ON THEVETIN

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The constituents of be-still nuts, the fruit of the plant Thevetia neriifolia, have recently been critically examined by Chen and Chen.1 They succeeded in isolating three crystalline substances: viz., thevetin, to which the formula \( C_{29}H_{46}O_{13} \) was given; ahouain, of the formula \( C_{18}H_{19}O_{10} \); and kokilphin, \( C_{32}H_{61}O_{30} \). Of these, thevetin was shown to possess the pharmacodynamic properties typical of the digitalis group of cardiac poisons.2 This relationship was further strengthened by the observation that thevetin gave the positive nitroprusside (Legal) reaction characteristic of the members of the digitalis-strophanthus group.

For the historical background, reference should be made to Chen and Chen's paper.1 Since its appearance, Ghatak3 has reiterated his opinion that thevetin can best be represented by the formula \( C_{29}H_{39}O_{6} \), and that on hydrolysis, glucose and a genin, \( C_{14}H_{20}O_{5} \), result.

Through the kindness of Dr. K. K. Chen of The Lilly Research Laboratories, Indianapolis, who has kindly supplied generous amounts of crystalline thevetin, it has become possible to characterize the glycoside more fully and to demonstrate more conclusively its intimate relationship to the other members of the digitalis group.

The sugar component of thevetin has been shown, after hydrolytic cleavage, to be glucose by its isolation as glucosazone and by the failure to obtain mannose phenylhydrazone. The aglycone

portion of the molecule would then possess the formula $C_{23}H_{36}O_8$ if the formulation for thevetin adopted by Chen and Chen be accepted. However, if, as is indicated by the positive nitroprusside reaction, the characteristic $\Delta^\beta,\gamma$-unsaturated lactone side chain is present, the formula for the aglycone, thevetogenin, should be revised to $C_{23}H_{44}O_8$ and that for thevetin would become $C_{23}H_{36}O_8$. Thevetogenin would thus be isomeric with ouabagenin, the hypothetical aglycone of ouabain.\(^4\) This revision is supported by the fact that thevetin on catalytic hydrogenation absorbs 1 mole of hydrogen, although it has not been possible to obtain dihydrothevetin in crystalline form, and by the formation of isothevetin when thevetin is subjected to the isomerizing action of alkali. These reactions again are characteristic of the unsaturated lactone side chain of the cardiac aglycones.

Attention was next directed to an examination of the aglycone. Because of the presence of the normal hexose, glucose, rather than a 2-desoxy sugar, hydrolysis of thevetin itself required sufficiently strenuous treatment to destroy the aglycone. However, after isomerization to isothevetin, it has been possible to obtain crystalline derivatives of thevetogenin. Manipulation was rendered exceedingly difficult by the tendency of the derivatives to crystallize in a highly hydrated state and frequently only in the presence of electrolytes which prevented their isolation in a state of purity.

When acetolysis was attempted with isothevetin by the procedure which had proved successful with isoouabain,\(^5\) only non-crystalline resins resulted. However, when isothevetin was carefully warmed with 2 per cent HCl, a less soluble crystalline substance separated from the solution. The analytical data indicated a monohydrate of isothevetogenin, but manipulation was made difficult by its unfortunate physical properties.

Better success attended the hydrolysis of the glycoside after oxidation with hypobromite. When isothevetin was oxidized in this manner after saponification of the lactone group, an acid, isothevetinic acid, was formed. This readily crystallized in the presence of electrolytes, such as NaCl, but when attempts were made to wash it free from salts, it assumed gelatinous properties. However, gentle acid hydrolysis of the crude isothevetinic acid

resulted in the formation of crystalline isothervetogenic acid which was brought to a state of analytical purity, although with difficulty. Attempts to prepare a methyl- or p-bromophenacyl ester of this acid resulted in gelatinous products. Benzoylation of the amorphous methyl ester led to an amorphous benzoate and acetylation gave an acetate which crystallized when strongly chilled, but melted at room temperature during manipulation. Likewise, attempts to characterize the hydroxyl groups by oxidation to carbonyl resulted in non-crystalline products.

These results confirm the placing of thevetin in the digitalis-strophanthus group of cardiac aglycones. The sugar has been shown to be glucose and the aglycone, while not isolated as such, has been shown to be isomeric with ouabagenin and exhibits the typical reactions of the other members of the group. While it is not possible, with the information at hand, to place the hydroxyl groups of thevetogenin definitely, analogy with the other members of the group warrants the assumption of the presence of a secondary hydroxyl group on carbon atom (3) and of a tertiary hydroxyl group on carbon atom (14).

**EXPERIMENTAL.**

Thevetin—The glucoside as received was recrystallized two times from 85 per cent isopropyl alcohol. It softened at 193–194° and slowly went to bubbles up to 210°. For analysis it was dried at 80° and 0.1 mm.

\[ \text{C}_{27} \text{H}_{36} \text{O}_{14} \]

Calculated. C 57.96, H 7.40

Found. " 57.90, 57.69, " 7.59, 7.46

Isothevetin—1 gm. of thevetin was dissolved in 10 cc. of absolute methyl alcohol and the solution chilled in ice. 10 cc. of a 20 per cent solution of potassium hydroxide in absolute methyl alcohol were added and the mixture was allowed to warm up to room temperature. After 30 minutes, the Legal test was negative. 100 cc. of water were added and the solution was made just acid to Congo red with HCl. After standing 4 hours at room temperature to complete lactonization, the solution was neutralized to Congo red with sodium acetate and concentrated in vacuo to copious crystallization. The crystalline material was centrifuged and recrystallized twice from water. Isothevetin as thus obtained
forms micaceous leaflets which were highly hydrated and extremely difficult to manipulate. For analysis it was recrystallized from isopropyl alcohol, from which it separates as microleaflets. After drying in a desiccator over P₂O₅, the substance still retained 0.5 mole of solvent. It shrinks at 180° and goes to a mass of fine bubbles at 200–210°.

