on the transformation of several pairs of the aglucones into identical substances by means of suitable chemical reactions. In this manner identity of the carbon nuclei in the following pairs was established (Table VI):

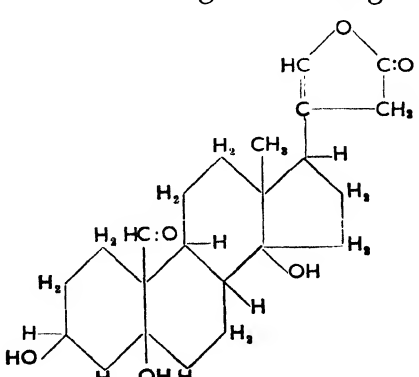
TABLE VI

Strophanthidin	— Periplogenin
Digitoxigenin	— Periplogenin
Digitoxigenin	— Gitoxigenin
Digitoxigenin	— Digoxigenin
Periplogenin	— Uzarigenin

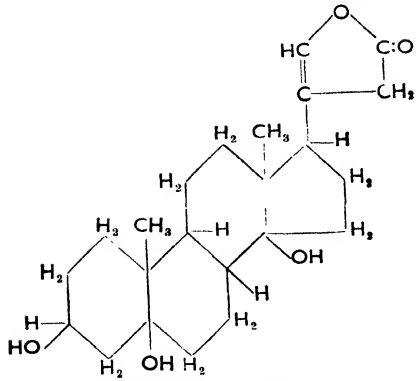
Moreover, Jacobs and Tschesche furnished evidence for the relation between the structure of the carbon skeleton common to the aglucones and that characteristic of the bile acids. By dehydrogenation of strophanthidin with selenium, Jacobs[79] obtained the aromatic hydrocarbon which had previously been prepared from sterols, and which was afterwards identified as methylcyclopentenophenanthrene. Tschesche[80] obtained the same hydrocarbon by dehydrogenation of anhydrouzarigenin.

After having thus determined the carbon nucleus of the aglucones, Jacobs[81] succeeded in degrading digitoxigenin, by a series of reactions, to a saturated acid, $C_{20}H_{32}O_2$, which proved to be identical with *aetio*cholanic acid. This degradation was accomplished, *mutatis mutandis*, by means of the method which Wieland had used in his determinations of the structure of the side chains of bile acids. In a similar way Tschesche[82] obtained *aetio*allocholanic acid, starting with uzarigenin.

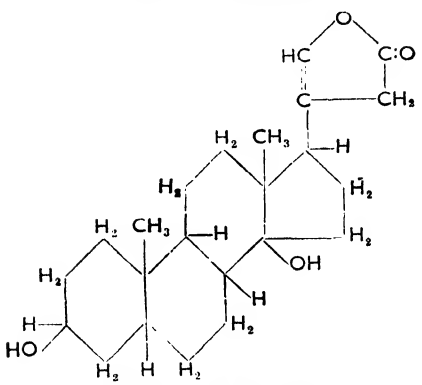
In consequence of these results the carbon nucleus and the position of the lactone ring have been established in all the above-mentioned, structurally related, aglucones. On the basis of reactions concerning the anhydro derivatives, and the oxidation and reduction of the aglucones and their isomeric derivatives, Jacobs[83] and Tschesche[84] proposed structural formulae for several aglucones. The same formulae were obtained by G. A. R. Kon,[85] as a result of theoretical deductions, and were supported by measurements of the X-ray diagrams of suitable derivatives by Bernal and Crawfoot.[86] The formulae proposed by the above authors for several of the aglucones are given below.



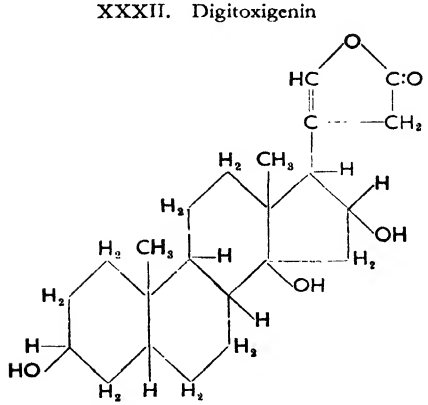
XXX. Strophanthidin



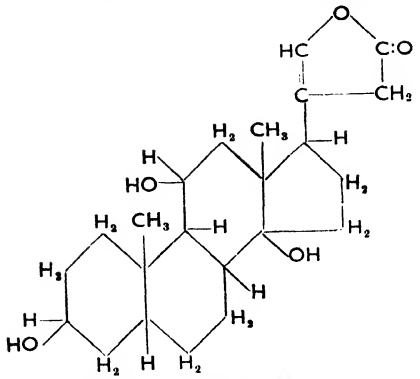
XXXI. Periplogenin



XXXII. Digitoxigenin

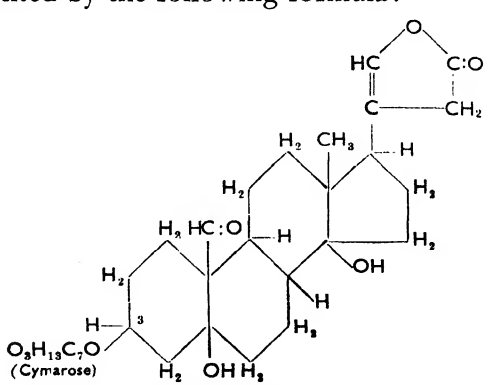


XXXIII. Gitoxigenin



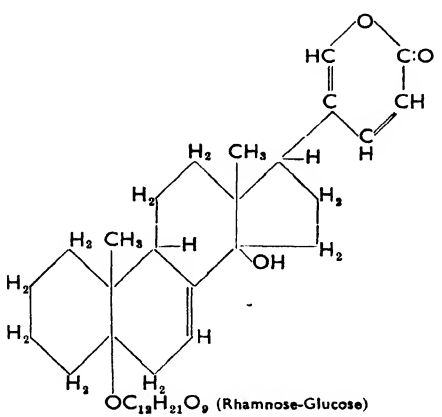
XXXIV. Digoxigenin[87]

In strophanthidin (XXX), one secondary and two tertiary hydroxyl groups and one aldehyde group will be noted in addition to the lactone ring. The hydroxyl group in position C_{14} is present in all these aglucones, and is responsible for the formation of the oxide ring during the production of the *iso*-compounds. The positions of the other hydroxyl groups vary in the different aglucones. The point of union of the sugars in the glycosides derived from these aglucones is assumed by the same authors to be at the secondary hydroxyl in position 3, and hence cymarin (XXXV) is represented by the following formula:



XXXV. Cymarin

The formula of scillaridin A, previously discussed, does not contain a hydroxyl in position 3, and it is unlikely that the additional hydroxyl of the corresponding glycoside, scillaren A, which is lost together with the sugar during hydrolysis, should be in this position. The fact that the hydrolysis of scillaren A proceeds readily under extremely mild conditions suggests the probability that the hydroxyl group concerned with the glycosidic link is tertiary. It follows that the following formula (XXXVI) for scillaren A may be advanced:

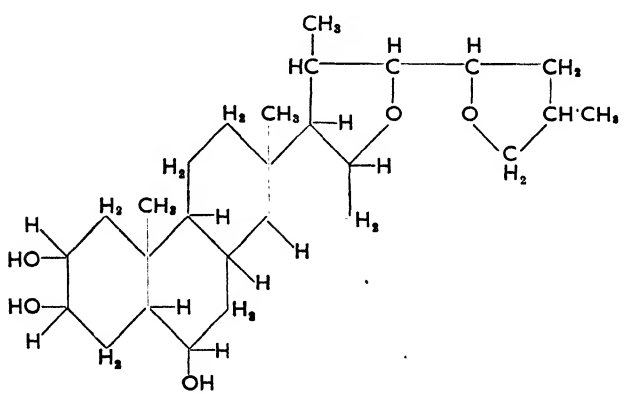


XXXVI. Scillaren A

Concluding this comprehensive discussion of the structure of cardiac glycosides and aglucones, it is interesting to consider their connections with related substances, which are characterised by their occurrence in the same plants, by similar physiological activities, or by the fact that their structures are known to be of the same type.

In many plants a series of saponin glycosides is found, which yield on hydrolysis sapogenins showing similarities to the aglucones and the sterols. The

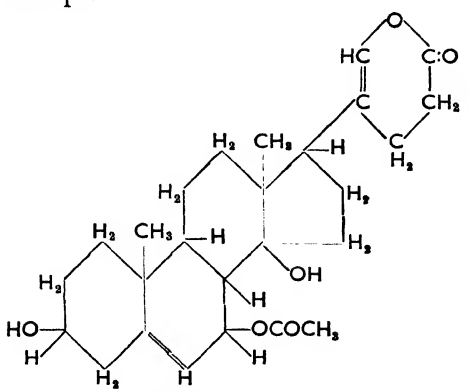
research work of the last few years has made it possible to form a conception of the structure of several digitalis sapogenins. According to Tschesche the formula of digitogenin (XXXVII), for example, shows that whereas the nuclei are fundamentally identical, the lactone ring of the aglucones has been extended to a longer side chain, in a way similar to that which marks the derivation of the sterols from the bile acids. Furthermore, the side chain of the sapogenins contains two oxide rings, and there are differences in the position of the hydroxyl groups.[88]



XXXVII. Digitogenin

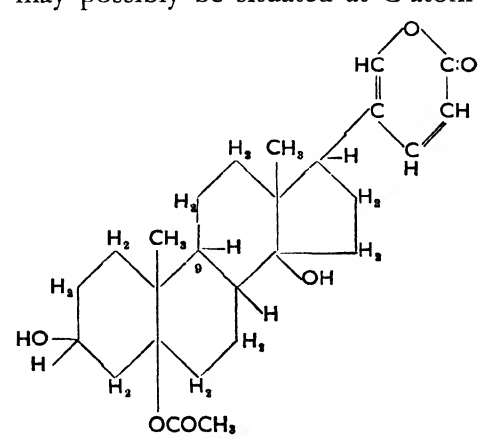
The secretions of certain parotid glands on the surface of toads contain poisonous nitrogenous substances with sterol-like constituents. These poisons, the so-called toad venoms, exert physiological activities similar to those of the cardiac glycosides. The researches carried out in the field of sterols have opened up the possibility of arriving at the correct structural formulae for this part of the toad poisons,

Wieland's[89] formula for bufotalin (XXXVIII) is shown as an example.



