THE GENUINE CARDIAC GLUCOSIDES.*

BY ARTHUR STOLL.[1]

Those glucosides which constitute the large class of cardiac glucosides, are derived from numerous plants belonging chiefly to the *Liliaceæ*, *Ranunculaceæ*, *Scrophulariaceæ* and *Apocynaceæ*. Several of these poisonous plants such as *Scilla maritima* and Convallaria majalis, Helleborus niger, Adonis vernalis and Nerium oleander, the principal species of Digitalis and Strophanthus, Periploca and Acocanthera and some others have been the subject of more or less profound chemical studies.

In discussing this subject, I shall attempt to deal only with a small part of this vast field of research, and more particularly with some of the more important types of cardiac glucosides, isolated in a pure state and studied in my laboratory. These include the glucosides of *Scilla maritima*, *Digitalis lanata*, *Digitalis purpurea* and *Strophanthus kombé*, all of which are medicinal plants largely employed in therapeutics; one of them, since the most ancient times.

From the chemical point of view the cardiac glucosides are, as is known, composed of an aglucon fraction and a sugar fraction. The aglucon is the real source of the cardiac action, while the sugar fraction determines the solubility of the glucosides in aqueous media.

As shown in the following table, the aglucons of the cardiac glucosides which have been thoroughly studied and analyzed up to the present time, present only slight differences in their chemical composition.

TABLE I.—FORMULAS OF THE AGLUCONS OF THE BETTER KNOWN CARDIO-ACTIVE GLUCOSIDES.

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	$C_{24}H_{30}O_3$

In the above tabulation it is interesting to note that *scillaridin A*, the aglucon of the glucoside of *Scilla maritima*, possesses one carbon atom more than the other aglucons.

During recent years the study of the constitution of the aglucons of the cardiac glucosides has shown that these substances are related to the large class of the sterols and bile acids and, therefore, have a constitution closely related to the sex hormones. This same class of substances includes also the cardio-active principles of animal origin, such as bufotalin which is a characteristic constituent of toad venom.

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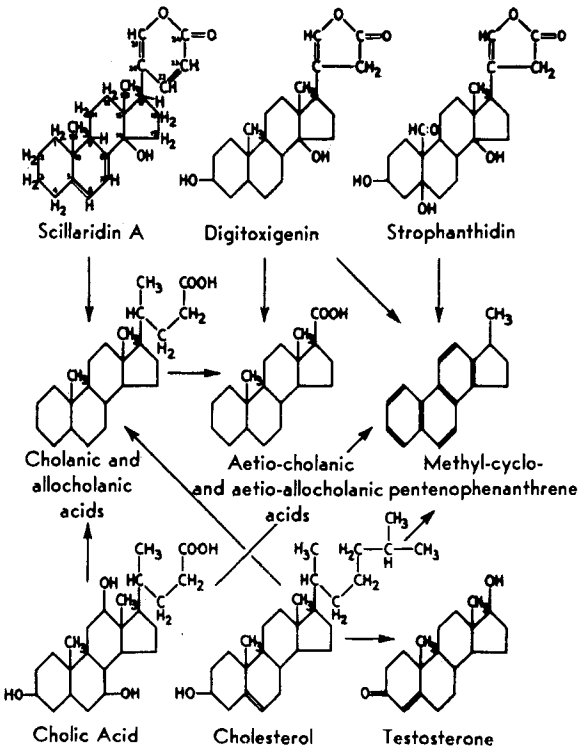
The relations of the aglucons of cardiac glucosides to the sterols and bile acids have been established by the production of identical degradation products from both groups of substances. Thus Jacobs and Elderfield obtained ætiocholanic acid from digitoxigenin and in a similar way Tschesche degraded uzarigenin to ætio-allocholanic acid. It has further been possible to transform *scillaridin A* by dehydration and, following total hydrogenation, without loss of carbon atoms and without modifying their structural arrangement, into allocholanic acid, which latter also may be obtained from biliary acids by an analogous method. In a very direct and clear way, a glucoside of vegetable origin and the bile acids occurring in animal products, have thus been linked.

TABLE II.—FORMULAS OF SOME DERIVATIVES OF CYCLO-PENTENO-PHENANTHRENE AND THEIR CORRELATIONS.