\[ [\alpha]_D^{\text{H}} = -60° \ (c = 0.366 \text{ in pyridine}) \]

For analysis the substance was dried at 100° and 0.2 mm.

\[
\text{C}_{12}\text{H}_{20}\text{O}_{13}. \quad \text{Calculated.} \quad \text{C 57.96,} \quad \text{H 7.40} \\
\text{Found.} \quad " \text{57.90, 58.05,} \quad " \text{7.63, 7.65}
\]

12.130 mg. of substance were refluxed with 1 cc. of alcohol and 3 cc. of 0.1 N NaOH for 4 hours and titrated back against phenolphthalein with 0.1 N HCl. 0.214 cc. of 0.1 N NaOH was consumed. Calculated for 1 equivalent, 0.202 cc.

Isothevetogenin—130 mg. of isothevetin were heated in 10 cc. of 2 per cent HCl on the steam bath for 5 minutes. During this time the isothevetin dissolved and was replaced by lens-shaped crystals of the isogenin. These were filtered from the cooled solution and recrystallized from dilute methyl alcohol. On drying in a desiccator, the crystals shrank and effervesced to a varnish. In this form it sintered at about 170° and decomposed at 220°, although these temperatures varied greatly with the rate of heating. The Molisch test was negative. For analysis it was further dried at 100° and 0.2 mm. over H₂SO₄. The figures obtained indicated that it still retained 1 mole of H₂O.

\[
\text{C}_{12}\text{H}_{20}\text{O}_{13} \cdot \text{H}_{2}\text{O}. \quad \text{Calculated.} \quad \text{C 66.49,} \quad \text{H 7.95} \\
\text{Found.} \quad " \text{60.33, 60.96,} \quad " \text{7.56, 7.77}
\]

Isothevetinic Acid—1 gm. of isothevetin was dissolved in 25 cc. of water containing 2.5 cc. of 10 per cent NaOH solution. 50 cc. of a solution of NaOBr (prepared from 50 cc. of N NaOH and 3.3 gm. of Br) were added. After standing 1 hour at room temperature, the alkaline solution was extracted with ether in order to remove the CBr₄ formed by oxidation of the isopropyl alcohol of crystallization of the isothevetin. The solution was then made acid to litmus with acetic acid and evaporated to about 15 cc. in a desiccator. It was then acidified to Congo red
with HCl. After 2 hours the fine prisms which separated were centrifuged off. The substance is very highly hydrated and crystallized only from diluted solvents in the presence of inorganic electrolytes. When attempts were made to wash it free of salts, the substance became gelatinous as the salts were removed. In the crystalline state it adsorbed too large an amount of salts to permit accurate analytical results. However, after removal of the sugar the resulting acid was purified, although with difficulty.

 Isothevetogenic Acid—The alkaline solution from the oxidation of 1 gm. of isothevetin, as described above, was made faintly but distinctly acid to Congo red with HCl and then heated in a beaker of actively boiling water for 10 minutes. During this time a turbidity formed and fine needles separated. The solution was cooled as rapidly as possible and the crystals were centrifuged. The substance appeared to crystallize only from aqueous solvents, best from dilute acetone. Addition of a few drops of acetic acid was found to facilitate crystallization. The crystals were apparently highly hydrated and very difficult to filter. Therefore, they were centrifuged and dried as such in a desiccator, during which large amounts of water were lost and the substance dried down to a resin. In this state it froths up at 120-130° and slowly decomposes.

\[ \alpha \] = -47° (c = 1.075 in pyridine)

For analysis it was further dried at 100° and 20 mm.

\[
\begin{align*}
C_{10}H_{18}O_6. & \quad \text{Calculated. C 60.76, H 7.54} \\
& \quad \text{Found. C 60.29, 60.48, H 7.74, 7.86}
\end{align*}
\]

The original acid mother liquor from the above was again heated at 100° for 10 minutes and an additional amount of isothevetogenic acid obtained. Further hydrolysis resulted in the formation of obscure dehydration products.

 Isolation of Glucosazone from Thevetin—0.5 gm. of thevetin was heated on the steam bath with 30 cc. of 3 per cent H₂SO₄ for 5 hours. The mixture was chilled and filtered from resinous hydrolysis products. Excess BaCO₃ was then added and the solution was again filtered. 15 cc. of 50 per cent NaHSO₃ solution, 15 cc. of a saturated solution of sodium acetate in 50 per cent acetic acid, and 1.5 cc. of phenylhydrazine were added to the
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filtrate, and the mixture was heated 1 hour on the steam bath. After cooling, the osazone was collected and recrystallized from methyl alcohol. It decomposed at 207° and showed no depression when mixed with glucosazone.

A 1 per cent solution of the osazone in 2:3 pyridine-alcohol solution at first showed \( \alpha' = -0.55° \) in a 1 dm. tube. After 24 hours, \( \alpha' = -0.35° \). This is in accord with the observed mutarotation of glucosazone.\(^6\)

\[
\text{C}_{18}\text{H}_{22}\text{O}_6\text{N}_4. \quad \text{Calculated. C 66.36, H 6.19} \\
\text{Found. C 66.22, H 6.31, H 6.31, 6.41}
\]

In another experiment, the sugar solution from the hydrolysis of 100 mg. of thevetin was similarly treated with phenylhydrazine and allowed to stand at room temperature. No mannose phenylhydrazone was formed.

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