THE STROPHANTHINS OF STROPHANTHUS EMINII

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In accordance with a plan to investigate as fully as possible the essential chemistry of the strophanthins and other cardiac glucosides, we have attempted to include for comparative purposes, as opportunity presented itself, a study of the digitaloid glucosides and aglucones which occur in the different species of Strophanthus plants. Our own and previous investigations have had to do principally with the glucosides of Strophanthus kombe, Strophanthus hispidus, Strophanthus gratus, and Strophanthus sarmentosus. And now a further opportunity has been offered to include a study from this standpoint of the seeds of Strophanthus eminii. Through the very generous cooperation of the Director of Agriculture of Tanganyika Territory, arrangements were made for the collection by field officers of his department of seeds of this Strophanthus species. 20 pounds of the cleaned seeds were procured in September, 1929, in the Shinyanga and Kahama areas of this territory. They were found by us to give uniformly a rose color with sulfuric acid. An earlier sample of such seeds collected in 1925 was stated by the Director to have been identified at the Royal Botanical Gardens, Kew, England, as being those of Strophanthus eminii.

As far as previous literature on the glucosides of Strophanthus eminii is concerned, the only reference which we have been able to find is the brief statement of Thoms¹ that the strophanthin of Strophanthus eminii is different from any strophanthins known at that time.

During the present study, we have found the glucoside mixture to be of complex character. It consists of easily hydrolyzable glucosides and of more stable glucosides. The former, which were

¹ Thoms, H., Ber. pharm. Ges., 14, 114 (1904).
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not isolated in crystalline form, are glucosides of an α-desoxy sugar because of the typical Keller-Kiliani reaction given by the crude mixture. At first the aglucone obtained on hydrolysis of the labile glucosides gave analytical figures which suggested a formula C_{23}H_{34}O_{6}. But on attempting to make certain derivatives, we found it to be a mixture of strophanthidin, the aglucone of _Strophanthus kombe_ and _Strophanthus hispidus_, and of periplogenin, which has heretofore been found only as the aglucone of the glucosides of _Periploca graeca_.2 On repeated recrystallization of the mixed aglucones, strophanthidin itself was finally obtained from the mixture. The presence of periplogenin, however, was shown by the isolation of derivatives such as dihydroperiplogenin and dihydroperiplogenin benzoate.

The more stable glucosides were isolated as a chloroform-soluble monoside, C_{30}H_{46}O_{9}, and a bioside, C_{36}H_{58}O_{14}, which was sparingly soluble in chloroform. Both of these glucosides proved to be derivatives of the same aglucone, C_{23}H_{34}O_{6}. The latter could not be isolated as such, because of the severe hydrolytic conditions needed for cleavage of the glucosidic linkage. By a special procedure in which methyl alcoholic hydrochloric acid was used, a trianhydro derivative, C_{23}H_{28}O_{2}, was obtained which, while resembling it closely, proved to be isomeric with trianhydroperiplogenin produced by similar treatment of periplogenin itself. Although this would indicate that the previous aglucone is not periplogenin but a closely related isomer, it is still possible that periplogenin in stable glucosidic union could give such an isomeric trianhydro derivative owing to an altered course of dehydration. However, the toxicity of both the monoside and bioside (0.5 mg. failed to kill a 33 gm. frog) was far less than that of periplocymarin. It so happened that the new glucosides were first obtained from seeds which were already practically 3 years old. This fact, together with the low toxicity of these glucosides, gives rise to the suspicion that a certain amount of allomerization, such as had already been shown in the case of _Strophanthus kombe_ seeds (allocyrmarin),3 may have occurred during this lapse of time and that the monoside and bioside may therefore be glucosides of an alloperiplogenin.

An attempt will be made to determine this point definitely by an early investigation of fresh seeds which are expected this autumn. In the above monoside, the aglucone appears to be conjugated with a methyl ether sugar, \( C_7H_{14}O_5 \), which is either identical or isomeric with digitalose, the sugar of \textit{digitalinum verum}. In the bioside there is an additional hexose, possibly glucose, attached to this sugar. Owing to the limited amount of material at our disposal, we have been forced to leave some of these points incomplete for the moment.

\section*{EXPERIMENTAL}

\textit{Isolation of the Glucosides and Aglucones—}2900 gm. of the seeds were finely ground and defatted with petroleum ether. The defatted seeds, which weighed 2200 gm., were thoroughly extracted with 95 per cent alcohol. The alcoholic extract was precipitated with basic lead acetate solution, and the excess lead was removed from the filtrate with hydrogen sulfide. After concentration to a syrup, the residue was taken up in 3 liters of water and the solution was extracted three times with chloroform. The extract, after concentration to about 100 cc. and dilution with petroleum ether, yielded a resinous precipitate which weighed about 15 gm. when dried. This material was then digested for about 15 minutes with boiling water. The aqueous extract was filtered hot from the water-insoluble resins. On cooling, about 0.5 gm. of the crude monoside crystallized out. The resinous residue was digested again with the mother liquor from this crystalline material. Concentration of the filtered solution gave 0.2 gm. more of crude monoside.