For purposes of comparison we have completed the above table by the formulas of cholic acid and cholesterol, and have indicated by arrows the chemical transformations that interlink the different members of these groups. All these substances possess a skeleton with 4 condensed carbon rings, the structure typical of cyclopentenophenanthrene. The carbon rings are all completely hydrogenated except where double bonds are indicated.

On closer examination we see that the formulas of digitoxigenin and strophanthidin contain a five-membered lactone nucleus with one double bond, which is typical for the aglucons with 23 carbon atoms. Strophanthidin is further char-

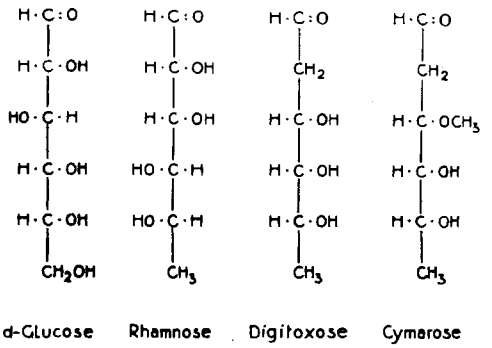
acterized by an aldehyde group attached to carbon atom 10. In *scillaridin A* the lactone nucleus is 6-membered and includes 2 double bonds.

The formulas of the aglucons show the hydroxyl groups to which the sugars are fixed by a glucosidic linkage. In the case of the strophanthins and Digitalis glucosides it is the hydroxyl group, attached to carbon atom 3, which is the carrier of the chain of sugars. In *scillaren A*, we do not know the position of the hydroxyl group which possesses the function of carrying the sugars, because this hydroxyl group, on hydrolysis, is eliminated with formation of a double bond. That is why the structural formula of *scillaridin A* is not yet definitely established.

This brief review of the chemistry of the aglucons, which has been mostly based on the extensive work of W. A. Jacobs and his collaborators, was intended to show the relations of the cardio-active constituents to other natural substances.

The sugars isolated from the cardiac glucosides are either widely occurring, such as *d*-glucose and rhamnose, or sugars specific for the cardiac glucosides, such as digitoxose and cymarose. These latter are characterized by the absence of a hydroxyl group at carbon atom 2 and for this reason they must be considered as 2-deoxysugars.

TABLE III.—SUGARS OF THE CARDIO-ACTIVE GLUCOSIDES.



The modifications undergone by the cardiac glucosides during storage or unsuitable manufacturing processes of the drugs do not, as a rule, concern the aglucon fraction which, with few exceptions, preserves its initial constitution even after successive elimination of the sugar molecules. The modifications of the sugar fraction are of first consideration. Before proceeding further, we must, first of all, define the expression "genuine glucosides." By this we understand the unchanged initial cardiac glucosides as they pre-exist in the fresh plant, carefully dried.

We know that in cardiac therapeutics many physicians prefer the powdered fresh Digitalis leaf to substances which have been isolated from it. In these circumstances, we were, therefore, justified in asking ourselves if the pure products isolated up to then, such as digitoxin or "digitaline cristallisée" and other glucosides obtained from galenical preparations, were different from the cardiac glucosides originally contained in the plant. I may state now that a difference actually exists. We have established in the course of our researches that it is the glycolytic enzymes which play the principal rôle during the transformation of the genuine cardiac glucosides into those previously known.

Biochemistry has shown that the many different chemical reactions of the living cell are achieved by the agency of enzymes, and one may accept that well-directed enzymatic reactions, *i. e.*, those which follow in sequence, and with an intensity appropriate to cellular life, constitute the chief distinctive characteristic, perhaps even the essence of the life of the cell.

The living cell, above all the vegetable cell, possesses special capacities, in view of the synthesis of the most complicated compounds. Thanks to the regulating mechanism of the enzymatic processes, the reactions of disintegration of matter which furnish the energy necessary to vital functions, follow each other gradually, thus making the best use possible of the energy liberated.

After the death of the cell, the enzymatic processes alter entirely. The mechanism controlling the enzymes ceases, the processes of synthesis retrogress and general decomposition begins.

