THE ASSOCIATION OF THE DOUBLE BOND WITH THE LACTONE GROUP IN THE CARDIAC AGLUCONES.

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By way of introduction a brief summary of the conclusions of a previous communication1 may be given. Strophanthidin and all of its derivatives which possess both the lactone group and the double bond of the parent substance reduce Tollens' reagent and also give a positive nitroprusside reaction. The same has been found to be true of ouabain, gitoxin, and digitoxin. On saponification or hydrogenation these substances in general no longer give these reactions. On relactonization of the saponified substances a reaction will be again obtained where regeneration of the original substance is possible. In the case of strophanthidin the nature of this apparent association of the double bond with the lactone group appeared to be definitely established by the fact that the ethylal of oxidodianhydrostrophanthidin2 yielded a keto acid on saponification which formed an oxime, thus characterizing it as a substituted crotonic lactone. It was therefore inferred that the unsaturated lactone group is present not only in strophanthidin but is in all likelihood a structural characteristic of all of the above "genins." The possible pharmacodynamic significance of this group was also considered in view of the fact that hydrogenation of the double bond had been shown to produce a marked effect upon the physiological behaviors of several of these substances.3 It was necessary however to carry

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3 In the recent beautiful work of M. Cloetta (Arch. exp. Path. u. Pharmakol., 1926, exii, 261) this investigator has reached the same conclusion
the investigation beyond the point of mere color reactions in order to obtain if possible verification of such a conclusion by more direct methods. Even in the case of strophanthidin itself it became desirable to test the case more fully since a few observations had been made with several of its derivatives which did not seem to fit with the assumption that they are lactones of keto acids.

As a first step and for confirmation of the preliminary observations made with the digitalis glucosides the study was extended to the aglucones themselves. Digitoxigenin and gitoxigenin like the parent glucosides were found to give strong positive nitroprusside reactions and when saponified this property was lost. In dilute pyridine solution they slowly reduced Tollens' solution. The hydrogenated substances no longer gave the Legal test and their action upon Tollens' reagent was decidedly less marked, thus paralleling completely the experience with strophanthidin and ouabain. Like the latter substances the digitalis aglucones therefore exhibit towards these reagents a behavior unquestionably due to a similar if not identical association of the lactone group with the double bond.

regarding the effect of hydrogenation on "gitaligenin" and "bigitaligenin" (probably identical with gitoxigenin) thus adding to the observations of Windaus, Bohne, and Schwieger (Ber. chem. Ges., 1924, Ivii, 1388) on the effect of hydrogenation on digitalinum verum and those of Jacobs and Hoffmann on ouabain.


In this article the case in point was the failure of the lactone acid obtained by oxidation of strophanthidin to give an oxime after saponification. This experiment is developed at greater length in the present communication. The formula for this acid previously given as C_{23}H_{32}O_{7} has since been changed to C_{24}H_{32}O_{7} (Jacobs, W. A., and Collins, A. M., J. Biol. Chem., 1925, lxv, 494).

5 Cloetta, M., Arch. exp. Path. u. Pharmakol., 1920, lxxxviii, 133.


7 The material used in this test was obtained by hydrogenation of gitoxigenin with palladium black in acetic acid solution. The substance obtained formed needles which melted at 165-167°. The amount was too small for analysis. Dihydrodigitoxigenin was prepared in methyl alcoholic solution with colloidal palladium and formed delicate needles from dilute alcohol which melted at 110-113° and probably contained solvent of crystallisation.
The attempt was next made to determine whether all of these substances would yield keto acids on saponification. In the case of strophanthidin the isomerizing effect of alkali compelled the choice of suitable derivatives, preferably not of the anhydrostrophanthidin series, the formation of which had apparently not involved the lactone group or the double bond of the parent substance. The lactone acid, \(C_{29}H_{34}O_7\), obtained by the oxidation of the aldehyde group of strophanthidin to carboxyl, was employed. All attempts to prepare an oxime from this substance after gentle saponification resulted in recovery of the unchanged lactone acid. Analogous experiments were then made with pseudostrophanthidin in which both hydroxylamine and semicarbazide were employed with similar negative results and with recovery of unchanged material. Although in both of these substances just as in the case of the dianhydrostrophanthidin derivative the lactone group and the double bond were essential for the positive outcome of the nitroprusside reaction it appeared that hydroxy acids and not keto acids were produced by saponification. This was particularly suggested by the facility with which relactonization could be accomplished in contradistinction to the failure of such attempts with the ethylal of oxidodianhydrostrophanthidinic acid.