XXXVIII. Bufotalin

However in a recent publication [*Ann.*, **524**, 203 (1936)], H. Wieland changed the distribution of the double bonds in his bufotalin formula, especially in the lactone ring. The new formula (XXXVIIIa) closely resembles our formula of scillaridin A (XXVIII) discussed above. According to Wieland the acetoxy group may possibly be situated at C-atom 9.



XXXVIIIa. Bufotalin

The formula of bufotalin again shows a carbon nucleus common to all the substances discussed here and a lactone ring with the same number of carbon atoms as found in the active principles of squill mentioned above. But it must be pointed out that a direct connection between the toad poisons and the sterols has not yet been established as the bufocholanic acid obtained by degradation was isomeric, but not identical, with the corresponding derivatives of bile acids. However, the great number of possible isomerides favours the occurrence of isomeric compounds.

This discussion of the structure of some cardiac glycosides and related compounds serves to emphasise the variety of substances which, in recent times, have been added to the class of physiologically interesting sterol-like compounds. After the many remarkable results obtained in the domain of hormones and vitamins, some of which, like the sex hormones and vitamin D, are sterols, the investigations of cardiac glycosides have provided a further contribution to the knowledge of this group of substances, which nature seems to have selected so successfully in order to produce interesting effects.

LECTURE III

The Genuine Digitalis Glycosides

The chemical investigation of the cardiac glycosides and of their aglucones, discussed in the first two lectures, has furnished a considerable amount of information concerning their structure, but a direct application of these results in practical medicine has hardly been possible. The explanation is to be found in the fact that chemical modifications of these substances are accompanied by a decrease in their activity: indeed, if such chemical transformations concern the group responsible for cardio-activity (i.e., the aglucone constituent), a complete loss of the physiological activity generally ensues. The researches of Jacobs[90] have shown that in the strophanthin glycosides the unsaturated lactone ring is indispensable for a strongly active substance: hydrogenation of the double bond of this ring reduces the toxicity of cymarin to one twenty-third of its original value, and dihydroouabain is sixteen times less active than ouabain. Again, conversion into the *iso*-compounds, with the formation of an oxide ring which brings about the disappearance of the double bonds, also causes complete loss of activity.

Jacobs' observations have been found to be applicable to the structurally somewhat different scillaren A.[91] The two double bonds of the lactone ring in this glycoside seem to be necessary for a powerful cardiac activity, but it must be emphasised that the presence of these double bonds does not alone

suffice, as they are still present in the *iso*-scillaren A compounds which are entirely inactive.

A remarkable result was obtained when testing the methyl ester of scillaridinic acid, in the form of its potassium enolate, for physiological activity, as although this compound does not possess the lactone ring, it exhibited a distinct, though feeble, cardiac activity. It appears, therefore, that the presence of the lactone ring is not an essential condition for this physiological effect, but it causes an enormous increase in activity.

The partial or complete elimination of the sugar from the glycosides originally present in the drug brings about a less pronounced decrease in activity. It has been observed, especially with glycosides of squill and digitalis, that, as a rule, a lower sugar content implies a lower solubility of the substances in aqueous solvents. Scillaridin A is so sparingly water soluble that, up to the present time, it has not been possible to test its physiological activity. Decreased solubility causes poor reabsorption in the intestinal tract and hence it is understandable that the activity of an extract decreases as the sugar content of the glycoside is diminished. Crude extracts are especially susceptible to loss of activity due to enzymatic action resulting in removal of sugars or in promoting isomerisation. Such processes have been observed by Jacobs in the conversion of cymarin into the completely inactive allocymarin.

Long before the causes responsible for the inactivation of preparations of cardiac medicines had been scientifically established it was a known fact that digitalis leaves which had been dried or stored by

inappropriate methods, as well as preparations made from such leaves, suffered a loss of activity. The preference shown by some physicians and pharmacists for the powder of carefully dried digitalis leaves is an attempt to use a preparation in which the cardiac glycosides are practically in their original condition, rather than depend upon galenical preparations which frequently contain the glycosides in a more or less decomposed state.

During the drying or storing of digitalis leaves without due care, or during unsuitable methods of extraction, decomposition, resulting in the loss of part of the carbohydrate constituents of the genuine glycosides, takes place so readily that it has not been realised until quite recently that the well-known glycosides digitoxin and gitoxin, as also the digoxin isolated in 1930 by Smith[33] from *Digitalis lanata*, are in reality products derived from the glycosides originally present which contained a higher carbohydrate content.

It is true that experienced clinicians have repeatedly pointed out that there exist marked differences between the activity of the crystallised glycosides and the drug itself, and between the powdered leaf or infusions prepared from it. These differences were frequently ascribed to the presence of inactive impurities, but a definite chemical discrepancy between the known cardiac glycosides and the genuine compounds, initially present in the drug, was not taken into consideration until Perrot and his associates[98] called attention to this possibility. The experimental demonstration of the truth of this view was first obtained in our laboratory by the isolation and description of the genuine digitalis glycosides and subsequent comparison with

those previously known. Our aim was to isolate the genuine digitalis glycosides in a chemically pure state, and then to apply these, not singly but as mixtures of the most important representatives, in suitable proportions in order to obtain a product of constant activity characteristic of the various glycosides of the drug.

More than fifteen years ago, attempts were made to prepare cardiac glycosides from the leaves of *Digitalis purpurea* and *Digitalis ambigua* by the application of mild conditions during extraction and further treatment, and highly active preparations were obtained, but their complex composition and failure to crystallise made it impossible to isolate chemically uniform substances. Attention was then directed to the investigation of squill, and the experience gained in the isolation of scillaren A furnished the key to the problem of how to isolate the genuine glycosides by preventing enzymatic decomposition. Nevertheless, on application of this procedure of extraction, elaborated for the squill glycosides, to the leaves of the red foxglove, only complex mixtures of amorphous glycosides were obtained. Success was finally achieved in isolating from this mixture a fairly pure, although amorphous glycoside which, on hydrolysis, yielded digitoxigenin, but which was characterised by a sugar content higher than that of digitoxin.

At this time *Digitalis lanata*, indigenous to the Balkans, made its appearance on the market. It was stated that the activity of this drug exceeded many times that of *Digitalis purpurea*, and that it was particularly suited for the preparation of digitoxin.

Digitalis lanata can also readily be grown in lime soils in Middle and Western Europe, and the cultivated

Pl. 8 Digitalis lanata

plant contains a large amount of cardioactive glycosides, whereas, as is generally known, the garden variety of *Digitalis purpurea* is characterised by a low glycoside content. The colour reproduction (Pl. 8) of a photograph taken in June 1934, shows two flowering plants of *Digitalis lanata* from our cultivation experimental station in the vicinity of Basle.

The perfected extraction process was then applied to *Digitalis lanata* leaves.[92] Fresh leaves are ground at low temperatures, with the addition of neutral salts, when the glycosides are precipitated together with the inactivated glycoside splitting enzymes, and thus the mash can be pressed out without injury of the former. The residue is exhaustively extracted with rather large amounts of ethyl acetate, the Keller-Kiliani test indicating when digitoxose-containing glycosides are no longer taken up by the solvent. The further treatment of the extract is carried out at low temperatures.

Since the glycosides, originally present as tannoids, are but sparingly soluble in ether a separation from chlorophyll, carotenoids, phytosterols and other accompanying impurities can be effected with only insignificant loss of glycosides by repeated extraction with this solvent.

From the tannoid fraction so purified the tannins are precipitated by the addition of an insoluble precipitant, such as lead hydroxide, to its aqueous alcoholic solution. The remaining glycoside solution yields on evaporation to a small volume two-thirds of the glycosides in the form of solids which can be recrystallised from aqueous methyl alcohol. The substances present in the mother liquor can also be partly crystallised by simple operations. If this method is carried out

properly with carefully selected raw material it is possible to isolate about one-half of the total content of the cardiac glycosides of *Digitalis lanata* in a crystalline state.

Repeated recrystallisations from aqueous methanol, or other solvents, soon yielded a main fraction which showed no further change of properties, and hence it was concluded that the product, which was called digilanid, was chemically pure. Hydrolysis led, however, to the conviction that it contained three different aglucones. All the types of digitalis aglucones then known, namely, digitoxigenin, gitoxigenin and digoxigenin have been isolated from the aglucone fraction obtained by the hydrolysis of the uniformly crystalline material.

The existence of the three aglucones together in one large molecule was excluded partly because the stoichiometric relationship of the three aglucones was much too complicated, but the assumption of the occurrence of mixed crystals of at least three similar isomorphous glycosides agreed with the observations.

Attempts to separate the components by fractional crystallisation or precipitation furnished negative results: it was not even possible to observe, in various fractions, a promising accumulation of one component. Finally, a separation was accomplished by distribution of the glycosides between an aqueous methanol layer and a solvent immiscible with water, such as chloroform.

