[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

STUDIES ON LACTONES RELATED TO THE CARDIAC AGLYCONES. III. THE PROPERTIES OF β-SUBSTITUTED $\Delta^{\alpha,\beta}$-BUTENOLIDES AND A SUGGESTED REVISION OF THE STRUCTURE OF THE SIDE CHAIN OF THE DIGITALIS-STROPHANTHUS AGLYCONES

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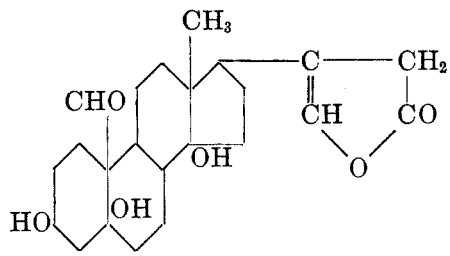
The main structural features of the digitalis-strophanthus group of cardiac aglycones rest on a reasonably firm foundation as a result of the investigations principally of Jacobs, Windaus, and Tschesche and their collaborators. The evidence on which these structures have been assigned has been adequately reviewed on several occasions (1). The main structural features of the molecule of strophanthidin, which may be taken as typical, are given in I.

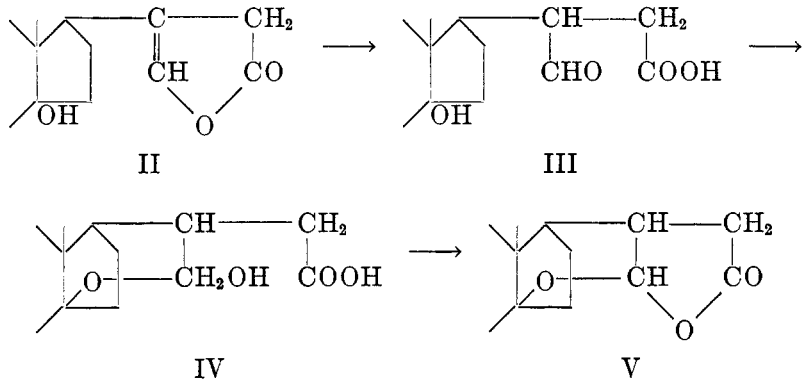


I

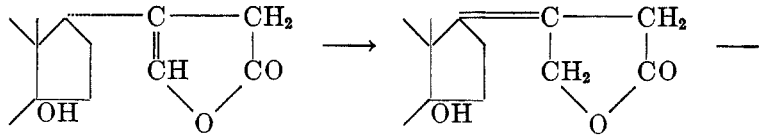
The other members of the group differ in the degree and position of hydroxylation, except that all known members of the group carry hydroxyl groups in positions 3 and 14, and in the nature of the group attached to carbon atom 10, which is aldehydic in strophanthidin but in the form of a methyl group in the other members which have been studied in detail. Perhaps the outstanding structural characteristic of these substances is found in the side chain which has been formulated as the lactone of an enolized β-aldehydopropionic acid to which the cyclopentanophenanthrene ring system is attached through the β-carbon atom. In the present communication we wish to present evidence which we believe suggests that these substances may be more satisfactorily formulated as reduced cyclopentanophenanthrene-$\Delta^{\alpha,\beta}$-butenolides, or as $\Delta^{\alpha,\beta}$-unsaturated lactones, rather than

as lactones of the $\Delta^{\beta,\gamma}$-type as hitherto suggested. For this purpose it will be necessary to review the evidence on which the $\Delta^{\beta,\gamma}$-structure has been based.

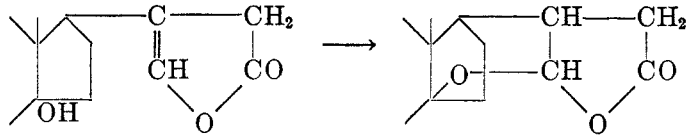
The evidence which indicates the presence of a $\Delta^{\beta,\gamma}$-structure rests largely on a study of the so-called iso-aglycones which result when the aglycones are subjected to the action of a methyl alcoholic solution of potassium hydroxide. For purposes of discussion, strophanthidin (2, 3) will be taken as typical of the other members of the group. For convenience such isomerizing action of alkali may be represented by the series of changes II–V. In this formulation the complete series of changes is



not necessary, as will be discussed below, although the case for the $\Delta^{\beta,\gamma}$-lactone can be more clearly presented on this scheme. The first action of the alkali consists in opening of the lactone ring in II which sets free an aldehyde group (III). This may exist as a lactal (IV) by virtue of the hydroxyl group in reactive proximity on carbon atom 14. The lactal can then undergo lactonization to yield isostrophanthidin (V). However convenient such an explanation may be, it does not accord with the experimental observation of Jacobs and Collins (2) that preliminary saponification of the lactone group of strophanthidin is not necessary for the formation of isostrophanthidin under the action of a methyl alcoholic solution of potassium hydroxide. In order to take this fact into account Jacobs and Elderfield (4) proposed a mechanism for the change in accordance with formulas VI–IX. In this interpretation, the side chain of strophanthidin is pictured as originally in a trans configuration to the hydroxyl group on carbon atom 14. The action of alcoholic alkali then consists in (a) a shift of the double bond to the 17,20-position (VII), (b) a shift of this $\Delta^{17,20}$-double bond back to its original position with an inversion of the configuration of the side chain at carbon atom 17 so that it has now become cis to the hydroxyl group on carbon atom 14 (VIII), and (c) forma-