The aqueous solution, which remained after the above chloroform extraction, was freed from chloroform and its reaction was made barely acid to Congo red with hydrochloric acid. Then sufficient hydrochloric acid was added to make its effective concentration 0.15 per cent. The solution was warmed to 75° and held at this temperature for 1 hour. After about 15 minutes, the aglucone mixture of strophanthidin and periplogenin began to separate, sometimes in the form of mixed crystals and sometimes as a thick oil which crystallized on standing. When the hydrolysis was complete, the solution was cooled and allowed to stand for several weeks in order to complete crystallization. The precipi-
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tate was then collected with water. About 20 gm. of crude mixed genins were thus obtained. The mother liquor was extracted three times with chloroform. The dried and concentrated chloroform extract, on dilution with petroleum ether, yielded about 3 gm. of an amorphous material which, on recrystallization from ethyl alcohol, proved also to be a mixture of strophanthin and periplogenin.

The aqueous mother liquor from the genin mixture was neutralized to Congo red with sodium acetate, and was then concentrated under diminished pressure to a volume of about 400 cc. Octyl alcohol was used to check persistent foaming. The concentrated solution was salted out with ammonium sulfate. The supernatant liquid was decanted from the gummy precipitate which was kneaded with a rod in order to free it from as much included mother liquor as possible. The mass was then dissolved in 1 liter of absolute alcohol. The filtrate from undissolved ammonium sulfate was concentrated under diminished pressure to a thick syrup. This was in turn taken up in about 300 cc. of water. A saturated solution of ammonium sulfate was then cautiously added to the solution until the curdy precipitate first formed barely redissolved. The solution was then seeded. After a week or so, the bioside very slowly crystallized. The suspension was allowed to stand at least a week more to insure complete crystallization. The crystals were best collected by centrifuging. The crude product was suspended in a small quantity of water and again centrifuged. After mixing again with water, it was readily collected on a filter. The crude bioside weighed 2.0 gm.

The mother liquor remaining after the above isolation of the active constituents was still quite rich in glucosides, but all attempts to isolate them in crystalline form have until now been unsuccessful.

The Monoside, C$_{50}$H$_{45}$O$_{9}$—The above monoside could be recrystallized only with difficulty, its behavior suggesting the presence of more than one component. After four recrystallizations from dilute ethyl alcohol, the substance was obtained as rosettes of fine needles which melted to a frothy mass at 174–180°, after preliminary sintering. It gave a positive Legal reaction. The Keller-Kiliani test for $\alpha$-desose was practically negative. In concentrated sulfuric acid, the substance gave a yellow-brown
solution which passed through a yellow-green to a dull blue-green.

\[ \alpha^\circ = +22^\circ \ (c = 0.995 \text{ in 95 per cent alcohol}) \]

4.045 mg. substance: 3.070 mg. H₂O, 9.725 mg. CO₂

4.034 " " : 2.990 " " 9.770 " "

3.775 " " : 1.210 " AgI

C₃₀H₄₆O₉. Calculated. C 65.41, H 8.42, OCH₃ 5.63

Found. " 65.59, " 8.49 " 66.07, " 8.29 " 6.03

The analysis of this glucoside shows it to be a derivative of an aglucone, C₂₃H₄₄O₉, and a methyl ether desoxy sugar, C₇H₁₄O₅. The latter is apparently not an \( \alpha \)-desoxy sugar, because of its resistance to the hydrolyzing action of mineral acids. Only a fraction of the glucoside was cleaved by a half hour’s treatment with 1 per cent hydrochloric acid in 40 per cent alcohol at 73°. 5 per cent hydrochloric acid in 50 per cent ethyl alcohol hydrolyzed the glucoside fairly rapidly in a half hour’s time, but the reaction product was not homogeneous and had poor physical properties. When treated by the following method, the sugar was cleaved and simultaneously 3 molecules of water were split out, with the formation of a trianhydroaglucone.

The Trianhydroaglucone, C₂₃H₂₈O₂, from the Monoside—0.3 gm. of the monoside was dissolved in 2 cc. of a 5 per cent solution of hydrochloric acid in absolute methyl alcohol. The solution was heated in a sealed tube at 100° for 30 minutes. On cooling, 8 mg. of a crystalline substance separated, which melted at 135–141°. After repeated recrystallization from alcohol, it formed lustrous platelets which melted at 154–156°. The melting point was not depressed when the sample was mixed with a similar substance obtained from the bioside.