In order to complete our data a similar test was made with the ethylal of oxidodianhydrostrophanthidin\(^8\) to see if this would parallel the experience with the dianhydro derivative. The acid obtained by its saponification proved indeed to be a keto acid which yielded an oxime without difficulty. Since this result could be obtained with substances only of the anhydrostrophanthidin series and which possessed at least one new double bond the possibility appeared that the appearance of this new double bond in the molecule may have caused a directed shift of the original olefinic linkage to a position \(\beta,\gamma\) within the lactone ring. That such a shift might have occurred with the participation of the alkali used for saponification was ruled out by the fact that when the new double bonds were removed by hydrogenation the crotonic lactone group could still be detected. For instance the previously described tetrahydrodianhydrodi-

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lactone, C\textsubscript{23}H\textsubscript{36}O\textsubscript{4},\textsuperscript{9} on gentle saponification which involves only the lactone group in question readily yielded an oxime. Therefore if the lactone double bond occupies a position in the anhydro derivatives different from that in strophanthidin the shift must have occurred simultaneously with their formation. It is doubtful that alcoholic hydrochloric acid, the reagent used for their preparation, was responsible since strong aqueous acid was used in the preparation of pseudostrophanthidin. We shall return later to a discussion of the possible explanation of what has occurred.

Careful saponification experiments with ouabain showed that this substance readily lactonizes upon reacidification and in agreement with this result no oxime or semicarbazone could be obtained from the saponified lactone. Digitoxigenin was found to behave in identical manner. Unfortunately in the case of gitoxigenin a complication was encountered. This substance when once saponified by the most gentle treatment was found to suffer further alteration. Although relactonization occurred on acidification the resulting material was amorphous but still gave a nitroprusside reaction. In a parallel experiment in which an attempt was made to prepare an oxime a neutral nitrogen-free substance was recovered. With these substances therefore as in the case of the strophanthidin derivatives it has not been possible to prove directly the presence of the crotonic lactone group.

As a way out we have turned to a study of the angelicalactones and of certain of their derivatives. In his classical studies on the unsaturated lactones Thiele\textsuperscript{10} described as a property characteristic of both the \( \Delta^{\alpha,\beta} \) and the \( \Delta^{\beta,\gamma} \) forms their immediate reducing action on alkaline silver solutions. Although the action of the cardiac aglucones on this reagent is much less marked and more gradual this might be explained by the complexity and the size of the molecule which might modify the speed of the reaction. Since no data were at hand with regard to the crotonic lactones and the nitroprusside test, we have investigated this point ourselves, and with very interesting and helpful results.

A very striking difference was at once observed in the behaviors

\textsuperscript{10} Thiele, J., Ann. Chem., 1901, ccxix, 152.
of Δ\textsuperscript{αβ}- and Δ\textsuperscript{βγ}-angelicalactone.\textsuperscript{11} The latter when treated with the reagent followed by a drop of alkali gave at once a deep red color which as usual gradually faded to a greenish yellow and on acidification changed to a deep violet. On the other hand the Δ\textsuperscript{αβ} form under the conditions employed developed a very faint color which slowly deepened and persisted much longer than in the case of the isomer. In this case the reaction was obviously due to gradual transformation into the Δ\textsuperscript{βγ}-lactone caused by the isomerizing effect of the alkali. The reaction could be explained as one between the reagent and 1 or 2 active hydrogen atoms of the α-methylene group lying between the unsaturated carbonyl and the ethylene groups as follows:

\[
\begin{align*}
\text{CH}_2 \cdot \text{C} &= \text{CH} \quad \text{CH}_2 \cdot \text{CO} \\
\text{Δ}^\text{βγ}\text{-Angelicalactone}. & \quad \text{Δ}^\text{αβ}\text{-Angelicalactone.} \\
\text{CH}_3\text{OCH}_2\text{CH} & \quad \text{CH}_3 \\
\text{CH}_2 \cdot \text{C} &= \text{CH} \quad \text{CH} \quad \text{CO} \\
\text{α-Anisenyl angelicalactone.} & \quad \alpha, \alpha\text{-Dimethylangelicalactone.}
\end{align*}
\]