VI VII



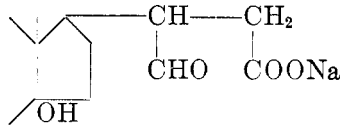
VIII IX

tion of the new oxidic bridge to yield isostrophanthidin (IX), presumably by direct addition of the hydroxyl group at position 14 to the double bond which has now become possible by virtue of the cis relationship of the side chain and the hydroxyl group in question.

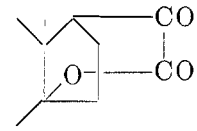
Whatever the mechanism of the formation of iso- derivatives of the aglycones, there is no doubt that the latter are represented by structures V or IX. In support of this, Jacobs and Gustus have succeeded in preparing carbonyl derivatives of saponified iso-aglycones (3, 5), and the lactal form of the saponified iso-aglycones has been oxidized to a lactone in many cases (2, 3, 5, 6, *inter alia*). Largely on the basis of the behavior of the iso-aglycones, the original aglycones have been formulated as $\Delta^{\beta,\gamma}$-butenolides, or as the lactones of enolized β-aldehydo acids. Additional points which have been interpreted as indicating such an arrangement will be developed shortly.

While the $\Delta^{\beta,\gamma}$-formulation satisfactorily accounts for the great majority of the observed experimental facts, a few remain which cannot be adequately explained on this basis. Among these may be cited the failure of certain aglycones, *e.g.* the aglycone of uzarin (7) and allostrophanthidin (8), to yield iso-aglycones under conditions where such a reaction would be expected. This has been ascribed to the occurrence of a trans configuration of the side chain with respect to the hydroxyl group on carbon atom 14, and, in the case of allostrophanthidin, experimental evidence has been obtained in support of this view (9). It is difficult to see why, on the basis of the Jacobs and Elderfield formulation of the formation of iso-aglycones, some such iso-compound should not be formed, since this mechanism postulates a disturbance and re-establishment of asymmetry on carbon atom 17, in which at least a portion of the substance would be expected to assume a cis configuration on the two asymmetric centers in question. A second fact which is difficult to reconcile with the $\Delta^{\beta,\gamma}$-formulation is the structure XI, which has been shown to represent the product of the oxidation of saponified strophanthidinic acid (X) (10). This com-

pound is much better formulated on the basis of a $\Delta^{\alpha,\beta}$-arrangement of the side chain. Indeed it is exceedingly difficult to account for XI on the



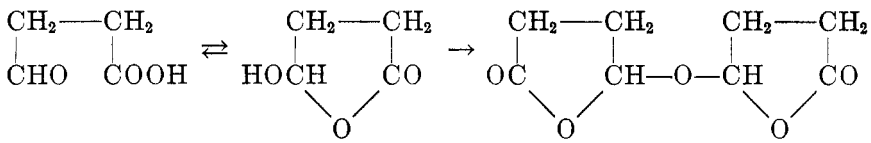
X



XI

basis of the $\Delta^{\beta,\gamma}$-arrangement unless one postulates a shift of the double bond to the $\Delta^{\alpha,\beta}$-position during saponification of the lactone.

Despite the almost overwhelming evidence of Jacobs and his co-workers in favor of the $\Delta^{\beta,\gamma}$-formulation, we were led to investigate the possible application of a $\Delta^{\alpha,\beta}$-formulation in the hope that it would be possible to explain some of the inconsistencies arising from the $\Delta^{\beta,\gamma}$-arrangement and at the same time to retain the unquestionably valid interpretation of the structure of the iso-aglycones. In this we have been guided by a few general observations on record concerning the nature of simple β-aldehydo acids and the products which are formed on attempted lactonization of their enolic forms. The simplest acid of this type, β-aldehydopropionic acid, has been the subject of intensive study by von Ungern-Sternberg (11), Harries and Alefeld (12), and Perkin, Jr. and Sprankling (13). In the course of their investigations, none of these workers was able to accomplish the lactonization of the acid, which they showed existed as an equilibrium mixture of the open aldehydo acid (XII) and the hydroxy lactone (XIII). All attempts at lactonization resulted either in the formation of derivatives of XIII in which the hydroxyl group was attacked, or in the formation of a bimolecular compound (XIV). A similar experience with α,α,β-trimethyl-β-formylbutyric acid and with α,α-dimethyl-β-phenyl-β-formylbutyric acid has been recorded by Blaise and Courtot (14). In this con-