The first important results obtained in the study of enzymes have already shown that the dead but fresh cellular substance, carefully treated, still contains the enzymes which, under appropriate conditions, exercise a hydrolytic action. The proteinases hydrolyze the albumins, the lipases and the esterases hydrolyze the fats, as well as the esters; the glucosidases hydrolyze the polysaccharides and the glucosides. In the living cell, a number of these enzymes may participate in the synthesis of the substances which they subsequently decompose.

In the light of these considerations, we now understand why substances which formerly passed as being genuine substances, are now revealed actually as products decomposed by enzymes. We may cite, in this connection, one of the earliest examples known, that of crystalline chlorophyll which, long considered as a natural product, was shown later to be a product of conversion due to an enzyme, chlorophyllase. This enzyme substitutes ethyl alcohol for the alcohol phytol. We can understand that Nativelle may have thought his crystalline digitalin to be a natural product, this supposition being current until recently. It is only during the past few years that crystalline digitalin has been recognized to be a decomposition product, hydrolyzed by a glycolytic enzyme from a glucoside originally richer in glucose, a genuine glucoside.

Knowledge of the chemistry of enzymatic action has only recently been used to advantage in pharmaceutical chemistry in the preparation of active products from the natural cellular substance. Many years ago Perrot and his school first drew attention to the probable presence of ferments with an oxidizing and diastatic action in certain drugs, belonging chiefly to the Digitalis group; they established that it is not possible to prepare crystalline digitalin from stabilized fresh leaves of Digitalis purpurea.

Later, in 1926, Jacobs and Hoffmann discovered that the seeds of certain varieties of Strophanthus contain a glycolytic enzyme which they called strophanthobiase. Starting from amorphous mixtures of cardiac glucosides isolated from the drugs, they made use of this enzyme to prepare crystalline glucosides with a lower sugar content. These experiments on enzymatic decomposition in the case of Strophanthus kombé, however, did not lead to the isolation of the genuine glucoside in crystalline form. On the other hand Jacobs has given us a typical example of enzymatic decomposition, with the reaction of elimination of a molecule of glucose,

starting from crystalline *k*-strophanthin-*β* which is transformed into cymarin under the influence of the enzyme. Still, as we now know, *k*-strophanthin-*β* may itself be derived by enzymatic hydrolysis from a glucoside richer in sugar.

In order to isolate the initial cardiac glucosides we adopted a procedure the reverse of that of the American authors, *i. e.*, we suppressed the action of the enzymes before extraction of the glucosides and thus facilitated the preparation, in a pure state, of the cardiac glucosides as they exist in the natural drug. We then systematically submitted these pure initial cardiac glucosides to hydrolysis by enzymes and by more or less powerful chemical agents. This procedure enabled us to isolate, describe and analyze the products corresponding to all stages of the hydrolysis. Thus were elucidated the relations between the initial glucosides, unknown until then, and the cardiac glucosides already used in therapeutics, all of which were shown to result from enzymatic decomposition.

The first drug treated successfully by our method was *Scilla maritima*. From it we isolated a pure product, two-thirds of which consisted of well-crystallized *scillaren A* (Plate 1), and the remaining third of amorphous, but still more active, *scillaren B*. We observed that *scillaren A* is only obtained in good yield from the bulb of fresh squill if the extraction is carried out very rapidly. If the extract is left in contact with the squill substance the initial *scillaren A* loses, owing to the action of an enzyme, a molecule of glucose, and is transformed into *proscillaridin A*, a well-crystallized product, but with a lower sugar content. My collaborator, Dr. Kreis, has demonstrated that the addition of salts, such as ammonium sulfate, prevents enzymatic action even more effectively than rapid extraction. The added salt causes coagulation of the cellular substance, and prevents completely the action of the enzyme which we have called *scillarenase*. Accordingly, without seeking to destroy the enzyme by drastic measures such as heating, which is damaging to *scillaren A*, we have been able to suppress enzymatic action and to obtain the glucoside in its initial state. Thus the basis for a convenient technical method, which permits the preparation of an initial glucoside in good yield, was created. I do not propose here to enter into more detail regarding the preparation of *scillaren A* and the glucosides of Digitalis and Strophanthus, still to be considered, because these methods are fully described in our experimental publications.