It will be recalled that, during the investigations on chlorophyll a separation of the two components *a* and *b*, which could not be carried out by fractional crystallisation or precipitation, was achieved by similar

means. In both cases chemical treatment of the material had to be avoided on account of the sensitivity of the substances to change.

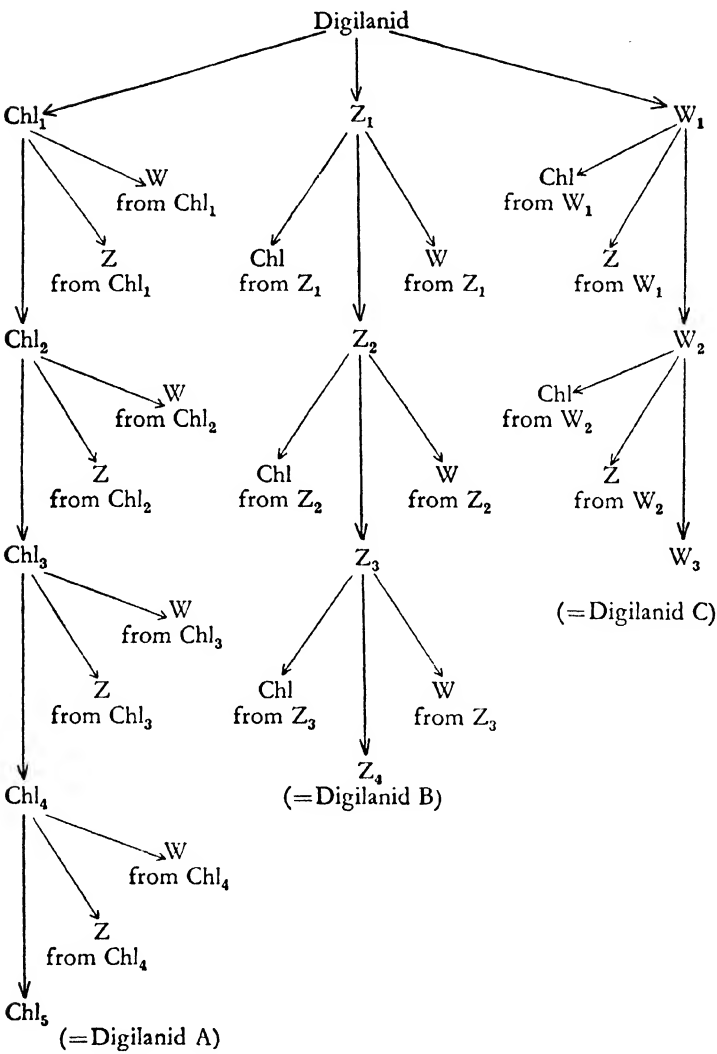
The digilanid preparation thus consists of a mixture of three isomorphous, closely related individual substances, the digilanids A, B and C. As a consequence, complete separation was by no means simple, and it required the great skill and persistence of my co-worker, Dr. Walter Kreis, to carry it to a successful conclusion. For full particulars of the method, reference should be made to the original publication.[92] At this juncture it will suffice to outline, with the aid of a few diagrams, the fundamentals of the various operations concerned so that other investigators may apply the method to the solution of similar problems.

If the mixed digilanid preparation is dissolved in aqueous methanol and the solution shaken with chloroform, at suitably chosen concentrations, digilanid A is found principally in the chloroform layer (Chl), whilst digilanid C remains in the aqueous layer (W), and digilanid B separates out as an intermediate layer (Z), still, of course, in an impure form. A fourfold repetition of the process with the chloroform fraction yielded a nearly pure preparation of digilanid A, a threefold treatment of fraction Z yielded digilanid B, and a twice repeated fractionation of fraction W yielded a high-grade digilanid C preparation (Table VII).

This method of procedure could only be carried out with considerable loss, but success was attained for the first time in obtaining the three components in a more or less pure form. These preparations were suited for the preliminary studies upon which to base a

rational procedure for the more efficient separation of the components in a high state of purity.

TABLE VII



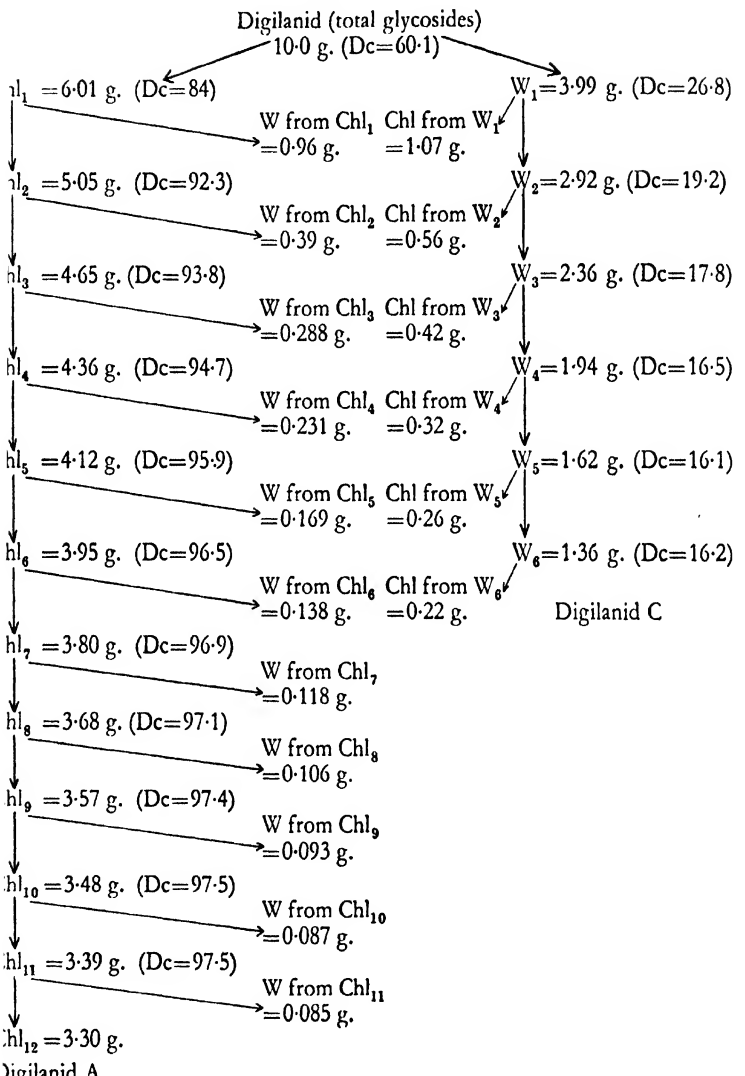
The improved process was characterised by the use of solutions diluted to such a degree that the separation of an intermediate layer containing the digilanid B fraction no longer occurs, whilst the components A and C can be obtained quite pure and in good yields by frequently repeated liquid extractions following the first separation of the two solvents. From ten grammes of a mixed digilanid preparation, 3·3 g. pure digilanid A and 1·36 g. pure digilanid C were obtained (Table VIII).

The residual fractions (W and Chl of Table VIII) were collected and separately worked up for the isolation of digilanid B, as shown in Table IX. It may be mentioned in passing that this involved forty liquid extractions with a periodic change of the solvents, chloroform and aqueous methanol.

Digilanid B is the most difficult of the three components to obtain as a pure substance, as it is present in the naturally occurring mixtures in amounts of less than 20 per cent., and its solubility in, and distribution between, solvents are intermediate between those of the digilanids A and C.

The isolation of the individual components in chemically pure form was necessary for two reasons. Only with chemical identities could the chemical composition, indicated by analysis and hydrolysis of the mixture, be definitely proved. This isolation of individual components was also necessary for the study of their physical properties, including their solubilities and distribution coefficients between different solvents. In this way the foundations were laid for a relatively simple method of determining the ratios in which the components occur in unknown mixtures of digilanids.

TABLE VIII



Dc=Distribution coefficient between chloroform and 16 per cent. aqueous methanol.)