In harmony with this view α-anisenylangelicalactone\textsuperscript{12} and α,α-dimethylangelicalactone\textsuperscript{13} in which the active hydrogen atoms are replaced gave no reaction. The question as to whether both hydrogen atoms of the methylene group are necessary is now under investigation.\textsuperscript{14}

\textsuperscript{11} Wolff, L., Ann. Chem., 1885, ccxxix, 249.
\textsuperscript{13} Pinner, A., Ber. chem. Ges., 1882, xv, 579.
\textsuperscript{14} It was of interest to determine whether the lactone ring itself was essential for the positive nitroprusside reaction since the cardiac aglucones, when once saponified, no longer give the reaction. In agreement with this observation γ-phenylisocrotonic acid, C\textsubscript{6}H\textsubscript{5}·\text{CH} = \text{CH}·\text{CH}_2·\text{COOH}, gave no reaction, but its methyl ester gave the characteristic deep red color. This demonstrates that the lactone ring itself is not essential, but that the α-CH\textsubscript{2} group becomes reactive when the carboxyl group is esterified or lactonized with simultaneous development of the unsaturated character of its CO group.
Thiele drew no distinction between the $\Delta^{\alpha,\beta}$- and the $\Delta^{\beta,\gamma}$-lactones in regard to their immediate reducing action on alkaline silver solutions but regarded this reaction as a common property of the two forms. We have reexamined this question however in view of the above observations and have found that if the aqueous solutions of the angelicalactones are chosen sufficiently dilute (0.2 per cent) a distinct difference in behavior is to be observed. Upon addition of a drop of Tollens’ solution to the $\Delta^{\beta,\gamma}$-lactone the expected immediate precipitate of silver was obtained as stated by Thiele but the $\Delta^{\alpha,\beta}$ form remained clear for at least 15 seconds and then gradually deposited silver. If instead of an alkaline solution an ammoniacal solution of silver was employed the difference in behavior was much more marked. $\Delta^{\beta,\gamma}$-angelicalactone gave an immediate silver precipitate while the isomer was without action upon it even on long standing. The action of alkaline silver solution upon the $\Delta^{\alpha,\beta}$ form is therefore due to its preliminary transformation into the active $\Delta^{\beta,\gamma}$ form and was due primarily to the active hydrogen of the $\alpha$-methylene group. In agreement with this conclusion $\alpha$-anisylangelicalactone and $\alpha,\alpha$-dimethylangelicalactone do not reduce Tollens’ reagent. Thiele noted that the ability to reduce silver solutions appeared to depend upon the presence of a hydrogen atom on the $\gamma$-carbon atom since diphenylmethoxy-$\Delta^1$-crotonic lactone and the acetate of diphenylhydroxy-$\Delta^1$-crotonic lactone did not reduce, whereas both of the $\beta,\gamma$-diphenyl crotonic lactones reduced the reagent.

The proper interpretation of this observation shows that the presence of the $\gamma$-carbon hydrogen is necessary merely to make structurally possible the conversion of the $\Delta^{\alpha,\beta}$ into the $\Delta^{\beta,\gamma}$ form and does not itself react with the silver solution.
The outcome of the study of the behavior of the crotonic lactones towards Tollens' reagent and nitroprusside is in harmony with the view that the cardiac aglucones belong to this group of substances. The promptness of the appearance of the nitroprusside color would indicate that these substances are $\Delta^{\alpha,\gamma}$-lactones while the gradual reduction of Tollens' reagent would suggest rather the $\Delta^{\alpha,\beta}$ form. But any conclusion must be made to explain the failure of the experiments on oxime formation after saponification and the facility of relactonization in contrast with the behavior of the $\gamma$-keto acids which are the normal saponification products of the crotonic lactones. To explain this inconsistency two possibilities appear. The acids resulting after saponification may remain as $\Delta^{\delta,\gamma,\gamma}$-hydroxy acids (the enolic form of the $\gamma$-keto acid) or perhaps as the $\Delta^{\alpha,\beta,\gamma}$-hydroxy acid. The stability in such a case would have to be referred to obscure structural influences. As an alternative explanation the assumption may be made that the double bond is attached to the $\beta$-carbon atom but outside of the lactone ring as follows:

\[
\begin{array}{c}
\text{CO} - \text{CH} - \text{C} - \text{CH} - \\
\text{O}
\end{array}
\]