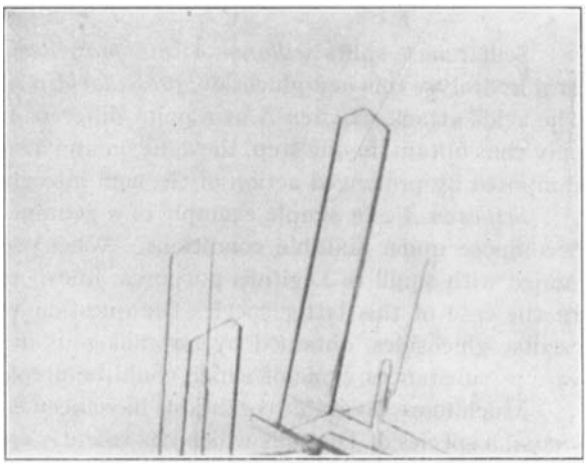
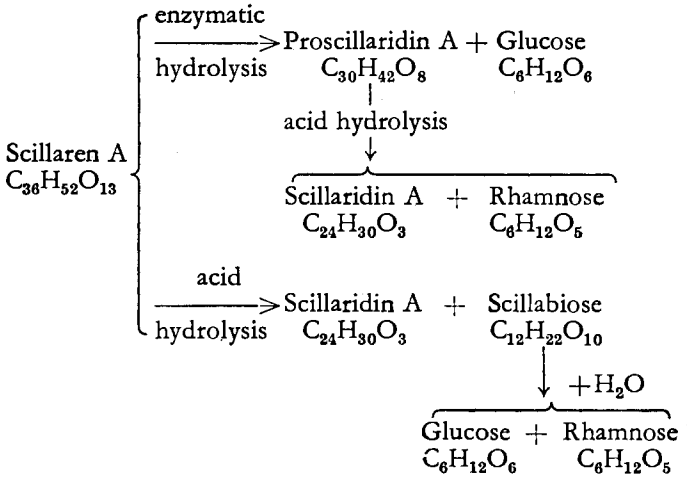


Plate 1.—Scillaren A (from 50% methanol).

Analytical investigations have shown that *scillaren A* is composed of an aglucon, *scillaridin A*, and the sugars, rhamnose and glucose. Table IV illustrates the successive enzymatic and chemical decomposition of the glucosides down to the aglucon.

TABLE IV.—THE HYDROLYSIS OF SCILLAREN A.



Scillarenase splits *scillaren A* into *proscillaridin A* and glucose; acids in their turn hydrolyze this new glucoside, *proscillaridin A*, into *scillaridin A* and rhamnose. The acids attack *scillaren A* at a point different from that of the enzyme, and one may thus obtain, in one step, the aglucon and a sugar, scillabiose, which is then decomposed by prolonged action of the acid into glucose and rhamnose.

Scillaren A is a simple example of a genuine glucoside which an enzyme may decompose under suitable conditions. When we came to apply the experiences gained with squill to Digitalis purpurea, unexpected difficulties were encountered. In the case of this latter species the question was more complicated, the initial cardiac glucosides, obtained by an analogous method, consisting of a mixture of various substances, none of which could be prepared in the crystalline state.

Much more favorable conditions have been encountered in the case of *Digitalis lanata*, a species of Digitalis which has recently enjoyed special favor in cardiotherapy. Having recourse again to the enzyme-inhibitor method in order to avoid the degradation of the glucosides, it was found possible to extract from *Digitalis lanata* a glucosidic complex in the form of beautiful crystals and representing more than half of the total quantity of the glucosides in the leaf. Further investigations showed that this complex to which we gave the name Digilanid (Plate 2) is composed of three separate glucosides, the *digilanids A*, *B* and *C*, which appeared to be crystallographically isomorphous.