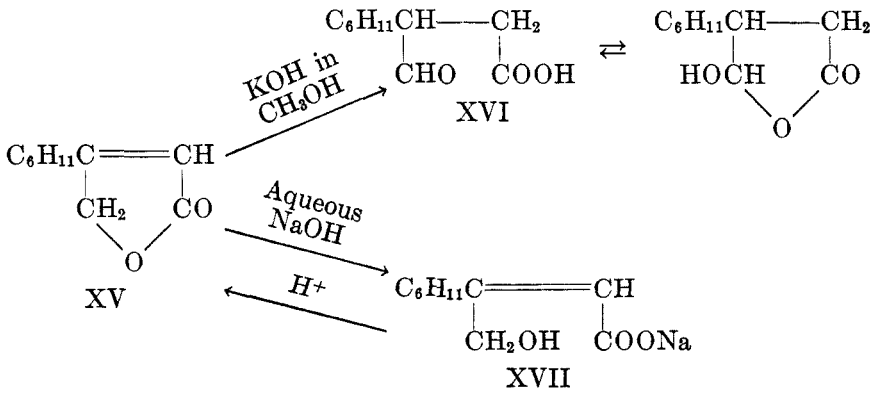
XII XIII XIV

nection it should be noted that the valuable studies of model unsaturated lactones carried out by Jacobs, Hoffmann, and Gustus (15) and by Jacobs and Scott (16) were all done with the lactones of enolized keto acids, with the exception of the lactones of the formyl acids described by Blaise and Courtot (14). The latter lactones were prepared by elimination of hydrogen bromide from saturated bromo lactones rather than by ring closure of the aldehydo acids. No proof of their structure was offered aside from the fact that they yielded aldehydo acids on hydrolysis. In the light of

observations presented in this paper, this proof can not be taken as conclusive. The lactone of enolized β-aldehydopropionic acid can be considered as the ketonic form of α-hydroxyfuran, and the consistent failure of investigators to obtain this from other furan derivatives occasions some doubt as to the existence of stable derivatives of α-hydroxyfuran (17) unless a substituent occurs in the other α-position, which would result in a keto lactone, or unless the molecule is heavily substituted elsewhere. Thus the reported relative stability of the Blaise and Courtot lactones can be explained satisfactorily on the basis of the observation of Boorman and Linstead (18), that the presence of an α-methyl group greatly increases the stability of α-methyl-γ-valerolactone compared to γ-valerolactone. The presence of two stabilizing α-methyl groups in the Blaise and Courtot lactones, therefore, would result in an enhanced stability over that predicted for the unsubstituted derivative, possibly because of the absence of α-hydrogen atoms.

In a preceding paper (19) the synthesis of model β-substituted $\Delta^{\alpha,\beta}$-butenolides has been described. A study of the properties of β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide has revealed information which has led us to consider more seriously the question of a $\Delta^{\alpha,\beta}$-formulation for the unsaturated lactones of the natural cardiac drugs. Catalytic reduction of the cyclohexyl lactone with the platinum oxide catalyst of Adams and Shriner resulted in the absorption of one mole of hydrogen and the formation of β-cyclohexyl butyrolactone. The reduction thus parallels that of the natural aglycones, and contrasts with the behavior of lactones which have a double bond at the point of lactonization. The latter give varying amounts of desoxy acids on catalytic reduction (16). From the natural substances, as well as from our model substance, no acidic products have been isolated.

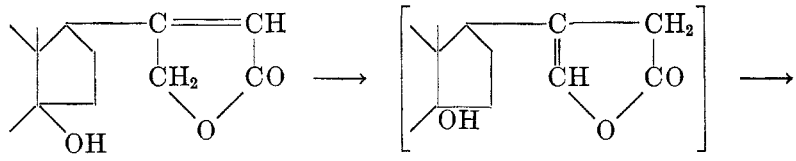
A study of the action of alkali on β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide has yielded information which lends strong support to the hypothesis for the presence of a $\Delta^{\alpha,\beta}$-lactone in the natural aglycones. Two distinct modes of action of alkali, depending on the experimental conditions used, have been noted. These may be represented by formulas XV–XVII:



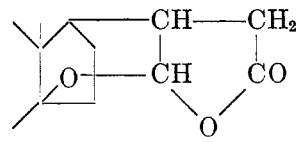
When the unsaturated lactone is subjected to the action of a solution of potassium hydroxide in absolute methyl alcohol at 0°, the characteristic nitroprusside (Legal) reaction disappears on allowing the reaction-mixture to stand for some hours. On working up the reaction-mixture, β-cyclohexyl-β-formylpropionic acid was isolated in practically quantitative yield and was characterized by the preparation of the semicarbazone of its methyl ester. The aldehyde acid showed no tendency to relactonize when subjected to the prolonged action of warm dilute hydrochloric acid. When it was heated in a sealed tube with acetic anhydride-acetyl chloride under conditions which normally lactonize a keto acid, the acetate of the hydroxy lactone form was obtained. Whether the aldehydo acid produced by the action of methyl alcoholic potassium hydroxide on the unsaturated lactone results through the formation of an intermediate methylal, or enol ether, which is decomposed on acidification of the reaction-mixture is a question which must be left open for the present. However the irreversibility of the reaction under these conditions is definite.