TABLE IX

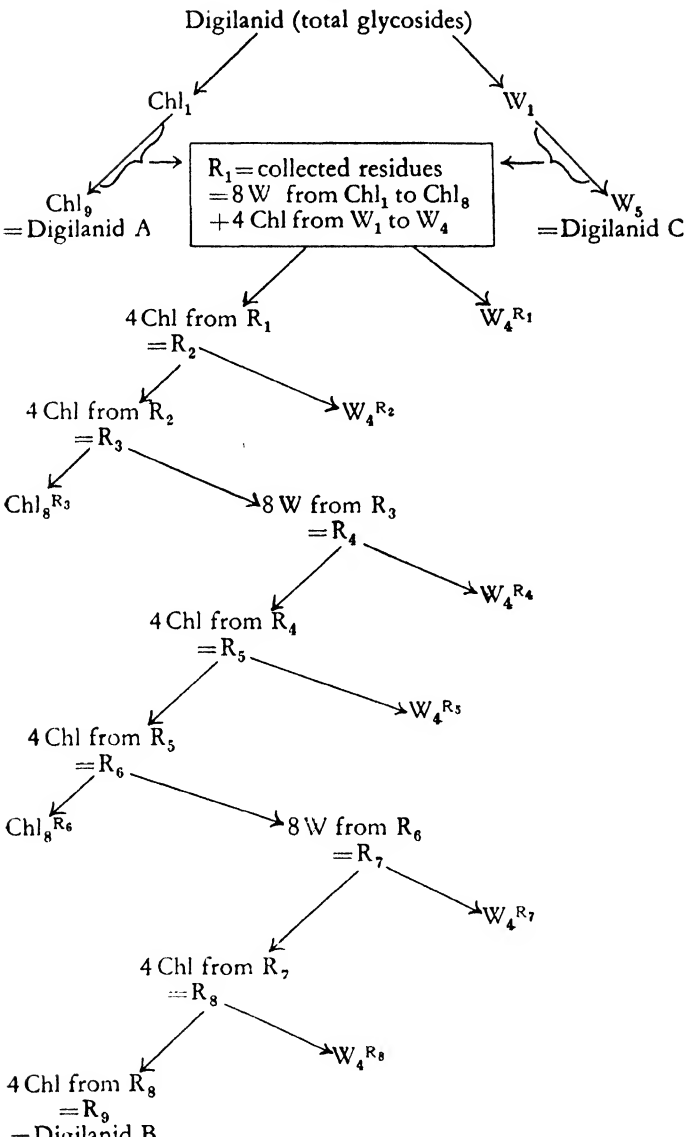
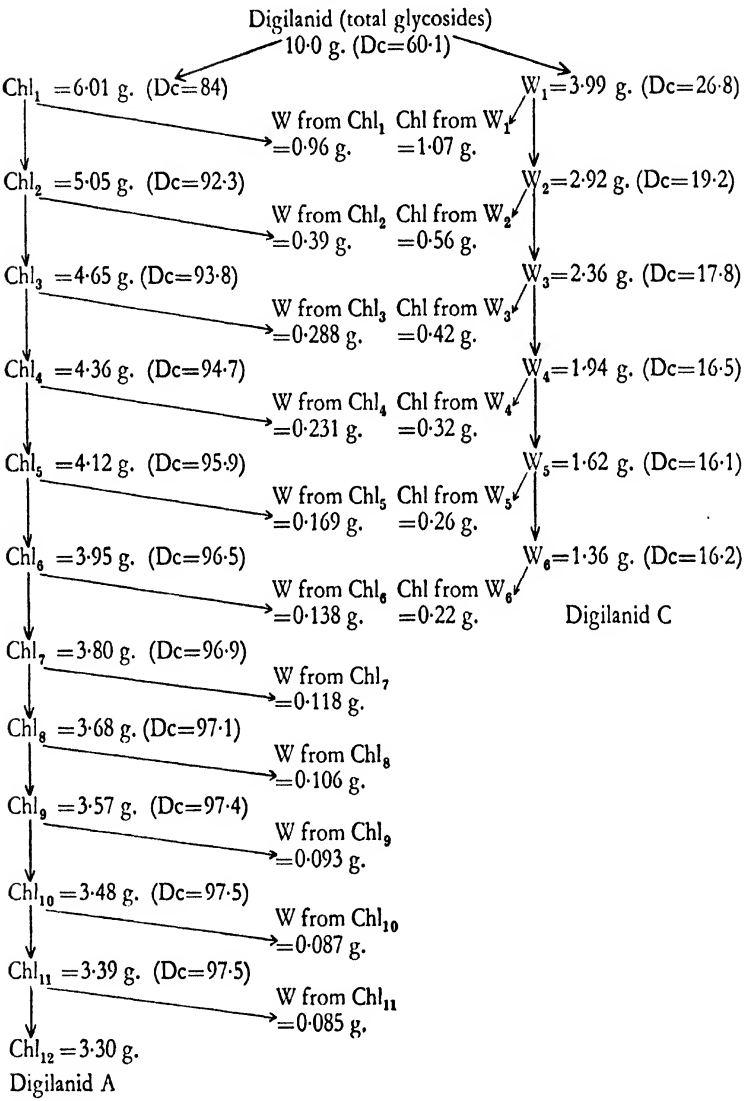
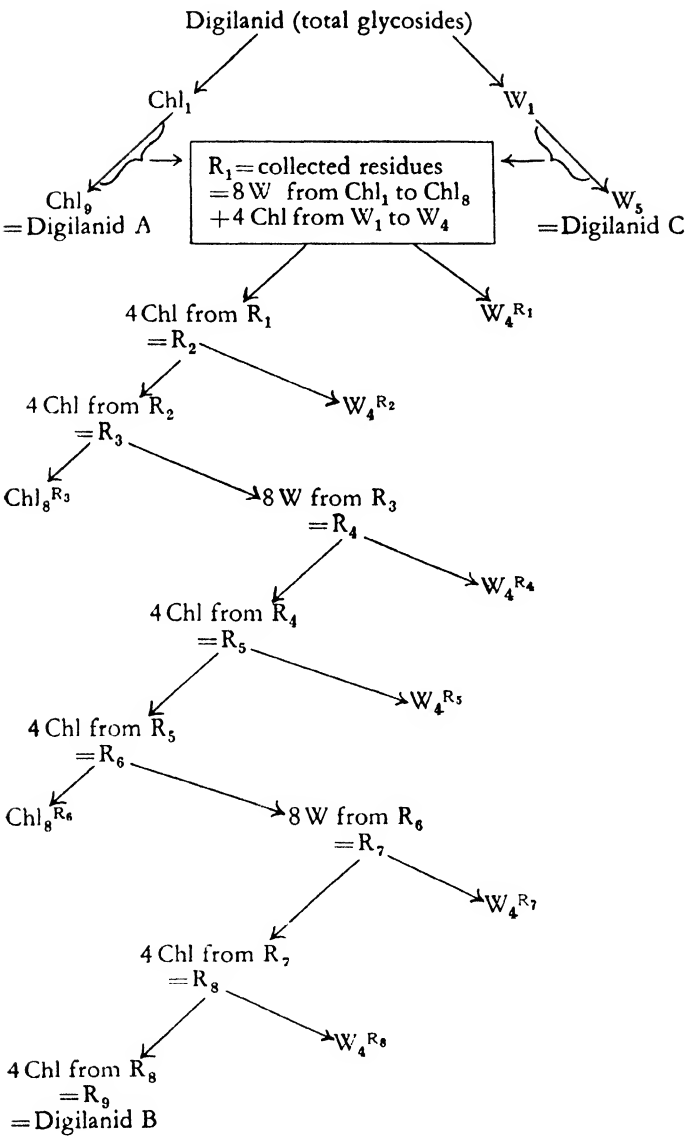

Digilanid (total glycosides)
Chl1
W1
R1 = collected residues
= 8 W from Chl1 to Chl8
+ 4 Chl from W1 to W4
Chl9
= Digilanid A
W5
= Digilanid C
4 Chl from R1
= R2
W4R1
4 Chl from R2
= R3
W4R2
Chl8R3
8 W from R3
= R4
W4R4
4 Chl from R4
= R5
W4R5
4 Chl from R5
= R6
Chl8R6
8 W from R6
= R7
W4R7
4 Chl from R7
= R8
W4R8
4 Chl from R8
= R9
= Digilanid B

TABLE VIII



(Dc=Distribution coefficient between chloroform and 16 per cent. aqueous methanol.)

TABLE IX


Digilanid (total glycosides)
Chl1
W1
R1 = collected residues
= 8 W from Chl1 to Chl8
+ 4 Chl from W1 to W4
Chl9
= Digilanid A
W5
= Digilanid C
4 Chl from R1
= R2
W4R1
4 Chl from R2
= R3
W4R2
Chl8R3
8 W from R3
= R4
W4R4
4 Chl from R4
= R5
W4R5
4 Chl from R5
= R6
Chl8R6
8 W from R6
= R7
W4R7
4 Chl from R7
= R8
W4R8
4 Chl from R8
= R9
= Digilanid B

This method has been published, and will not be discussed here,[93] but its practical importance warrants emphasis. It is possible to check the manufactured preparations and to standardise them to a definite composition by the addition or removal of single components only when an accurate method for the estimation of these ratios exists. Fluctuations in this respect would lead to uncertainty in the activity of the preparations in therapeutic use, and hence if a mixture of the three glycosides corresponding to the one which occurs in the fresh leaves is to be introduced into therapy, then the uniform composition of the preparation must be guaranteed.

The digilanid mixture, which has been tested clinically and introduced into medicine, contains about 46 per cent. digilanid A, 17 per cent. digilanid B, and 37 per cent. digilanid C. If the various factors, especially temperature and concentration of solvents, are rigorously observed, the method for estimation of the component ratios yields figures which are accurate to about 2 per cent.—a much greater accuracy than could be obtained by physiological tests.

The distribution of glycosides between different solvents can be used as an interesting test of the purity of glycoside preparations. Within certain limits of concentration a pure substance must always distribute itself between immiscible solvents in the same ratio, whilst mixtures of components with different distribution coefficients will reveal their complex nature because the distribution ratios undergo changes during consecutive liquid extractions. The uniformity of the digilanids was tested in this way and, in many cases, this method might be advantageously applied

in testing the homogeneity of complex natural substances.

The position with regard to the sugars of the mixed digilanid preparation is less complicated than that of the aglucones. Acid hydrolysis, carried out with special care to prevent decomposition of the digitoxose, yielded results which can be expressed in a relatively simple equation. Firstly, the presence of two molecules of digitoxose could be established and, afterwards, an unknown, well-crystallised disaccharide, digilanidobiose, could be isolated. This carbohydrate, which is not readily hydrolysed, consists of one molecule of digitoxose and one molecule of glucose. The presence of this disaccharide accounts for the fact that the digilanids, although containing about the same amount of digitoxose as do the glycosides of the digitoxin type, give a much fainter response to the digitoxose test than the latter. The digitoxose molecule in combination with glucose is not detectable by the Keller-Kiliani test, and hence this reaction is negative for digilanidobiose.