The separation of the two components, *a* and *b*, of chlorophyll, realized by a purely physical method, *i. e.*, by fractional separation from a fat solvent and a mixture of alcohol and water, had already presented a serious problem. The separation by analogous methods of these three digilanids, so closely resembling each other in all their properties, was still more complicated. I shall not attempt to describe this method in detail here. It consists in systematic fractionation in non-miscible solvents such as chloroform and aqueous methyl alcohol. More than fifty fractionations were necessary in order to obtain *digilanid B*, the proportion of which is lowest, in a pure state. When the leaves treated come from a good crop, freshly gathered and perfectly stored, the Digilanid obtained consists of about 46%

digilanid A, 17% *digilanid B* and 37% *digilanid C*. It is in these proportions that Digilanid has been introduced into therapeutics and these proportions are maintained strictly constant.

Analytical researches have shown that three different aglucons, digitoxigenin, gitoxigenin and digoxigenin, correspond to the three *digilanids A*, *B* and *C*. In so far as the sugar fraction of their molecules is concerned, the three glucosides have the same constitution. To each of the three aglucons are attached three molecules of digitoxose, a molecule of glucose and an acetyl group. It is a curious fact that this acetyl group determines the isomorphism of the crystals of the three digilanids. If this group is eliminated, which may readily occur when one works without precautions in an alkaline medium, the deacetyldigilanids are obtained, of which only the *deacetyldigilanid C* crystallizes, the other two, *A* and *B*, being amorphous.

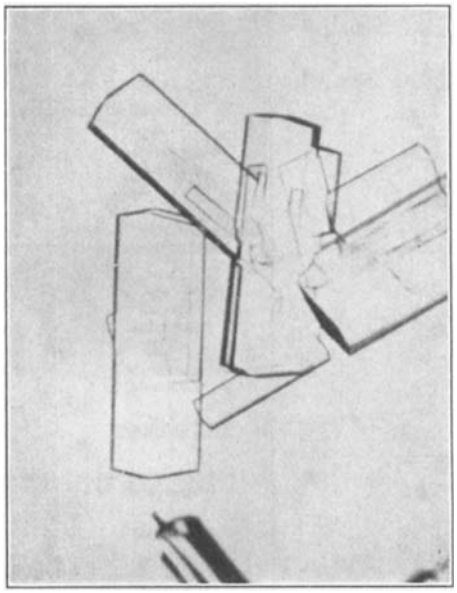


Plate 2.—Digilanid (total glucosides from methanol).

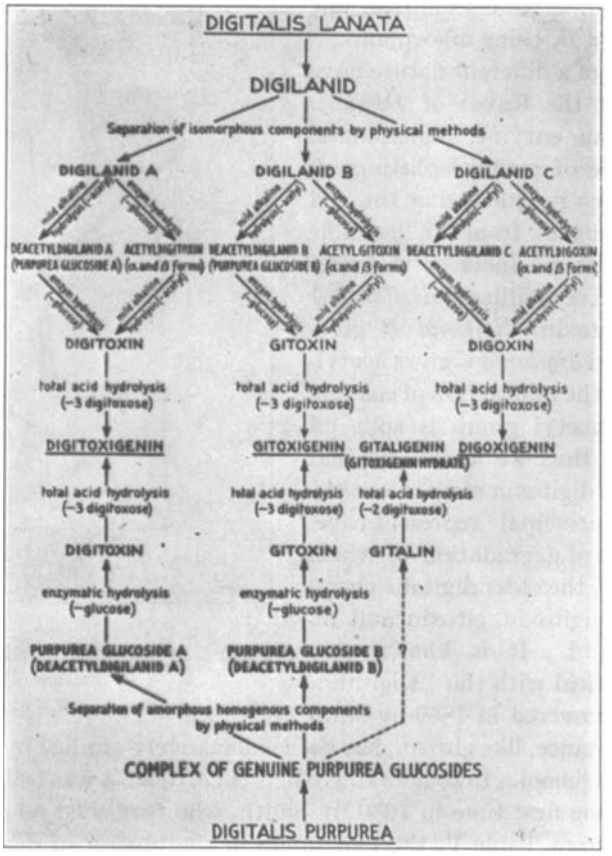
Researches of a different nature have established that the leaves of *Digitalis lanata* contain an enzyme, digilanidase, which is capable of readily splitting off the glucose, which is attached at the end of the chain of sugars, from the "genuine glucosides." The products of this degradation are well crystallized; *digilanid A* gives acetyldigitoxin, *digilanid B* gives acetylgitoxin and *digilanid C* gives acetyldigoxin. Under the mild action of calcium hydroxide the acetyl group is split off in its turn and thus we arrive at what we may call the digitoxin stage, from the name of the principal representative. This is the stage of degradation to which the glucosides of the older digitalis chemistry known as digitoxin, gitoxin and digoxin, correspond. It is known that digitoxin is identical with the "Digitaline crystallisée" discovered in 1869 by Nativelle. This substance, like gitoxin, has been exhaustively studied by Cloetta and by Windaus and his school. In so far as digoxin is concerned, it was isolated from *Digitalis lanata* for the first time in 1930 by Smith, who furnished its description.