On the other hand, when the unsaturated cyclohexyl lactone was treated with alkali in 50% alcoholic solution, the reaction took a different course. While the above aldehydo acid was formed to a greater or less extent, at the same time direct hydrolysis of the lactone to the unsaturated hydroxy acid (XVII) also occurred. The hydroxy acid, in contrast to the aldehydo acid (XVI), readily relactonized on gentle acidification with regeneration of the original $\Delta^{\alpha,\beta}$-lactone. The amount of aldehydo acid formed under these conditions varied with the temperature at which the reaction was carried out. When the unsaturated lactone was shaken with a solution of sodium hydroxide in 50% alcohol at room temperature, the product was almost exclusively the sodium salt of the unsaturated hydroxy acid, with but a trace of the aldehydo acid; on the other hand, when the reaction was carried out at the boiling point of the solvent, both products resulted in the ratio of about one part of the sodium salt of the hydroxy acid to two parts of the aldehydo acid.

We believe that, if the observed experimental facts concerning the varying action of alkali on the natural cardiac aglycones be re-interpreted on the basis of the above behavior of the model $\Delta^{\alpha,\beta}$-unsaturated lactone, a more logical explanation for these findings results. The formation of the iso-aglycones under the influence of a solution of potassium hydroxide in methyl alcohol, thus becomes due merely to an irreversible shift of the $\Delta^{\alpha,\beta}$-double bond to yield an aldehyde acid, possibly through the intermediate transitory formation of a $\Delta^{\beta,\gamma}$-lactone, followed by establishment of the new oxidic bridge on carbon atom 14 (XVIII–XX), in some manner yet to be determined.



XVIII XIX



XX

The possible intermediate formation of XIX is indicated by the transitory character of the positive nitroprusside test shown by the substances in question. In such a formulation for the iso change, no disturbance of the asymmetry about carbon atom 17 is necessary, from which it follows that a trans configuration of the hydroxyl group on carbon atom 14 and the side chain, such as apparently exists in allostrophanthidin, will effectively prevent the formation of an iso compound.

Another observation which can be explained more satisfactorily on the basis of the above experiments is the ready relactonization of saponified pseudostrophanthidin and digitoxigenin when these substances are saponified by shaking with aqueous alcoholic alkali at room temperature (15). If an aldehydo acid were liberated by saponification of a $\Delta^{\beta,\gamma}$-lactone under such circumstances, it is difficult to explain the extremely easy relactonization in the light of our own experience, as well as that of others (11, 12, 13, 14) on the lactonization of aldehydo acids. On the other hand, the isolation of carbonyl derivatives from saponified derivatives of dianhydrostrophanthidin (20, 15) when the saponification was carried out hot is paralleled by the behavior of our model lactone under similar conditions.

Finally, as already indicated, the observed degradation of the side chain when saponified strophanthidinic acid (10) is oxidized by permanganate can only be explained by the presence of a $\Delta^{\alpha,\beta}$-double bond. A completely satisfactory picture of the reported isomonoanhydrostrophanthidin (21) is not possible at present. We are studying a model lactone containing a double bond in the same relative positions to the side chain as have been suggested for monoanhydrostrophanthidin.

Unfortunately, it has not been possible to strengthen the argument for the $\Delta^{\alpha,\beta}$-formulation of the side chain of the natural aglycones by direct oxidative rupture of the double bond, except in the previously reported case of saponified strophanthidinic acid (10). Attempts to cleave the

side chain of representative aglycones using such reagents as ozone, osmic acid, hydrogen peroxide and osmic acid, and Prevost's reagent have led either to the recovery of the original material or to non-crystallizable material. However, the ultraviolet absorption curves for strophanthidin