In addition to the usual products of hydrolysis, namely, the aglucone and the carbohydrate, the digilanids yield a molecule of acetic acid. This was characterised as the silver salt and had not been encountered previously as a constituent of digitalis glycosides. Its presence accounts for one of the two molecules of alkali used in the titration of the digilanids—the other molecule being required by the lactone ring.

Further investigations revealed the identity of all the constituents of the mixed digilanid preparation: its complete acid hydrolysis gave an aglucone fraction

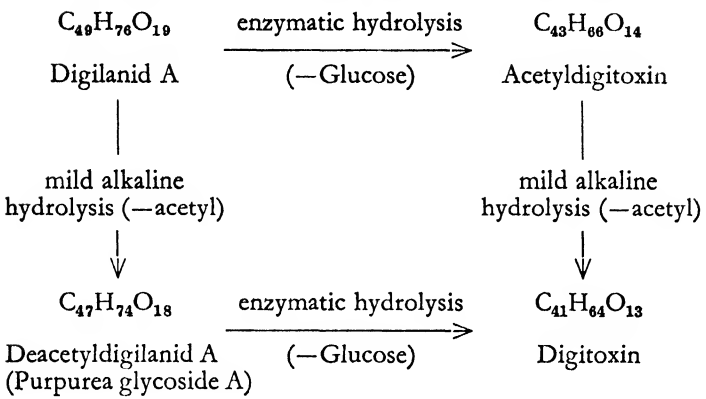
containing digitoxigenin, gitoxigenin and digoxigenin, and, in simple stoichiometric ratio, three molecules of digitoxose, one molecule of glucose and one molecule of acetic acid. Subsequent hydrolysis of the individual components, the pure digilanids A, B and C, yielded quantitatively the expected constituents (see Table XII) and furnished corroboration for the assumption that the mixed preparation consisted of an isomorphous mixture of three similarly built glycosides.

Of particular interest was the partial hydrolysis of the digilanids to yield the already known glycosides of the digitoxin type. In order to accomplish this conversion it was necessary to remove the acetyl group, as well as the glucose, whilst preventing any further change in the molecule. It is possible to remove the acetic acid from the glycosides with calcium hydroxide solution under mild conditions[94] with the formation of deacetyldigilanids A, B and C, in which the lactone ring is still intact. So far, only deacetyldigilanid C has been obtained in crystalline form both the other deacetyldigilanids remaining amorphous.

The selective removal of the glucose can be achieved only by the use of enzymes. The leaves of *Digitalis purpurea* and *Digitalis lanata* contain enzymes analogous to those present in squill, which will decompose the genuine glycosides or their deacetyl derivatives with the liberation of one molecule of glucose. Thus one obtains, in very good yields, digitoxin from deacetyldigilanid A, gitoxin from deacetyldigilanid B, and digoxin from deacetyldigilanid C. The same result is obtained if the order of the two degradations is reversed. Table X illustrates the conversions involved in the case of digilanid A.

TABLE X

DEGRADATION OF DIGILANID A TO DIGITOXIN



Careful elimination of the acetyl group, followed by the removal of glucose with the aid of enzymes, leads to the formation of uniform products but, in contrast, the enzymatic hydrolysis of the pure digilanids (A, B and C) produces, in addition to glucose, the α- and β-acetylglycosides.[95] Consequently acetyldigitoxin, acetylgitoxin, and acetyldigoxin each exist in two modifications. It has not yet been decided whether the more optically active β-form, which also seems to be the more stable, originates from the α-form. In so far as it has been possible to test these substances for physiological activity the two isomers possess the same toxicity, although the solubilities and other physical properties show distinct differences. The equation representing the hydrolysis of the acetyl glycosides is identical for the α- and β-forms.

As a result of the experience gained during the investigation of the glycosides of *Digitalis lanata* our

researches on the genuine glycosides of *Digitalis purpurea* offered a better prospect of success. In spite of this, several factors greatly increased the difficulties of this investigation.[96] In the first place the glycosidal content of this plant is smaller whilst the enzyme activity is relatively greater than that of *Digitalis lanata*; secondly, all attempts to obtain crystalline glycoside preparations failed; and finally, the isolation of chemically pure, genuine purpurea glycosides was greatly handicapped by the presence of large quantities of inactive impurities. As a result the isolation of the pure components, with the simultaneous removal of inactive impurities, was made possible only by the use of a tedious process of purification involving considerable loss of material.

However, success was attained in isolating the genuine precursors of the two most important glycosides obtained from *Digitalis purpurea* in a chemically pure state. These precursors of digitoxin and gitoxin have been called the purpurea glycosides A and B—the former being obtainable more easily in larger quantities as the latter occurs in much smaller amounts and is therefore more difficult to isolate. Investigation of their physical, chemical and physiological properties showed that the purpurea glycoside A is identical with deacetyldigilanid A, and purpurea glycoside B with deacetyldigilanid B. Thus the genuine glycosides of *Digitalis purpurea*, the amorphous purpurea glycosides A and B, can be obtained from the genuine glycosides of *Digitalis lanata*, the crystalline digilanids A and B, by the removal of the acetyl group.

Derivatives of digilanid C could not be found in the

leaves of *Digitalis purpurea*, although their detection would have been facilitated by the fact that digoxin, as well as deacetyldigilanid C, crystallises well. It would appear that the C series is not represented in *Digitalis purpurea*.

The identity of the purpurea glycosides A and B with the deacetyldigilanids A and B was supported on obtaining from them, by enzymatic hydrolysis with powdered leaf of *Digitalis purpurea*, digitoxin and gitoxin respectively.

The genuine digitalis glycosides, in common with scillaren A and *k*-strophanthin-β, react with the appropriate enzymes in such a way that the terminal glucose molecule is split off from a deoxysugar; in the case of scillaren A from rhamnose, in *k*-strophanthin-β from cymarose, in the digitalis glycosides from digitoxose. All these glycosides possess one molecule of glucose as a terminal constituent of the sugar chain, in spite of other differences in structure and origin.

Both the enzymes[97] capable of catalysing the above hydrolysis—the one contained in the leaves of *Digitalis lanata*, digilanidase, and that of *Digitalis purpurea*, digipurpidase—have been more thoroughly investigated and proved to be, according to Willstätter, desmo-enzymes. Quantitatively they show a certain specificity towards the substrate with which they occur in the leaf: digilanidase splits off glucose more readily from the digilanids than from deacetyl glycosides, whilst digipurpidase hydrolyses the acetyl-free purpurea glycosides more easily. Qualitatively, however, the enzymes and the substrates are mutually interchangeable. Again, both enzymes hydrolyse scillaren A, but the contrary does not hold as the

genuine digitalis glycosides are unattacked by scillarenase: all three enzymes mentioned have no action upon the pure, isolated disaccharides, scillabiose and digilanidobiose.

Table XI summarises the reactions of graded hydrolysis leading to the isolation of the aglucones from the various cardiac glycosides so far isolated as chemically pure substances from *Digitalis lanata* and *Digitalis purpurea*. It illustrates clearly the similarity of the reactions and the general relationships of the genuine glycosides of the two *Digitalis* species and portrays equally well the main differences between the two series of degradations, namely the complication introduced by the presence of the acetyl group in the lanata glycosides and the absence from the mixed purpurea glycosides of any analogue of digilanid C.

On account of the presence of both an acetyl and a glucose group in the digilanids A, B and C, the degradation of these substances to the digitoxin type requires two consecutive steps which can be accomplished in two ways. One procedure is characterised by the initial hydrolysis of the acetyl group by mild alkaline treatment, followed by the enzymatic elimination of glucose. In the other the enzymatic hydrolysis precedes the saponification of the acetyl group. Complete hydrolysis of the glycosides of the digitoxin type with acid leads to the three well-known aglucones digitoxigenin, gitoxigenin and digoxigenin, together with the liberation of three molecules of digitoxose per molecule of glycoside.

For the sake of completeness gitalin, which has been isolated from *Digitalis purpurea*, has been included in Table XI. It is not yet certain, but probable, that

gitalin is originally contained in the leaves of this plant in the form of a compound richer in sugar. In favour of this view is the fact that gitalin, although known to crystallise comparatively easily, could not be separated from the complex mixture of the genuine glycosides yielded by the special extraction process which precluded the possibility of enzyme action.

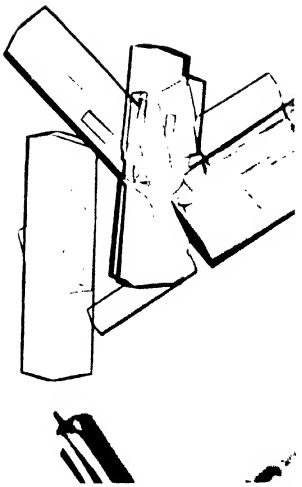
The following series of illustrations portray the various stages of the degradation of *Digitalis lanata* glycosides in the form of crystalline products. Plate 9 shows digilanid, the isomorphous mixture of digilanids A, B and C, and the next three illustrations are photomicrographs of the mutually identical crystal structures of its individual components (Pl. 10, 11, 12). Enzymatic decomposition of digilanid A leads to α- and β-acetyldigitoxin (Pl. 13 and 14), which on hydrolysis with dilute alkali yield digitoxin (Pl. 15), from which by intensive treatment with acid the aglucone digitoxigenin (Pl. 16) is obtained. Similarly digilanidase converts digilanid B into acetylgitoxin (Pl. 17), of which the β-modification has been obtained in a chemically pure state; elimination of the acetyl group furnished gitoxin (Pl. 18), and complete acid hydrolysis gitoxigenin (Pl. 19).