As is seen from Table V, the two stages of the process of degradation of the initial glucosides to the glucosides of the digitoxin stage may also be carried out in reverse order, *i. e.*, the acetyl group of the digilanids may be split off first by chemical action and then the glucose fraction of the deacetyldigilanids may be separated by the action of the enzymes. If, then, the hydrolysis is completed by the action of an acid, we obtain in each of the three series, three molecules of digitoxose, plus the corresponding aglucon.

After the chemical relationships between the three most important initial glucosides of *Digitalis lanata* and the known glucosides of the digitoxin stage had thus been clearly determined, an attempt to obtain the initial glucosides of *Digitalis*

purpurea, in a pure state, had greater chance of success. However, the difficulty was appreciably increased here, owing to the small quantity of the glucosides in *Digitalis purpurea* as compared with *Digitalis lanata;* there are also many more secondary substances in the drug of the purpurea species. Finally, the initial glucosides of purpurea have not yet been obtained in a crystalline state. It was necessary for us to perfect the methods of extraction and separation of the glucosides. On the other hand, owing to the number of operations necessary for the purification of the products, the yields were always very small. Nevertheless, it was

TABLE V.—THE INTERRELATIONSHIP OF THE LANATA AND PURPUREA GLUCOSIDES.



eventually possible to isolate two essential principles and to establish that one, the *purpurea glucoside A*, is identical with *deacetyldigilanid A*, and the other, *purpurea glucoside B*, with *deacetyldigilanid B*.

The table shows how these two glucosides are related to the others, and how they are decomposed by the action of digipurpidase, an enzyme present in the drug. The molecule of glucose attached at the end of the chain of sugars, is then readily split off, giving digitoxin and gitoxin. These glucosides, as already mentioned, have been known for a fairly long time, and were first extracted from

Digitalis purpurea. It has not yet been possible to isolate an initial glucoside, corresponding to gitalin, also obtained from *Digitalis purpurea.*

A survey of the various well-defined digitalis glucosides, arranged according to the aglucons and carbohydrate contents, is presented in Table VI. The equation

TABLE VI.—DIGITALIS GLUCOSIDES ARRANGED IN THE ORDER OF THEIR AGLUCON AND THEIR SUGAR CONTENT.

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

for the complete hydrolysis of each individual glucoside is given, and the large brackets include those groups of glucosides which are derived from the same aglucon, the lowest member of each group corresponding to the digitoxin type.

In comparing the glucosides of the two species of digitalis, it is quickly realized that *Digitalis lanata* represents, from various points of view, a drug of superior

value. That does not mean that the invaluable services which *Digitalis purpurea* has rendered to medicine since Withering introduced it into therapeutics, should be underestimated. But *Digitalis purpurea* possesses only a fraction of the activity of *Digitalis lanata*. Further, this latter species gives three different types of aglucons, all three being present in Digilanid, which makes for synergy in their action. The component C built up with digoxigenin is lacking in *Digitalis purpurea*.

Thanks to the precise analytical study of each of the three *digilanids, A, B* and *C*, it has been possible to find a quantitative method of analysis for determining the proportions of the three components in the total products. The three constituents of Digilanid, may, therefore, be accurately dosed by weight, thus guaranteeing constancy in composition and action. Digilanid, therefore, not only possesses the total action of the natural drug, but is free from the dangers of the instability and inconstancy of cardioactivity characteristic of *Digitalis purpurea*. The search for an analogous method for determining the proportions of the constituents of the total product obtainable from *Digitalis purpurea* has, up to now, met with insurmountable difficulties. The following shows how easily the glucosides of Digitalis decompose and, consequently, the importance to be attached to their quantitative determination. On extracting, by careful methods, crystalline digilanid from an infusion freshly prepared according to the method of the Swiss Pharmacopœia, only 50% of the Digilanid originally present in the leaves is obtained intact, the other half being decomposed. It is well known that, once prepared, the infusion rapidly loses its activity—in the hands of the patient, so to speak.