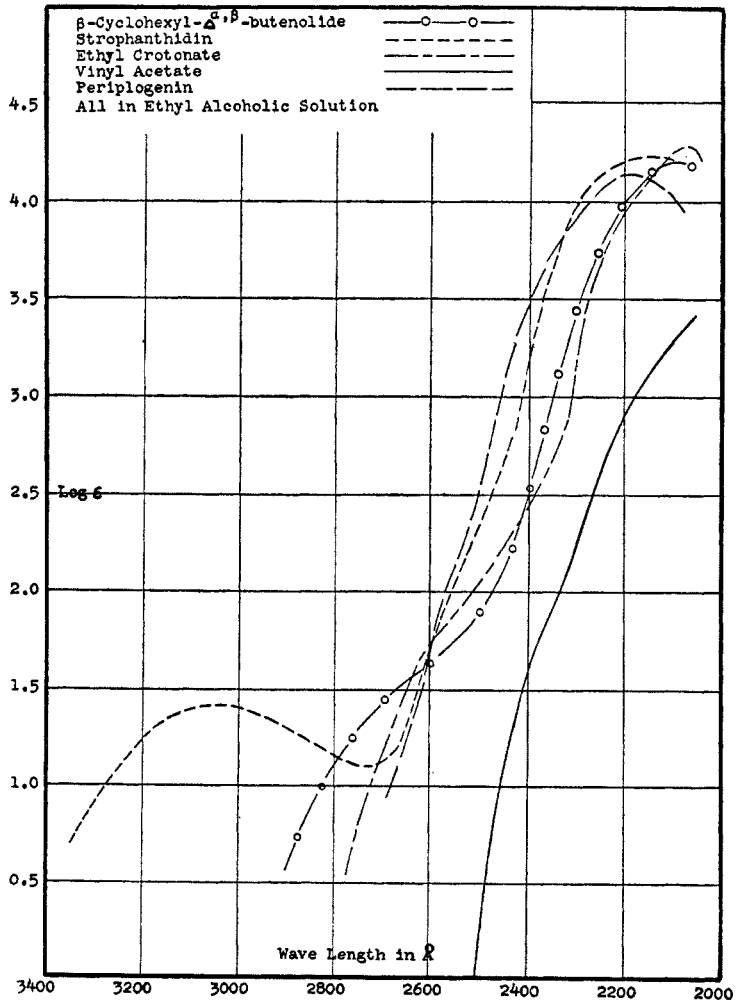


FIG. 1

and for periplogenin supply evidence for the $\Delta^{\alpha,\beta}$-arrangement. In Fig. 1 are shown the curves for the two aglycones together with similar curves for β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide, ethyl crotonate and vinyl acetate. The curves for the first four substances are closely similar with strong maxima between 2100–2150 Å, which are due to the presence of the conjugated

system. Vinyl acetate, which possesses the same arrangement of ethylenic and carbonyl double bonds as would obtain in a $\Delta^{\beta,\gamma}$-lactone furnished a curve radically different from those of the $\Delta^{\alpha,\beta}$-derivatives.

The presence of an apparently reactive methylene group in the aglycones has been offered as supporting evidence for the $\Delta^{\beta,\gamma}$-formulation. Such would, indeed, be expected by virtue of the position of the hydrogen atoms on the α-carbon atom which is situated between the carbonyl double bond and the ethylenic double bond. The evidence in favor of the presence of such a reactive methylene group rests first on the nature of the characteristic nitroprusside (Legal) and Tollens reactions, and secondly on the apparent formation of a mole of methane under the conditions of the Zerewitinoff procedure.

In a study of the model α- and β-angelica lactones, Jacobs, Hoffmann, and Gustus (15) noted a well defined difference between the $\Delta^{\alpha,\beta}$- and $\Delta^{\beta,\gamma}$-angelica lactones both with regard to the speed of development of the Tollens test and to the speed of development and duration of the Legal test. From their observations the promptness of the appearance of the nitroprusside test indicated that the aglycones are $\Delta^{\beta,\gamma}$-lactones, while the gradual reduction of Tollens' reagent suggested the $\Delta^{\alpha,\beta}$-form. On the logical assumption, based on Thiele's observations (22) that the $\Delta^{\alpha,\beta}$-isomer is transformed into $\Delta^{\beta,\gamma}$-angelica lactone under the influence of the reagents used in the tests, they interpreted the positive tests given by the $\Delta^{\alpha,\beta}$-angelica lactone as in reality being due to a small amount of the other isomer formed during the tests. While these studies of the angelica lactones are of value, the fact must be emphasized that the two unsaturated lactones are in equilibrium. On the other hand, as is now shown, the corresponding $\Delta^{\beta,\gamma}$-lactone of an aldehydo acid presumably is capable of but short life, at best, and the equilibrium between the $\Delta^{\alpha,\beta}$- and $\Delta^{\beta,\gamma}$-forms in such a case is irreversible. In contrast to the reported behavior of $\Delta^{\alpha,\beta}$-angelica lactone, we now find that β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide shows exact resemblance to strophanthidin in both the Tollens and Legal tests. In applying the nitroprusside color test, we have noted that the intensity and duration of the color produced is closely dependent on the acidity or alkalinity of the solution. By employing a technique slightly different from that usually used in these tests, we have succeeded in bringing out more sharply the similarity of the tests exhibited by strophanthidin and the cyclohexylbutenolide, and the contrast shown by $\Delta^{\beta,\gamma}$-angelica lactone in comparison with the two former substances. Inasmuch as the production of the color in the nitroprusside test is apparently due to an oxidation phenomenon, it was felt that a still sharper differentiation could be obtained if a reagent of slightly lower oxidation potential could be found. This has been done by using potassium ferricyanide. In alkaline solution no

color is obtained with $\Delta^{\beta,\gamma}$-angelica lactone, whereas both strophanthidin and β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide give a reddish-brown color which was indistinguishable in the two cases.