The trianhydro derivative dissolves in concentrated sulfuric acid with an orange-brown color that turns through a dull red-purple to a dull violet-blue. It gives a positive nitroprusside reaction.

1.977 mg. substance: 1.540 mg. H₂O, 5.945 mg. CO₂

C₂₃H₂₈O₂. Calculated. C 82.09, H 8.39

Found. " 82.01, " 8.69
The Bioside, \(C_{36}H_{56}O_{14}\)—The crude bioside was recrystallized first by dilution of its alcoholic solution and finally from hot water. It formed large, irregular leaflets which melted not sharply at 195-200°, after preliminary sintering. The Legal test was positive. The Keller-Kiliani test was practically negative. The substance dissolved in concentrated sulfuric acid to give a yellow-brown solution, a little darker than that of the monoside, passing in a few hours time through a dull purple to a purple-black.

\([\alpha]_b^N = +8^\circ \ (c = 0.950 \text{ in 95 per cent alcohol})\)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Weight (mg)</th>
<th>Water (mg)</th>
<th>CO (mg)</th>
<th>AgI (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioside</td>
<td>3.182</td>
<td>2.725</td>
<td>8.420</td>
<td>1.530</td>
</tr>
<tr>
<td></td>
<td>4.246</td>
<td>3.000</td>
<td>9.390</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.548</td>
<td>1.530</td>
<td>9.390</td>
<td>1.530</td>
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\(C_{36}H_{56}O_{14}\). Calculated. C 60.64, H 7.92, OCH, 4.35

<table>
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<th>Found</th>
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<tr>
<td>C 60.24,</td>
<td>60.24</td>
</tr>
<tr>
<td>H 8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>OCH 4.44</td>
<td>4.44</td>
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This bioside, from the analysis, is a derivative of a methyl ether deoxy bioside, \(C_{13}H_{24}O_{10}\), and an aglucone, \(C_{23}H_{34}O_{6}\). As far as can be determined with the small quantity of material available, this aglucone is identical with that contained in the monoside. As in the case of the latter, only a trianhydrogenin could be obtained, owing to the resistance of the bioside to hydrolysis. A satisfactory cleavage was obtained by the use of the method successfully employed with the monoside, as follows:

The Trianhydroaglucone from the Bioside—0.5 gm. of the bioside was heated in 3 cc. of 5 per cent absolute methyl alcoholic hydrogen chloride at 100° for 30 minutes. 40 mg. of the trianhydro derivative crystallized out. Recrystallization from ethyl alcohol yielded irregular platelets which melted at 154-156°, and agreed in all properties with the substance obtained from the monoside.

\([\alpha]_b^N = -84.6^\circ \ (c = 1.015 \text{ in pyridine})\)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Weight (mg)</th>
<th>Water (mg)</th>
<th>CO (mg)</th>
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<tr>
<td>Bioside</td>
<td>4.290</td>
<td>3.248</td>
<td>12.882</td>
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\(C_{23}H_{34}O_{2}\). Calculated. C 82.09, H 8.39

<table>
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<tr>
<th>Calculated</th>
<th>Found</th>
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<tr>
<td>C 81.93,</td>
<td>81.93</td>
</tr>
<tr>
<td>H 8.47</td>
<td>8.47</td>
</tr>
</tbody>
</table>

This substance is definitely different from trianhydroperiplogenin, which will be described in a subsequent communication.

The Aglucone Fraction—This consisted roughly of equal amounts of strophanthidin and periplogenin. They appeared to form
mixed crystals, the fractionation of which proved to be difficult. 2 gm. of the crude mixture yielded, after seven recrystallizations from 95 per cent alcohol, 40 mg. of a substance which melted at 174–178° and appeared as the characteristic rhombs of strophanthinidin.

\[ \alpha^0 = +35.6^\circ \ (c = 1.005 \text{ in 95 per cent ethyl alcohol}) \]

3.897 mg. substance: 2.840 mg. H₂O, 9.620 mg. CO₂
\[ C_{23}H_{27}O_4 \cdot 0.5H_2O. \text{ Calculated. } C 66.79, H 8.05 \]
\[ \text{Found. } " 67.33, " 8.15 \]

The presence of strophanthinidin in the crude mixture was directly substantiated by the production of the methylal of dianhydrostrophanthinidin, as follows:

A solution of 0.2 gm. of mixed aglucones in 2 cc. of a 5 per cent solution of hydrogen chloride in absolute methyl alcohol was heated in a sealed tube at 100° for 15 minutes. On cooling, the crude product crystallized spontaneously. After two recrystallizations from methyl alcohol, 38 mg. of the methylal, melting at 240–244°, were obtained. An additional recrystallization sharpened the melting point to 242–244°, and the substance gave no depression when mixed with an authentic sample of the methylal of dianhydrostrophanthinidin.*

\[ \alpha^0 = -124.7^\circ \ (c = 1.000 \text{ in chloroform}) \]

4.132 mg. substance: 2.905 mg. H₂O, 11.400 mg. CO₂
\[ C_{24}H_{26}O_4. \text{ Calculated. } C 75.35, H 7.91, \text{OCH}_3 8.12 \]
\[ \text{Found. } " 75.23, " 7.85 \]


Hydrogenation of the Mixed Aglucones—A solution of 0.4 gm. of the mixed aglucones in 40 cc. of ethyl alcohol was hydrogenated in the presence of 0.2 gm. of the platinum oxide catalyst of Adams and Shriner. After reduction of the catalyst, approximately 1 mol of hydrogen was absorbed in about 40 minutes. Absorption continued after this, but too slowly to make the measurement reliable. At the end of 24 hours, the solution was filtered from the catalyst and concentrated to a syrup. When treated with ether, a portion of the product crystallized. This fraction, after repeated
recrystallization from boiling methyl alcohol, melted constantly at 170–173°. A mixed melting point with a sample of dihydrostrophanthidol showed no depression. The melting point of dihydrostrophanthidol, when recrystallized from dilute methyl alcohol, has been reported in a previous communication as 160–163°. It has been found more recently that after recrystallization from a small volume of methyl alcohol it melts at 172–175°. Shortly after, it resolidifies and melts again at about 195°.

In all other properties, the new substance proved to be identical with dihydrostrophanthidol. \([\alpha]_b^{20} = +35.0° (c = 1.030 \text{ in } 95 \text{ per cent ethyl alcohol})\).

An authentic sample of dihydrostrophanthidol, recrystallized from methyl alcohol, showed a comparable rotation.

\([\alpha]_b^{20} = +31.8° (c = 0.980 \text{ in } 95 \text{ per cent ethyl alcohol})\)

4.577 mg. substance: 3.620 mg. H₂O, 11.420 mg. CO₂

C₂₃H₃₈O₁₁. Calculated. C 70.35, H 9.25

Found. “ 70.39, “ 9.40

That portion of the crude hydrogenation product which remained soluble in ether was freed from solvent and dissolved in cold methyl alcohol. A small amount of dihydrostrophanthidol, insoluble under these conditions, was filtered off. The filtrate was brought to crystallization by the cautious addition of water. The crude product weighed 180 mg. After several recrystallizations, a substance which melted at 201–203° was obtained. This substance proved to be dihydroperiplogenin. A mixed melting point with dihydroperiplogenin showed no depression. In concentrated sulfuric acid, the substance gave a clear light orange color finally changing to a bright blue identical with that given by an authentic specimen of dihydroperiplogenin. The identity of the substance was confirmed by other properties. \([\alpha]_b^{27} = +24.8° (c = 1.01 \text{ in } 95 \text{ per cent alcohol})\).

Authentic dihydroperiplogenin gave a rotation of \([\alpha]_b^{25} = +25° (c = 1.000 \text{ in } 95 \text{ per cent alcohol})\).

4.390 mg. substance: 3.690 mg. H₂O, 11.330 mg. CO₂

C₂₃H₃₈O₁₁. Calculated. C 70.35, H 9.25

Found. “ 70.39, “ 9.40

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In the course of checking the identity of the ether-soluble hydrogenation product, its benzoyl derivative was also prepared. Benzylation was accomplished by the usual procedure with benzoyl chloride in pyridine solution. A monobenzoate was formed, which after recrystallization from methyl alcohol, melted at 214–216°.

\[
[\alpha]_{D}^{20} = +46^\circ \text{ (c = 1.000 in pyridine)}
\]

4.210 mg. substance: 3.060 mg. H₂O, 11.237 mg. CO₂
C₂₀H₄₈O₆. Calculated. C 72.54, H 8.12

Benzoyldihydroperiplogenin similarly prepared from authentic dihydroperiplogenin also melted at 214–216°.

\[
[\alpha]_{D}^{20} = +47.1^\circ \text{ (c = 1.015 in pyridine)}
\]

4.387 mg. substance: 3.230 mg. H₂O, 11.665 mg. CO₂
C₂₉H₄₆O₆. Calculated. C 72.54, H 8.12
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