After these studies of squill and the two species of Digitalis, it was of great interest to proceed to researches on some important members of the large strophanthus family. As already mentioned, the researches of Jacobs demonstrated that strophanthus seeds contain glycolytic enzymes. It was to be anticipated, therefore, that by suppressing enzyme action prior to extraction, we should find glucosides richer in sugars.

Our first attempts were deceptive. The total glucosidic product extracted from the seeds of *Strophanthus gratus*, gave more than 80% ouabain, a well-crystallized substance known for a very long time, of which the constituents are ouabagenin and a single sugar, rhamnose. The question which still remains unanswered is whether glucosides, richer in sugar, exist in the seeds of *Strophanthus gratus*.

On the other hand, we have been able to extract from the seeds of *Strophanthus kombé* a very beautiful specimen of a genuine glucoside, unknown until now in a pure state. The amorphous mixture of the glucosides of this drug is, as we know, widely used in therapeutics under the name of *k*-strophanthin. Side by side with cymarin, a compound of strophanthidin and cymarose, Jacobs isolated *k*-strophanthin-β, which contains a molecule of glucose more than cymarin. He succeeded in transforming *k*-strophanthin-β into cymarin by splitting off the molecule of glucose, by the action of an enzyme, strophanthobiase.

In the course of experiments recently carried out in our laboratory by Dr. Renz, the seeds of *Strophanthus kombé* have given singularly weak proportions of cymarin and of *k*-strophanthin-β. We have succeeded in extracting from them, in well-crystallized form, a new glucoside representing more than three-fourths of the amorphous mixture of glucosides. It contains one glucose molecule more than *k*-strophanthin-β and was named *k-strophanthosid* (Plate 3). In order to prepare

this new glucoside the essential is to prevent enzymatic glycolysis, but special fractionation processes must be applied in order to obtain it in such a state of purity that it crystallizes.

It will be noted from the above table that *k*-strophanthosid is the glucoside with the largest sugar content.

Among all the known initial cardio-active glucosides *k*-strophanthosid furnishes the most typical example of a degradation by successive stages. Treated with strong acid it decomposes partly into its aglucon, strophanthidin, and partly into a well-crystallized triose until now unknown, strophanthotriose. This triose is composed of a molecule

Plate 3.—*k*-Strophanthosid (from methanol-chloroform).

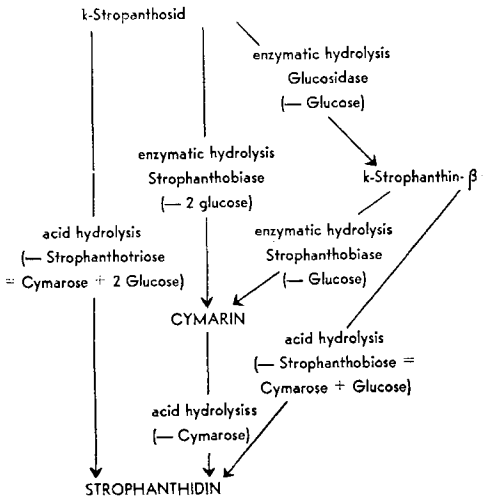
TABLE VII.—STROPHANTHUS GLUCOSIDES AND SOME RELATED CARDIAC GLUCOSIDES.

$C_{30}H_{44}O_9$ 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

of cymarose and two molecules of glucose. It gives quantitatively these three sugars when submitted to strong acid action.