At first glance the reported formation of a mole of methane from the side chain of the aglycones in the Zerewitinoff test (23) appears difficult to re-interpret on the basis of the $\Delta^{\alpha,\beta}$-formula, although, from time to time certain abnormalities have occurred with certain aglycone derivatives. For convenience the necessary data for re-interpretation of this point are shown in Table I. At the outset, a discrepancy between the reported behavior of the intact side chain of the aglycones and the observed production of about 0.4–0.5 moles of methane from the analogous cyclohexyl

TABLE I

SUBSTANCE	MOLES CH_4 PER MOLECULE
Digitoxigenin (26)	3.04
Strophanthidinic acid methyl ester (26)	4.27
Digitoxigenin acetate (26)	2.09
Dianhydrodilactone from strophanthidin (26)	1.09
Dihydrostrophanthidin (26)	2.92
Isostrophanthidin (26)	2.07
α-Anhydrodigitoxigenin (26)	1.42
β-Anhydrodigitoxigenin (26)	1.42
Tetrahydrodilactone from strophanthidin (26)	0.57
Dihydromonoanhydrostrophanthidin (27)	2.1
Cyclohexyl benzoate (27)	0.06
Cyclohexyl acetate (27)	.70
β-Cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide (27)	.43
β-Cyclopentyl-$\Delta^{\alpha,\beta}$-butenolide (27)	.39
β-Phenyl-$\Delta^{\alpha,\beta}$-butenolide (27)	.55
Strophanthidin *p*-bromobenzoate (27)	2.45

model appears. This discrepancy may be rationalized on the basis of observations dealing with the behavior of various classes of compounds under the conditions of the Zerewitinoff procedure which indicate that extreme caution must be used in interpreting data obtained by this method. Thus, in substances where enolization is possible, one frequently observes the formation of a fraction of a mole of methane. For example, catechol diacetate gives 0.24 moles of methane, benzoin acetate gives 0.58 moles, and acetic anhydride gives 0.54 moles (24). We have confirmed this trend for cyclohexyl acetate. On the other hand, where enolization is impossible, as with cyclohexyl benzoate, no significant amount of methane was observed. Likewise the presence of a hydrogen atom on a carbon atom situated between two ethylenic double bonds has been found to be

responsible for the formation of a mole of methane from cyclopentadiene, indene, and fluorene (25). We have been unable to find information on the behavior of an open system of the type C:C·C·C:C in the Zerewitinoff determination. However it seems reasonable to expect somewhat less activation of the hydrogens in question in such a substance than is the case with the similar highly activated cyclic compounds. With the aid of this information we believe that it is now possible to reconcile the observed active hydrogen data with the $\Delta^{\alpha,\beta}$-lactone structure in the majority of cases as shown in Table II.

Dihydrostrophanthidin and isostrophanthidin need not be considered in this connection, except as controls, since the side chain double bond is absent in these substances. In justification of the assumption of an

TABLE II

SUBSTANCE	MOLES CH_4 PER MOLECULE OBSERVED	SOURCE OF CH_4
Digitoxigenin acetate	2.09	1 OH; 0.43 moles from side chain; 0.7 moles from acetate. Total: 2.13 moles.
α-Anhydrodigitoxigenin	1.42	1 OH; 0.43 moles from side chain. Total: 1.43 moles.
β-Anhydrodigitoxigenin	1.42	Same as for α-derivative.
Dianhydrodilactone from strophanthidin	1.09	0.43 Moles from side chain; balance from activated nuclear double bond.
Tetrahydrodilactone from strophanthidin	0.57	All from side chain.
Strophanthidin *p*-bromobenzoate	2.45	2 OH; 0.45 from side chain. Total: 2.45 moles.

activated nuclear methylene group in the dianhydrodilactone from strophanthidin, the following available information may be cited. The nuclear double bonds in dianhydrostrophanthidin show no evidence of conjugation from the absorption spectra and color test with diazotized *p*-nitroaniline (28). One of these double bonds may logically be placed in the $\Delta^{5,6}$-position by analogy with other steroids and on the basis of the value for the optical rotation (28b), and the second double bond can be assigned the $\Delta^{8,14}$-position on the basis of the observations of Jacobs and Elderfield (29). Such an arrangement leaves the hydrogen atoms on carbon atom 7 activated. The active hydrogen values for digitoxigenin and for strophanthidinic acid methyl ester are obscure and can perhaps be accounted for by retention of solvent by these substances, which are notoriously difficult to dry. Finally we have subjected strophanthidin *p*-bromobenzoate to the Zere-

witinoff determination. This derivative was chosen in preference to the benzoate because it can be readily dried, whereas strophanthidin benzoate retains water of crystallization very tenaciously. The result obtained with the *p*-bromobenzoate is in accordance with the prediction based on the $\Delta^{\alpha,\beta}$-formulation.

One other piece of evidence is at hand in favor of the revised structure. Jacobs, Hoffmann, and Gustus (15) report bromine titrations on a number of the aglycones and derivatives. In these no noticeable absorption of bromine could be detected. The substances thus exhibit the behavior of $\Delta^{\alpha,\beta}$-unsaturated esters.