Both routes of partial degradation of digilanid C result in beautifully crystallised products—even the deacetyl derivative (Pl. 20) crystallises readily. Commencing the degradation with enzymatic hydrolysis the crystalline acetyldigoxins α- and β- (Pl. 21 and 22) are obtained. Digoxin (Pl. 23) is the next stage in the hydrolysis of either deacetyldigilanid C or the acetyldigoxins, and finally digoxin yields the aglucone digoxigenin (Pl. 24).

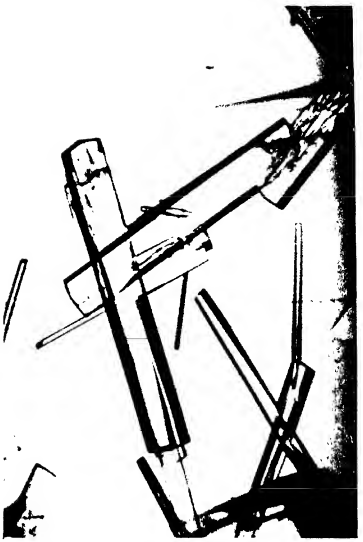
A survey of the various well-defined digitalis glycosides, arranged according to the aglucones and carbohydrate contents, is presented in Table XII. The equation for the complete hydrolysis of each individual glycoside is given, and the large brackets include those groups of glycosides which are derived from the same aglucone, the lowest member of each group corresponding to the digitoxin type.

Tables XI and XII indicate the various possibilities for a more or less extensive decomposition of the genuine glycosides during the manufacture of galenical preparations by the usual methods. To what extent such decompositions actually occur can be gathered from the following observation: an infusion of *Digitalis lanata* from powdered leaf was prepared in accordance with the directions given in the Pharmakopoeia Helvetica IV and analysed immediately: only about one-half of the amount of the genuine glycosides originally present could be detected. When such admittedly unstable infusions are stored, the decomposition will probably proceed further. It is common knowledge among physicians that digitalis infusions must always be freshly prepared.

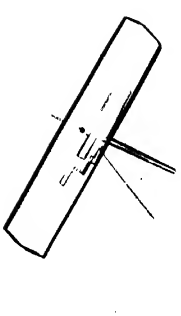
On the basis of our knowledge of the enzymatic nature of the hydrolysis of the glycosides, it might seem possible to avoid the uncertainty of the composition of galenical preparations by stabilising the drugs by treatment with alcohol vapour according to Perrot and Goris.[98] However, other causes for such fluctuations exist, the most important of which being the individual variations of the glycoside content of raw material of different origin. In a hitherto unpublished investigation several samples of *Digitalis*



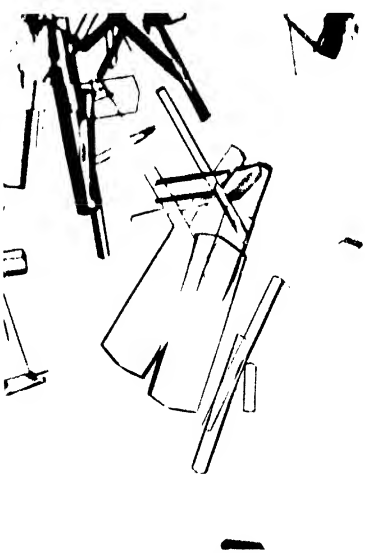
Pl. 9. Digilanid
(total glycosides from methanol)



Pl. 10. Digilanid A
(from methanol)



Pl. 11. Digilanid B
(from methanol)

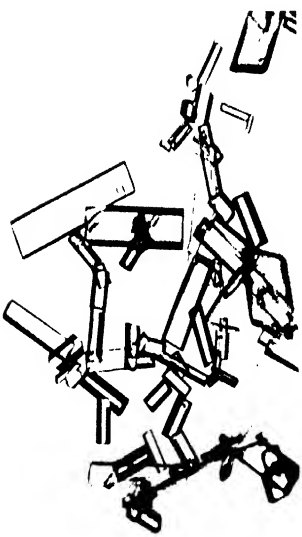


Pl 12. Digilanid C
(from methanol)

Pl. 13. α-Acetyldigitoxin
(from chloroform + ether)

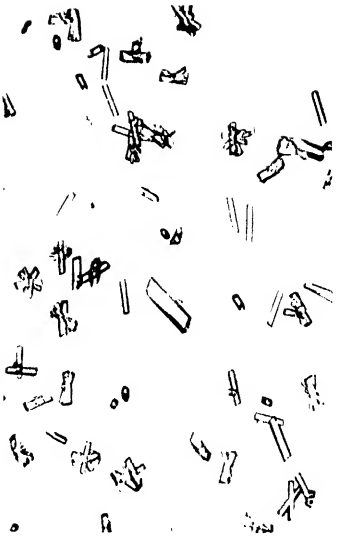
Pl. 14. β-Acetyldigitoxin (above, from aqueous methanol; below, from methanol)

Pl. 15. Digitoxin
(from aqueous ethanol)



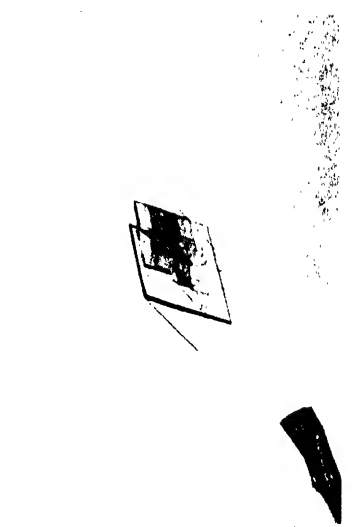
Pl. 16. Digitoxigenin
(from aqueous ethanol)

Pl. 17. β-Acetylgitoxin
(from aqueous methanol)



Pl. 18. Gitoxin
(from aqueous ethanol)

Pl. 19. Gitoxigenin
(from absolute ethanol)

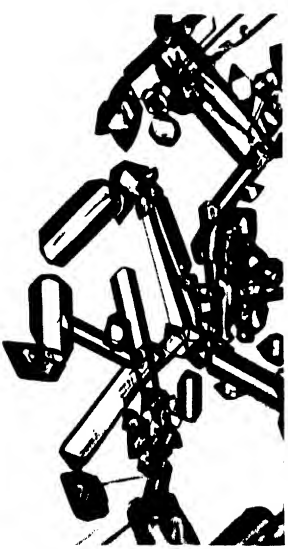


Pl. 20. Deacetyldigilanid C
(from aqueous methanol)

Pl. 21. α-Acetyldigoxin
(from chloroform—methanol—ether)

Pl. 22. β-Acetyldigoxin
(from aqueous methanol)

Pl. 23. Digoxin
(from aqueous ethanol)



Pl. 24. Digoxigenin
(from aqueous methanol)

TABLE XII

DIGITALIS GLYCOSIDES

arranged in the order of their aglucones and their sugar content

$C_{49}H_{76}O_{19}+5\ H_2O$ Digilanid A $C_{47}H_{74}O_{18}+4\ H_2O$ Deacetyldigilanid A (Purpurea glycoside A) $C_{43}H_{66}O_{14}+4\ H_2O$ Acetyldigitoxin (α and β) $C_{41}H_{64}O_{13}+3\ H_2O$ Digitoxin (1871)	$C_{23}H_{34}O_4+$ Digitoxigenin	3 $C_6H_{12}O_4$ Digitoxose + $C_6H_{12}O_6$ Glucose + CH_3COOH Acetic acid 3 $C_6H_{12}O_4$ Digitoxose + $C_6H_{12}O_6$ Glucose 3 $C_6H_{12}O_4$ Digitoxose + CH_3COOH Acetic acid 3 $C_6H_{12}O_4$ Digitoxose
$C_{49}H_{76}O_{20}+5\ H_2O$ Digilanid B $C_{47}H_{74}O_{19}+4\ H_2O$ Deacetyldigilanid B (Purpurea glycoside B) $C_{43}H_{66}O_{15}+4\ H_2O$ Acetylgitoxin (α and β) $C_{41}H_{64}O_{14}+3\ H_2O$ Gitoxin (1925)	$C_{23}H_{34}O_5+$ Gitoxigenin	3 $C_6H_{12}O_4$ Digitoxose + $C_6H_{12}O_6$ Glucose + CH_3COOH Acetic acid 3 $C_6H_{12}O_4$ Digitoxose + $C_6H_{12}O_6$ Glucose 3 $C_6H_{12}O_4$ Digitoxose + CH_3COOH Acetic acid 3 $C_6H_{12}O_4$ Digitoxose
$C_{35}H_{56}O_{12}+2\ H_2O$ Gitalin	$C_{23}H_{36}O_6+$ Gitoxigenin-hydrate	2 $C_6H_{12}O_4$ Digitoxose
$C_{36}H_{56}O_{14}$ Digitalinum verum	$C_{23}H_{30}O_3+$ Dianhydrogitoxigenin	$C_7H_{14}O_5$ Digitalose + $C_6H_{12}O_6$ Glucose
$C_{49}H_{76}O_{20}+5\ H_2O$ Digilanid C $C_{47}H_{74}O_{19}+4\ H_2O$ Deacetyldigilanid C $C_{43}H_{66}O_{15}+4\ H_2O$ Acetyldigoxin (α and β) $C_{41}H_{64}O_{14}+3\ H_2O$ Digoxin (1930)	$C_{23}H_{34}O_5+$ Digoxigenin	3 $C_6H_{12}O_4$ Digitoxose + $C_6H_{12}O_6$ Glucose + CH_3COOH Acetic acid 3 $C_6H_{12}O_4$ Digitoxose + $C_6H_{12}O_6$ Glucose 3 $C_6H_{12}O_4$ Digitoxose + CH_3COOH Acetic acid 3 $C_6H_{12}O_4$ Digitoxose

purpurea were examined and yields obtained varying from 0·1 g. or less to 0·5 to 0·6 g. of digitoxin per kg. Samples giving a poor yield of digitoxin usually showed a higher gitoxin content. Thus it is clear that even by stabilising the drug a product with a predictable physiological activity cannot be expected.