In this arrangement the $\alpha$-carbon atom lies between a carbonyl and an ethylene group. Its attached hydrogen should be active and exhibit the properties of a $\Delta^{\alpha,\gamma}$-crotonic lactone with the exception that an unsaturated hydroxy acid would result on saponification. The formation of the true crotonic lactones of the anhydrostrophanthidin series could be attributed to the directive influence of newly formed double bonds in sufficiently close proximity to cause a shift of the original double bond to a position within the ring and apparently $\beta,\gamma$. The final solution of such a problem with substances of such complexity will be difficult. Although further investigations in this direction are being continued we have presented our observations in the present form because of the great importance which the association of the lactone group and the double bond have now assumed in the structural problem of the cardiac glucosides.
A further matter of interest has been the identical behavior shown by these substances on titration with bromine for double bonds by Winkler's method. Strophanthinid, ouabain, gitoxigenin, and digitoxigenin in spite of the presence of double bonds absorbed negligible amounts of bromine. On the other hand dianhydrostrophanthinid and its ethylal which possess two new double bonds add two mols of bromine. The tetrahydrodianhydrodilactone $\text{C}_{22}\text{H}_{32}\text{O}_{7}$, a derivative of dianhydrostrophanthinid in which these new double bonds have been hydrogenated, does not add bromine. Similarly digitaligenin which Windaus and Schwarte have shown to be a dianhydrogitoxigenin was found to add two mols of bromine. In all of these substances therefore the lactone double bond does not add bromine. This is a property which also distinguishes the $\Delta^\alpha\gamma$- from the $\Delta^\alpha\beta$-lactones and might perhaps support the assumption that these aglucones are of the former series. But it is also conceivable that very complex $\Delta^\beta\gamma$-lactones might fail to add bromine, just as there are complex $\Delta^\alpha\beta$-lactones which cannot be oxidized by permanganate to dihydroxy acids. The above titrations can therefore be given only as an additional point in the resemblance of these substances.

**Experimental.**

Attempts to Prepare Oximes after Saponification.

Since the attempt to prepare oximes and semicarbazones from saponified pseudostrophanthinid, the lactone acid, $\text{C}_{22}\text{H}_{32}\text{O}_{7}$, ouabain, and digitoxigenin proved in each case to result in recovery of unchanged material, we shall give only the following examples.

15 In this connection trianhydrostrophanthinid (Jacobs, W. A., and Collins, A. M., J. Biol. Chem., 1925, lxiii, 126) was found to have lost all tendency to add bromine possessed by dianhydrostrophanthinid. This is a most interesting confirmation of the previous experience (loc. cit.) of the behavior of this substance on hydrogenation. The new double bond as previously explained must participate in the formation of a conjugated system of three double bonds or a benzenoid structure.

16 $\Delta^\gamma, \beta, \gamma$-Diphenylcrotonic lactone in contrast to other $\Delta^\alpha$-lactones was found by Thiele and Straus (Ann. Chem., 1901, ccxxix, 150) to be affected by permanganate only with difficulty.
of our procedure. In the case of gitoxigenin it was found impossible to recover the substance unchanged in a preliminary saponification experiment due to the altering effect of the alkali.

0.5 gm. of pseudostrophanthidin was shaken in a machine in 20 cc. of alcohol and 10 cc. of 0.5 N sodium hydroxide until dissolved. The mixture was carefully titrated against phenolphthalein to neutrality which showed saponification to be complete. 0.3 gm. of hydroxylamine hydrochloride was dissolved in a small volume of water and carefully treated with sodium hydroxide solution until alkaline to phenolphthalein. This solution was then mixed with the saponified pseudostrophanthidin solution and was allowed to stand 24 hours, in some cases for a week at room temperature. The mixture was carefully acidified with acetic acid and allowed to evaporate slowly at ordinary temperature. A neutral substance slowly crystallized which was found to be recovered pseudostrophanthidin after recrystallization from dilute alcohol and possessed the required properties. In another experiment semicarbazide was used with similar results.