In summary, we here present evidence which we believe suggests that the cardiac aglycones of the digitalis-strophanthus group may be more satisfactorily formulated as $\Delta^{\alpha,\beta}$-unsaturated lactones rather than as $\Delta^{\beta,\gamma}$-lactones as heretofore accepted. The application of the proposed new formula to gitoxigenin and its derivatives will be presented at another time.

EXPERIMENTAL

Action of methyl alcoholic potassium hydroxide solution on β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide. Ten and two-tenths grams of the unsaturated lactone was dissolved in a solution of 6 g. of potassium hydroxide in 250 cc. of absolute methyl alcohol which had been previously chilled to 2°. After standing for 6 hours at 2–3° the mixture was allowed to come to room temperature, and after about 13 hours the nitroprusside test was negative. The solution was then made faintly acid by addition of the calculated amount of acetic acid, diluted with water, and extracted with ether. The ether solution was washed with water and dried with anhydrous magnesium sulfate. After removal of the solvent, the residue was distilled and practically all boiled at 152–154° at 1 mm. The yield was practically quantitative. n_D^{25} 1.4928; d_4^{25} 1.1290; M_D calc'd for the hydroxy lactone: 47.27; for the aldehydo acid: 47.88; found: 47.34.

Anal. Calc'd for $C_{10}H_{16}O_3$: C, 65.2; H, 8.8.
Found: C, 65.7; H, 8.9.

The above acid (1.7 g.) was treated with an ethereal solution of diazomethane. After removal of the solvent, the residue was refluxed for 15 min. with an alcoholic solution of semicarbazide prepared from 1.4 g. of semicarbazide hydrochloride and 1.03 g. of fused sodium acetate. After concentration to about 5 cc. the solution was diluted and the *semicarbazone of the methyl ester* crystallized on rubbing. After recrystallization from dilute alcohol it melted at 120°.

Anal. Calc'd for $C_{12}H_{21}N_3O_3$: C, 56.4; H, 8.3.
Found: C, 56.5; H, 8.5.

Hydrolysis of β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide in aqueous alkali at room temperature. A suspension of 0.549 g. of the unsaturated lactone in 49.8 cc. of 0.1060 *N* sodium hydroxide solution and 10 cc. of neutral alcohol was shaken at room temperature for 11 hours at the end of which time the nitroprusside test was negative. The solution was then back-titrated against phenolphthalein with 0.1108 *N* hydrochloric acid and 32.9 cc. of acid was consumed. Calculated for 1 equivalent: 33.1 cc. Saponification of the lactone was therefore complete. The neutral solution was then acidified to Congo red with hydrochloric acid and a faint nitroprusside test was immediately apparent. The intensity of the test increased on standing as relactonization oc-

curred. In order to complete the relactonization, the acid solution was warmed at 50° for 4 hours after which it was cooled and extracted with ether. The material left after removal of the ether and thorough pumping off to remove traces of solvent showed n_D^{25} 1.5043. n_D^{25} for β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide is 1.5042; n_D^{25} for β-cyclohexyl-β-formylpropionic acid is 1.4928. The recovered substance, therefore is the original lactone.

When the above crude product was treated successively with diazomethane and semicarbazide, a faint trace of semicarbazone was obtained. This was too small in amount for a melting point determination.

Action of hot aqueous alkali on β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide. A mixture of 0.5 g. of the unsaturated lactone, 50 cc. of 0.1 *N* sodium hydroxide solution, and 12.5 cc. of alcohol was refluxed for 1 hour. The cooled solution was made just acid to Congo red, warmed at 50° for 4 hours, and extracted with ether. After drying with anhydrous magnesium sulfate, the ether was removed from the extract, leaving a thick viscous residue with the characteristic odor of the above described aldehydo acid. The material was pumped off at 0.5 mm. and 100° in order to remove traces of solvent, and then showed n_D^{25} 1.4973. If the relative amounts of aldehydo acid and unsaturated lactone in mixtures can be approximated by assuming a linear relationship in the refractive indices, this corresponds to about two-thirds aldehydo acid. The presence of some unsaturated lactone was shown by the positive nitroprusside reaction given by the mixture. On treatment of the crude reaction-product successively with diazomethane and semicarbazide, a copious amount of the semicarbazone of the methyl ester of the aldehydo acid was obtained. This melted at 119° and the melting point was not depressed on admixture with a known sample.

Anal. Found: C, 56.0; H, 8.2.

Action of lactonizing agents on β-cyclohexyl-β-formylpropionic acid. A solution of 4 g. of β-cyclohexyl-β-formylpropionic acid in 36 cc. of acetic anhydride and 4 cc. of acetyl chloride was heated in a sealed tube at 110° for 14 hours. The acetic anhydride and acetyl chloride were removed from the reaction-mixture under reduced pressure. The residue was distilled at 1 mm., and boiled at 163–167°. The analytical figures corresponded to the acetate of the saturated hydroxy lactone.

Anal. Calc'd for $C_{12}H_{18}O_4$: C, 63.8; H, 8.0.
Found: C, 64.2; H, 8.4.