The question whether the leaves of *Digitalis purpurea* or *Digitalis lanata* contain still other genuine glycosides besides those which have so far been isolated seems justified and should be answered in the affirmative. Thus another important question arises: do the hitherto isolated glycosides include so much of the entire activity of the drug that by an appropriate combination of the chemically pure components an effect can be obtained similar to that which the physician observes when using the plant itself? This is certainly the case as regards *Digitalis lanata*. It has been possible to isolate in the form of the mixed isomorphous digilanids A, B and C a preparation of the glycosides which accounts for the greater part of the total activity of the drug itself. The mixed digilanid preparation has been applied in therapy quite extensively during the past few years, yet not a single case is known in which the physician by using the pure crystalline substances has obtained a therapeutic effect inferior to that which might have resulted had he used the plant itself or an infusion. Thus it seems that the digilanid preparation, composed of the three major types of glycosides, does not lack a single ingredient which might play a part in assuring an optimal digitalis activity. The crystalline preparation possesses many big advantages, such as its injectability, its stability and accurate dosage, and the fact that it is

easily tolerated when administered orally. These cardiac glycosides, containing a higher sugar content than any previously isolated, are more closely related to those naturally occurring in the plant than any of the pure substances hitherto used.

The yield of pure genuine glycosides from *Digitalis purpurea*, the purpurea glycosides A and B, is very much smaller than that from *Digitalis lanata*, probably on account of their amorphous character and the presence of large quantities of interfering substances. Thus the isolation of the genuine cardiac glycosides of *Digitalis purpurea* has so far only a scientific interest, and it would seem to us that, on account of its considerably greater glycoside content alone, *Digitalis lanata* should most certainly be preferred to *Digitalis purpurea* for the technical manufacture of crystalline products. Analytical considerations point in the same direction. A determination of the ratio in which the digilanids occur is readily carried out, whereas a method for the estimation of the purpurea glycosides has not yet been developed—and such a method will not easily be worked out. A physiological test is not a satisfactory substitute as it yields practically no information other than that concerning the toxicity of the preparations, and qualitative differences in their cardioactivity can only be established in rare cases and with little accuracy. Tests on experimental animals also fail to give a clear idea of the tolerance of the human body for oral administration of these preparations. Consequently the use of accurately determinable, crystalline, and well-tolerated genuine glycosides, as furnished by *Digitalis lanata*, should be preferred in cardiac therapy, the more so since the additional

presence of component C, lacking in *Digitalis purpurea*, provides a completion of the therapeutic activity. It goes without saying that the great services which *Digitalis purpurea* has rendered during the century and a half since its introduction into medicine by Withering remain unimpaired.

A few data from the extensive, comparative pharmacological investigations which Professor E. Rothlin[99] has carried out with the total digilanid preparation, with the individual components A, B and C, with the genuine purpurea glycosides A and B, and with the previously known glycosides, such as digitoxin and digoxin, will not be out of place. These investigations furnished the basis for the first clinical experiments. The following table (XIII) summarised the toxicity of the glycosides for cats and frogs:

TABLE XIII

TOXICITY OF DIGITALIS GLYCOSIDES

	FROG (medium lethal dose, sub-cut. inj., timeless method). Frog units per mg.	CAT (intravenous infusion according to Hatcher). Cat unit = mg. per kg.
Digilanid (total glycosides)	620	0·343
Digilanid A	690	0·380
Digilanid B	540	0·400
Digilanid C	640	0·281
Purpurea glycoside A	690	0·368
Purpurea glycoside B	315	0·369
Digitoxin	400	0·420
Digoxin	650	0·280

Digilanid contains collectively the two components A and B, which are eliminated rather easily, and the

more powerful and more permanently active digilanid C. It was to be expected that the application of the whole complex in therapy would cause an accelerated effect, accompanied by less risk as regards accumulation than would be the case if only digitoxin or digilanid C were administered. The practical value of a new preparation can, of course, only be judged on the basis of extensive clinical experience.

Digilanid is the only preparation of chemically pure, genuine digitalis glycosides of constant composition which has yet been introduced in cardiac therapy. Its therapeutic dose for man, when administered orally, amounts to a daily average of 0·75 mg. In severe cases this may be increased to 1·5 mg. divided into three separate doses. By intravenous or intramuscular injection 0·8 mg. or two doses of 0·4 mg. are given respectively.

By reason of their large molecular size these digilanids are the most active cardiac glycosides isolated from digitalis. The clinical experiences confirm the assumption that the total glycoside preparation adequately replaces the plant, offering at the same time the advantages of accurate dosage, etc., of pure substances. E. E. Bauke[100] in a recent publication on the clinical studies of this preparation found the normal dose for oral application to be 3 × 15 drops of digilanid solution, whereas a calculation on the basis of the number of frog units shows that a quantity of 3 × 30 drops would be equivalent to the usual 3 × 0·1 g. "folia digitalis titrata." This difference may be due principally to the fact that the digilanids are much more easily reabsorbed than are glycosides which are still enclosed in the cell material of the leaf where, in

addition, the possibility of partial enzymatic decomposition exists. The remarkable tolerance of the digestive tract for the lanata glycosides in this form is probably a result of the ease of reabsorption.

When injected intravenously digilanid does not exert its effect as rapidly as strophanthin. Nevertheless it acts rapidly and it appears to be less dangerous than strophanthus glycosides.

The indications for and therapeutic action of digilanid do not differ from those of digitalis, and are especially associated with cases of mitral disease and auricular fibrillation.

No matter how excellent a cardiac glycoside preparation may be the physician will always play an important and often difficult rôle in view of the great variability in patients suffering from cardiac diseases. He must depend upon his art to adapt himself, diagnostically and therapeutically, to every single case and its medicinal treatment. His work is, however, facilitated and rendered more certain, if he has at his disposal preparations of constant composition, which are readily reabsorbed, rapidly and strongly active, well tolerated, and whose action, owing to their composition, may be deemed optimal. Thus the pharmacist and the chemist, in exercising their function of the preparation and investigation of drugs, assist the physician in his difficult task and contribute to the advancement of medical science and its application to disease, which, after all, is the principal aim of all pharmaceutical research.

BIBLIOGRAPHY

1. N. Wattiez, *Journ. Pharmac. Belg.*, **11**, 597 (1929).
2. H. Colin and E. Guéguen, *C. r. Ac. Sci.*, **190**, 653 (1930); *ibid.*, **191**, 163 (1930).
3. F. Marino-Zuco and V. Pasquero, *Gazz. chim. ital.*, **41**, II, 368 (1910).
4. H. Schiff, *Ann.*, **206**, 159 (1881).
5. *Ibid.*, **154**, 14 (1870).
6. F. Tiemann and W. Haarmann, *Ber.*, **7**, 609 (1874).
7. F. Wöhler and J. Liebig, *Ann.*, **22**, 1 (1837); W. N. Haworth and B. Wylam, *Soc.*, **125**, 3120 (1923).
8. R. Seka and P. Kallir, B. **64**, 622 (1931).
9. G. Barger and F. D. White, *Biochem. J.*, **17**, 836 (1923).
10. J. Herzig, *Monatsh. f. Chemie*, **6**, 863 (1886); J. Herzig and Th. von Smoluchowski, *ibid.*, **14**, 53 (1893).
11. R. Willstätter and T. J. Nolan, *Ann.*, 408, 1 (1915); R. Robinson and A. R. Todd, *Soc.*, **1932**, 2488.
12. E. T. Jones and A. Robertson, *Soc.*, **1933**, 1167.
13. A. Robertson, *Soc.*, **1927**, 1937; A. Robertson and R. B. Waters, *Soc.*, **1933**, 30.
14. O. Hesse, *Ann.*, **309**, 32 (1899); C. A. Naylor and J. H. Gardner, *Am. Soc.*, **53**, 4114 (1931).
15. P. C. Mitter and D. Banerjee, *J. Indian Chem. Soc.*, **9**, 375 (1932).
16. E. Fischer and B. Helferich, *Ann.*, **383**, 77 (1911).
17. R. Kuhn and A. Winterstein, *Ber.*, **67**, 344 (1934).
18. P. Karrer and H. Salomon, *Helv. Chim. A.*, **11**, 514 (1928); P. Karrer and K. Miki, *Helv. Chim. A.*, **12**, 985 (1929), P. Karrer, F. Benz and M. Stoll, *Helv. Chim. A.*, **16**, 297 (1933).
19. F. B. Power and A. H. Salvay, *Soc.*, **103**, 399 (1913), *Shu Furukawa, Scient. Papers Inst. Physical Chem. Res.*, **19**, 27 (1932).
20. The chemistry of the cardiac glycosides, and especially of the Strophanthus glycosides, has been treated adequately by W. A. Jacobs, *Physiological Reviews*, **13**, 222 (1933), and R. C. Elderfield, *ibid.*, **17**, 187 (1935).
21. Nativelle, *J. pharm. chim.* (4) **9**, 255 (1869).
22. M. Cloetta, *Arch. exptl. Path. Pharmacol.*, **88**, 113 (1920).
23. A. Windaus and G. Stein, B. **61**, 2436 (1928).
24. M. Cloetta, *Arch. exptl. Path. Pharmacol.*, **112**, 261 (1926).
25. F. Krafft, *Arch. Pharm.*, **250**, 126 (1912).