In the case of the lactone acid C_{23}H_{32}O_{7} (the oxidation product of strophanthidin) the reaction was carried on at water bath temperature but again unchanged starting material was obtained after reacidification.

In another experiment 0.1002 gm. of digitoxigenin was dissolved in a mixture of 8 cc. of 0.1 N NaOH and 8 cc. of alcohol and left 24 hours at room temperature. The solution no longer gave a nitroprusside reaction. When titrated back 2.33 cc. of 0.1 N NaOH had been consumed. Calculated 2.57 cc. A carefully neutralized solution of 0.2 gm. of hydroxylamine hydrochloride was added to the mixture and with a drop of dilute alkali the whole made faintly pink to phenolphthalein. In the course of several days the color deepened as if alkali had been set free due to relactonization. Long prismatic needles then separated from the solution which were neutral and nitrogen-free and gave a strong nitroprusside test. The melting point was 246–248° and the substance recovered was therefore digitoxigenin. The mother liquor on acidification with acetic acid and concentration at room temperature gave a second crop of neutral crystals which also proved to be somewhat less pure starting material.
Cardiac Aglucones

Anhydrostrophanthin Derivatives.

Oxime of the Ethylal of Oxidoanhydrostrophanthinic Acid.—0.5 gm. of the ethylal of oxidoanhydrostrophanthinic acid was suspended in 5 cc. of 50 per cent alcohol and dissolved by careful addition of dilute sodium hydroxide solution. A concentrated solution of 0.3 gm. of hydroxylamine hydrochloride which was made alkaline to phenolphthalein was added and the mixture was left overnight at 20°. When acidified with acetic acid the oxime separated rapidly. Recrystallized from dilute alcohol it formed long thin lustrous leaflets which melted at 153–155°.

Air-Dried Substance. Dried at 100° and 15 mm. over H₂SO₄.

Anhydrous Substance.
C₃₅H₃₇O₆N. Calculated. C 67.07, H 8.34, N 3.13, O 10.06.

Oxime of the Tetrahydrodianhydrolactone Acid.—The tetrahydrodilactone was saponified as previously described and carefully neutralized to phenolphthalein. On concentration in vacuo a lustrous crystalline deposit separated which was collected and proved to be a sodium salt. This was dissolved in alcohol diluted with an equal volume of water and acidified with acetic acid. The crystalline acid which immediately separated was collected with 25 per cent alcohol and was found to melt at about 200° after preliminary softening. However when this was dissolved in hot acetic acid and diluted carefully it crystallized again in a form which melted at 262° although with another sample recrystallized in a similar manner the melting point 275–276° was obtained.

0.3 gm. of this acid was suspended in 10 cc. of 50 per cent alcohol and brought into solution with a slight excess of alkali. To this an alkaline solution of 0.2 gm. of hydroxylamine hydrochloride was added and the mixture was left at 20° for 24 hours. On acidification with acetic acid the crystalline oxime slowly separated. Recrystallized from dilute alcohol it melted at 248–249°.

Bromine Titrations.

For the double bond titrations we have employed the method of Winkler. The results obtained with the substances examined were as follows:

0.2104 gm. of strophanthidin used 0.10 cc. of 0.1 N KBrO₃ or a negligible amount.
0.1113 gm. of ethylal of oxidodianhydrostrophanthidin used 12.13 cc. of 0.1 N KBrO₃. Calculated for 2Δs, 11.22 cc.
0.1011 gm. of dianhydrostrophanthidin used 10.81 cc. of 0.1 N KBrO₃. Calculated for 2Δs, 10.98 cc.
0.0696 gm. of tetrahydrodianhydrodilactone, C₂₃H₃₀O₄, used no KBrO₃ solution.
0.1187 gm. of gitoxigenin used 0.62 cc. of 0.1 N KBrO₃ or a negligible amount.
0.1111 gm. of dianhydrogitoxigenin (digitaligenin) used 11.63 cc. of 0.1 N KBrO₃. Calculated for 2Δs, 12.08 cc.
0.0510 gm. of digitoxigenin used no KBrO₃ solution.
0.1219 gm. of ouabain used no KBrO₃ solution.

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