Saponification equivalent calc'd: 113; found: 116.

The aldehydo acid was recovered unchanged after warming in dilute hydrochloric acid at 50°, and showed no tendency to lactonize under these conditions.

Catalytic reduction of β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide. A solution of 4.183 g. of the unsaturated lactone in glass-distilled alcohol was shaken in an atmosphere of hydrogen with the platinum oxide catalyst of Adams and Shriner. After 558 cc. of hydrogen at 0° and 760 mm. had been absorbed, reduction ceased. Calc'd for 1 mole: 566 cc. The nitroprusside test on the product of the reduction was negative. The product was distilled, and boiled at 121.5–123° at 1 mm.; n_D^{25} 1.4794. Analyses corresponded to β-cyclohexylbutyrolactone.

Anal. Calc'd for $C_{10}H_{16}O_2$: C, 71.4; H, 9.6.
Found: C, 71.5; H, 9.7.

Two-tenths of a gram of the above lactone was heated on the steam-bath with 5% sodium hydroxide solution for 20 min., at the end of which time the lactone was completely saponified. On careful acidification to litmus with nitric acid, the solution became turbid and deposited prisms on standing. After crystallization from water this material melted at 94.5–95°. Analyses corresponded to β-cyclohexyl-γ-hydroxybutyric acid.

Anal. Calc'd for $C_{10}H_{18}O_3$: C, 64.5; H, 9.8.
Found: C, 64.7; H, 9.8.

The silver salt of the above hydroxy acid was prepared as usual. It darkened above 127° and did not exhibit a sharp melting point.

TABLE III

	$\Delta^{\beta,\gamma}$-ANGELICA LACTONE	β-CYCLOHEXYL-$\Delta^{\alpha,\beta}$-BUTENOLIDE OR STROPHANTHIDIN
Color reactions with sodium nitroprusside		
At the start the solutions are strongly acid		
1st drop NaOH	Immediate deep red color fading slowly to orange-red which persists	Immediate deep red color; lasts less than 1 sec. and fades to colorless solution
2nd drop NaOH	Intensity of color increases slightly	Immediate deep red color; lasts less than 2 sec.
Both solutions at this point are acid to Congo red		
3rd drop NaOH	Same intense red color	Immediate deep red color; fades in 15–30 sec. if on alkaline side
4th drop NaOH	Same intense red color	No further color developed
1st drop nitroprusside	No change; intense red which persists	Intense red color which fades in 15–30 sec.
Successive drops of nitroprusside	No change	Transient intense red color until finally no return of color when lactone is all oxidized
Acidification	Blue-green color	Pure blue color
Color reactions with potassium ferricyanide		
At the start the solutions are strongly acid		
1st drop NaOH	Pale pink	Pale brown
2nd and 3rd drop NaOH	Pale pink	Pale brown
7th drop NaOH	Clear solution	No effect; pale brown
1st drop ferricyanide	Clear solution; no effect	Red-brown color
2nd drop ferricyanide	Clear solution	Color increases
3rd drop ferricyanide	Clear solution	Color persists

Anal. Calc'd for $C_{10}H_{17}AgO_3$: Ag, 36.8. Found: Ag, 36.8.

When β-phenyl-$\Delta^{\alpha,\beta}$-butenolide was similarly reduced, 4 moles of hydrogen were absorbed and the same cyclohexylbutyrolactone was obtained. As thus prepared it boiled at 122–123° at 1 mm., n_D^{25} 1.4792.

Comparative color tests on the unsaturated lactones. If the Legal test is carried out in a slightly different fashion from that ordinarily used, the similarities between the synthetic $\Delta^{\alpha,\beta}$-lactone and strophanthidin, and the contrast between them and $\Delta^{\beta,\gamma}$-angelica lactone are more pronounced. The Legal test as now developed is carried out as follows: The substance to be tested (10–12 mg.) is dissolved in 1 cc. of alcohol or pyridine. To this solution are added 3 drops of 10% hydrochloric acid and 1 drop of 0.5% aqueous solution of sodium nitroprusside. Ten per cent sodium hydroxide solution is now added dropwise to the test solution, an interval of 2 min. being allowed between addition of successive drops, until the solution is on the alkaline side. Sodium nitroprusside solution is then added in the same manner with a 2 min. interval between addition of successive drops.

The same technique was employed in the tests using potassium ferricyanide except that the test solutions were made strongly alkaline before the second addition of the ferricyanide solution.

The results of these tests are tabulated in Table III.

The Zerewitinoff determinations were carried out in dry pyridine in an atmosphere of dry nitrogen according to the procedure given in Hans Meyer, "Analyse und Konstitutionsermittlung organischer Verbindungen," 6th Ed. Berlin, 1938.

The ultraviolet absorption spectra measurements were done with a Hilger rotating sector quartz spectrophotometer using Eastman special ultraviolet plates, type 111-O-UV for the far ultraviolet region.

The microanalyses here reported were performed by Mr. Saul Gottlieb of these laboratories.

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