26. A. Windaus, K. Westphal and G. Stein, *Ber.*, **61**, 1847 (1928).
27. A. Windaus, *Arch. exptl. Path. Pharmacol.*, **135**, 253 (1928).
28. O. Schmiedeberg, *Arch. exptl. Path. Pharmacol.*, **3**, 27 (1875).
29. A. Windaus, *Nachr. Ges. Wiss. Göttingen Math. phys. Kl.*, **1927**, 422.
30. W. A. Jacobs and N. M. Bigelow, *J. Biol. Chem.*, **96**, 647 (1932).
31. A. Stoll, E. Suter, W. Kreis, B. B. Bussemaker and A. Hofmann, *Helv. Chim. A.*, **16**, 703 (1933); A. Stoll, A. Hofmann and J. Peyer, *Helv. Chim. A.*, **18**, 1247 (1935).
32 A. Stoll and A. Hofmann, *Helv. Chim. A.*, **18**, 401 (1935).
33. S. Smith, *Soc.*, **1930**, 508; *Soc.*, **1931**, 23.
34. Em. Perrot, P. Bourcet and Raymond-Hamet, *Bull. Sc. pharm., Paris*, **38**, 7 (1931).
35. C. Mannich, P. Mohs and W. Mauss, *Arch. Pharm.*, **268**, 453 (1930).
36. P. Mohs, *Arch. Pharm.*, **271**, 393 (1933).
37. C. Mannich, *Arch. Pharm.*, **272**, 5 (1934); C. Mannich, *Helv. Chim. A.*, **17**, 789 (1934).
38. A. Stoll and W. Kreis, *Helv. Chim. A.*, **17**, 790 (1934).
39. W. A. Jacobs and A. Hoffmann, *J. Biol. Chem.*, **67**, 609 (1926).
40. A. Windaus and L. Hermanns, *Ber.*, **48**, 993 (1915).
41. W. A. Jacobs and A. Hoffmann, *J. Biol. Chem.*, **69**, 153 (1926).
42. W. A. Jacobs, *J. Biol. Chem.*, **88**, 519 (1930).
43. W. A. Jacobs and A. Hoffmann, *J. Biol. Chem.*, **79**, 531 (1928).
44. W. A. Jacobs and M. Heidelberger, *J. Biol. Chem.*, **81**, 765 (1929).
45. W. A. Jacobs and N. M. Bigelow, *J. Biol. Chem.*, **99**, 521 (1933).
46. Arnaud, *C. r. Ac. Sci.*, **106**, 1011 (1888); **107**, 1162 (1888); **126**, 346, 1208, 1654 (1898).
47. W. A. Jacobs and N. M. Bigelow, *J. Biol. Chem.*, **96**, 647 (1932).
48. W. A. Jacobs and A. Hoffmann, *J. Biol. Chem.*, **79**, 519, 1928.
49. A. Windaus and K. Westphal, *Nachr. Ges. Wiss. Göttingen Math. phys. Kl.*, **1925**, 78.
50. K. K. Chen and A. L. Chen, *J. Pharm.*, **49**, 561 (1933); *J. Biol. Chem.*, **105**, 231 (1934), cf. R. Tschesche, B. **69**, 2368 (1936).
51. R. Tschesche, *Z. physiol. Chem.*, **222**, 50 (1933).

52. W. Karrer, *Helv. Chim. A.*, **12**, 506 (1929).
53. R. Tschesche and W. Haupt, B. **69**, 459 (1936).
54. R. C. Elderfield, *Science*, **81**, 440 (1935); *J. Biol. Chem.*, **111**, 527 (1935).
55. F. Micheel, *Ber.*, **63**, 347 (1930).
56. W. A. Jacobs and N. M. Bigelow, *J. Biol. Chem.*, **96**, 355 (1932).
57. O. T. Schmidt and H. Zeiser, *Ber.*, **67**, 2127 (1934).
58. J. Markwalder, *Schweiz. Med. Woch. schr.*, **1922**, 560 and *Klin. Woch. schr.*, **1**, 212 (1922).
59. C. Focke, *Arch. Pharm. u. Ber. Deutsch. Pharm. Ges.*, **1927**, 91.
60. G. Sharp, *Pharmac. J. and Pharmacist*, **84**, 136 and 170 (1910).
61. E. Hirschfeld, *Kyklos Jahrb. d. Inst. f. Gesch. d. Med. II*, 163 (1929).
62. G. L. B. van Swieten, *Commentaria in Hermanni Boerhaave Aphorismos de cognoscendis et curandis morbis*, Vol. IV (1764).
63. W. Withering, "An Account of the Foxglove, and some of its Medical Uses, with practical remarks on dropsy and other diseases," Birmingham, 1785.
64. F. Home, *Chemical experiments, histories and dissections*, Edinburgh, 1780, p. 357.
65. F. Mendel, *Ther. d. Gegenw.*, **1918**, 16 and continued.
66. E. Merck, *Pharm. Ztg.*, **24**, 286 (1879).
67. E. von Jarmersted, *Arch. exptl. Path.*, **11**, 22 (1879).
68. F. Kurtz, *Diss, Erlangen*, 1894.
69. S. Waliszewski, *l'Union Pharm.*, **34**, 251 (1894).
70. W. Kopaczewski, *C. r. Ac. Sc.*, **158**, 1520 (1914) and *Biochem. Z.*, **66**, 501 (1914).
71. A. I. Ewins, *J. Pharmacol.*, **3**, 155 (1912).
72. A. Stoll, *Verhandl. der Schweiz. Naturf. Ges.*, **1920**, 190; K. Spiro and A. Stoll, *ibid.*, p. 235; *Schweiz. Med. Wochenschr.*, **1921**, No. 23; A. Stoll, *Schweiz. Apotheker-Ztg.*, **60**, No. 26–28 (1922).
73. *Schweiz. Pat.*, No. 79,578 (1918).
74. A. Stoll, W. Kreis and A. Hofmann, *Z. physiol. Chem.*, **222**, 24 (1933).
75. A. Stoll, A. Hofmann and A. Helfenstein, *Helv. Chim. A.*, **17**, 641 (1934).
76. A. Stoll, A. Hofmann and W. Kreis, *Helv. Chim. A.*, **17**, 1334 (1934).
77. A. Stoll, A. Hofmann and A. Helfenstein, *Helv. Chim. A.*, **18**, 644 (1935).
78. W. A. Jacobs and E. L. Gustus, *J. Biol. Chem.*, **74**, 811 (1927).

79. R. C. Elderfield and W. A. Jacobs, *J. Biol. Chem.*, **107**, 143 (1934); *Science*, **79**, 279 (1934).
80. R. Tscheche and H. Knick, *Z. physiol. Chem.*, **222**, 58 (1933).
81. W. A. Jacobs and R. C. Elderfield, *Science*, **80**, 434 (1934).
82. R. Tscheche, *Z. angew. Chem.*, **47**, 729 (1934); *Ber.*, **68**, 7 (1935).
83. W. A. Jacobs and R. C. Elderfield, *J. Biol. Chem.*, **108**, 497 (1935).
84. R. Tscheche, *Z. physiol. Chem.*, **229**, 219 (1934).
85. G. A. R. Kon, *J. Soc. Chem. Ind.*, **53**, 593 and 956 (1934).
86. J. D. Bernal and D. Crowfoot, *J. Soc. Chem. Ind.*, **53**, 953 (1934).
87. R. Tscheche and K. Bohle, *Ber.*, **69**, 793 (1936).
88. R. Tscheche and A. Hagedorn, *Ber.*, **69**, 797 (1936).
89. H. Wieland and G. Hesse, *Ann.*, **517**, 22 (1935).
90. W. A. Jacobs and A. Hoffmann, *J. Biol. Chem.*, **74**, 787 (1927); *Physiol. Rev.*, **13**, 241 (1933).
91. A. Stoll and A. Hofmann, *Helv. Chim. A.*, **18**, 401 (1935).
92. A. Stoll and W. Kreis, *Helv. Chim. A.*, **16**, 1049 (1933); Preliminary communications: *Verh. Schweiz. Naturforsch. Ges.*, **1932**, 331 and 435; A. Stoll, *Ein Gang durch biochemische Forschungsarbeiten*, Berlin, 1933; A. Stoll and W. Kreis, *Münch. Med. Woch. schr.*, **80**, 723 (1933); *C. r. Ac. Sci.*, **196**, 1742 (1933); *Bull. Sci. pharmacol.*, **40**, 321 (1933).
93. *Ibid.*, **16**, 1085 (1933).
94. *Ibid.*, **16**, 1390 (1933).
95. *Ibid.*, **17**, 592 (1934).
96. *Ibid.*, **18**, 120 (1935).
97. A. Stoll, A. Hofmann and W. Kreis, *Z. physiol. Chem.*, **235**, 249 (1935).
98. Em. Perrot and A. Goris, *Bull. Acad. Méd.* (3e S.), **61**, 681 and **62**, 97 (1909); *Bull. Sci. pharmacol.*, **38**, 7 (1931); *Progrès méd.*, **1933**, 354.
99. E. Rothlin, *Verhandlungen der Schweiz. Naturf. Ges. Thun.*, **1932**, 437; *Münch. Med. Wschr.*, **1933**, 727; *Praxis*, **1933**, *Beilage*, No. 51, 13; *Helv. Med. A.*, **1934**, 460; *Verhandl. Schweiz. Naturf. Ges., Zürich*, **1934**, 422; *Annals de l'hospital de la Santa Creu i San Pau*, **1935**, 586; *Schweiz. Med. Wschr.*, **1935**, 1162.
100. E. E. Bauke, *Deutsche Med. Wschr.*, **61**, 371 (1935).