If *k-strophanthosid* is subjected to the action of the enzyme of Strophanthus two molecules of glucose are eliminated, and cymarin is obtained, which decomposes into strophanthidin and cymarose on acid hydrolysis. This enzymatic cleavage is similar to one already performed by Jacobs with the amorphous mixtures of strophanthus glucosides, but now starts with a pure crystalline substance. If, on the other hand, *k*-strophanthosid is acted upon by the *a*-glucosidase of yeast, a single molecule of glucose is split off and *k*-strophanthin-β is obtained. This splitting off of a molecule of glucose, under the action of *a*-glucosidase, proves that this molecule, situated at the end of the chain, is fixed in the form of *a*-glucose. Under the action of strophanthobiase *k*-strophanthin-β gives cymarin, and by direct acid hydrolysis it gives strophanthobiose and strophanthidin.

TABLE VIII.—HYDROLYSES OF K-STROPANTHOSID.



The series of successive degradations of *k*-strophanthosid shows in a striking manner to what numerous risks of hydrolytic degradation a genuine cardio-active glucoside is exposed, if special precautions are not taken in the storage and treatment of the drug. In addition to the enzymes and the natural acids of the plant, capable of exercising a hydrolytic degrading action, there exists also other kinds of injurious enzymes. Thus Jacobs has found, in the seeds of *Strophanthus kombé*, an enzyme which has the effect of isomerizing the aglucon part after the seeds have been ground. By slow extraction Jacobs obtained, instead of cymarin, allocymarin, which is practically inactive.

When the amorphous mixture of glucosides of *Strophanthus kombé* is utilized in therapeutics, it is necessary to reckon with the manifold transformations which are always possible. One may now eliminate these hazards, thanks to the discovery of *k*-strophanthosid, a well-crystallized substance which represents more than three-quarters of the glucoside content of the drug.

It is only during the last few months that it has been possible to extract *k-strophanthosid* from *Strophanthus kombé*, and to isolate it in a pure state. A pharmacologic study of this substance now in progress has already demonstrated its very great activity. Clinical testing of the new product has been undertaken, but some years must pass before we can formulate a definite opinion as to its therapeutic value. At all events, it offers to the experimenter one advantage, that of being an absolutely pure substance, capable of exact dosage and always constant in activity. As with any new medicament, it is necessary to guard against forming an opinion too hastily.

The following table (IX) gives a view of the activity of some of the cardio-active

glucosides that we have discussed. These values, which have served as a starting point for clinical investigation, are the result of the extensive pharmacologic researches of Professor E. Rothlin.

TABLE IX.—TOXICITY OF DIGITALIS GLUCOSIDES.

Glucoside.	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 complex)	620	0.343
Digilanid A	690	0.380
Digilanid B	540	0.400
Digilanid C	640	0.281
Purpurea-glucoside A	690	0.368
Purpurea-glucoside B	315	0.369
Digitalin cryst.	400	0.420
Digoxin	650	0.280
k-Strophanthosid	1850	0.126
Cymarin	1500	0.146
Ouabain (*g*-Strophanthin)	2400	0.100
Scillaren A	1200	0.145

Without entering into further detail, I wish to emphasize the considerable difference in toxicity between the Digitalis glucosides on the one hand and the strophanthins on the other. The values in frog units and cat units show parallel variations from one glucoside to another. Nevertheless, the proportion is far from being constant, which shows once more the importance of a profound clinical study of the pure glucosides.

Like the physician, the biochemist knows that, with the preparation and the clinical study of the medicament, the essential work is far from complete. Cardiac diseases are so diverse in origin, and the reaction of the patient is so individual that even to-day, when there is available a series of valuable cardiotonics, the essential value of a treatment still depends upon the art of the physician. In every case it is necessary to choose carefully the medicament and the dosage best suited, but medical treatment is facilitated if the physician has at his disposal cardiotonics of constant activity, and as complete in action as possible.

The objects in biochemical research on drugs are not simply and purely scientific; there is not only the question of determining the constitution and the chemical relationships of the various, physiologically interesting substances. Such researches also have a practical significance, since their aim is to place the contribution of chemical science in the hands of the physician.

CONCLUSION.

This discussion has dealt with attempts to isolate the active principles of drugs in pure form without destroying their natural initial state. Only a few examples in the very specialized and limited field of cardiac glucosides have been given. There are doubtless many other cases where the genuine active principles of drugs are not yet known and, therefore, a vast field for investigation still remains.