ON CRYSTALLINE KOMBE'-STROPHANTHIN.

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(Concluded from page 724)

The results with crystalline Kombe strophanthin on the heart of frogs are seen in Table III:

TABLE III. Focke Method.

9/23/10 Crystalline Kombe strophanthin.

Dose per gm. of frog.	0.000001 gm.	0.0000015 gm.	0.000002 gm.
Intervals in minutes until ventricles stopped beating.	11 Min. 14 " 13 " 16 " 10 "	13 Min. 14 " 12 " 12 " 10 "	9 Min. 10 " 11 " 10 " 11 "
Average	13 Min.	12 Min.	10 Min.

9/27/10

Dose per gm of frog.	0.000001 gm.	0.000002 gm.	0.000004 gm.
Intervals in minutes.	19 Min. 18 " 15 " 9 " 10 "	16 Min. 13 " 10 " 15 "	10 Min. 10 " 8 " 6 " 10 "
Average	16 Min.	13.5 Min.	9 Min.

The lack of uniformity in the above table is not surprising to anyone who has observed the gradual and erratic cessation of the ventricle beat. The personal factor largely enters on account of their being no sharp end point. After the ventricle has stopped beating, a slight jar or noise will often induce muscular movements of the frog sufficient to force blood into the ventricle, when it will start up and beat again for some time.

We found the method very unsatisfactory and agree with Edmunds and Hale[38] when they say, "We believe that this method allows of greater variations and inaccuracies than any other method we employed."

If the frogs are examined at the end of one hour for complete stoppage of the heart in systole, a more uniform result is obtained, as appears from Table IV:

TABLE IV.

Crystalline Kombe strophanthin (fr. identified seed.)

Dose per gm. of frog.	0.0000011 gm.	0.0000012 gm.	0.0000013 gm.
Conditions of heart at 1 hour.	3 beating 0 stopped	6 beating 1 stopped	0 beating 7 stopped

We believe any physiologic method for the assay of heart tonics should have as a basis some definite standard, e. g., Tr. Strophanthus, or better, crystalline strophanthin. The sharper and more definite the end point the more exactly can physiological reactions be compared.

[38] Bulletin 48, Hygienic Laboratory, p. 45.

Protocol of the toxicity tests of November 1, 1910:

TABLE V.

Cry. K. strophanthin.				Amor. acid strophanthin from cry. K. strophanthin.				Standard Average Tincture.			
	Dilution 1 in 50,000				Dilution 1 in 12,500				Dilution 1 in 500		
Wgt. frog.	Dose in gm. per gm.	Full dose	Result.	Wgt. frog.	Dose in gm. per gm.	Full dose	Result.	Wgt. frog.	Dose in cc. per gm.	Full dose	Result.
13.5	.00000090	.605 cc.	L.	21.5	.0000026	.70 cc.	L.	16.5	.000066	.545 cc.	L.
14.0	.00000090	.63 cc.	L.	17.0	.0000028	.895 cc.	L.	16.5	.000066	.64 cc.	L.
16.0	.00000090	.72 cc.	L.	17.0	.0000028	.595 cc.	D.	17.5	.000069	.60 cc.	L.
14.0	.00000095	.665 cc.	D.	17.5	.000003	.655 cc.	D.	20.5	.000069	.70 cc.	D.
14.0	.00000095	.665 cc.	D.	17.5	.000003	.655 cc.	D.	17.5	.000072	.65 cc.	D.
14.5	.00000095	.69 cc.	D.	18.0	.000003	.675 cc.	D.	20.5	.000072	.74 cc.	D.

In the case of warm blooded animals the toxicity cannot logically be translated into heart action, since death is with them generally due to failure of respiration.

The toxicity for small warm blooded animals, when injected subcutaneously, is given in Table VI:

TABLE VI.

Animals used.	Crystalline Kombe strophanthin. Lethal dose per gram of animal.	Amorphous acid Kombe strophanthin. Lethal dose per gram of animal.	Standard Tr. K. Strophanthus. Lethal dose per gram of animal.
Guinea-pigs	.0000004 gm.	.0000010 gm.	.00095 cc.
Tame mice.....	.000009 gm.	.000025 gm.	.0005 cc.
Tame rats.....	.00006 gm.	.00005 gm.	.....
Wild rats.....	.000009 gm.	.00008 gm.	.0008 cc.

The guinea-pigs were invariably killed by failure of respiration and the heart was found to be in diastole. The rats and mice generally showed a failure of respiration before the heart action ceased, and the heart stopped in moderate systole.

Crystalline Kombe strophanthin, in aqueous solution, is changed into an amorphous acid strophanthin when boiled. As the toxicity of this amorphous acid strophanthin is only one-third that of the crystalline glucoside, it seemed important to obtain exact figures of this change.

A solution of crystalline Kombe strophanthin in pure water (1 in 2000) was vigorously boiled over a free flame for fifteen minutes, cooled and made up to volume. This tested against the unboiled portion showed a loss of about 15%.

A solution of gratus strophanthin (Ouabain Merck) (1-25,000) in pure water was vigorously boiled over a free flame for fifteen minutes, cooled and made up to volume. This tested against the unboiled portion showed about 10% loss.

As such a change is to be expected in the cold, although, of course, much more slowly, it is important to determine the keeping qualities under different conditions.

A water solution (1 in 2000) of this crystalline Kombe strophanthin was made up and kept in a cork-stoppered flask at room temperature. At the end of two weeks a beautiful growth of mould was present, and an assay showed it to have lost 50 percent of its activity.

Crystalline Kombe strophanthin and Kombe strophanthin (Merck) were then made up (1 to 200) with water containing 4/10% Trikresol and kept at room temperature in sealed containers; no loss appeared with the crystalline glucoside in three months. However, the amorphous preparation of Merck showed a loss of nearly 10 percent within the limits of error.

It was noted that a small amount of alcohol prevented the conversion into the amorphous acid strophanthin, and was taken advantage of in concentrating solutions.

We also found that at room temperature 10 percent alcohol preserves the activity of crystalline Kombe strophanthin.

The marked permanency of the tincture of Strophanthus has long been recognized. It seemed desirable, therefore, to examine this crystalline glucoside from identified Kombe seed in this respect.

Solutions of crystalline Kombe strophanthin (1 in 1000) were made up from time to time with 70% alcohol (and 2 cc. portions sealed in glass containers) and tested on frogs against the old solutions, with the results given in Table VII:

TABLE VII.

Permanency of crystalline Kombe strophanthin in 70% Ethyl Alcohol, kept at room temperature in sealed containers.

Date of Tests	Dose in gm. per gm.	Lived	Died	
March 13 and 15, 1911...	.000,001,0	3	1	
	.000,001,1	2	1	Solutions of June 27, 1910.
	.000.001,2*	0	3	
	.000,001,0	4	0	
	.000,001,1	2	1	Solution freshly made from crystals.
	.000,001,2*	0	3	
October 28	.000,000,70	4	0	
November 2	.000,000,75	6	2	Computed from standard average tincture.
	.000,000,80	4	3	
	.000,000,85*	2	10	
	.000,000,70	2	0	
	.000,000,75	5	2	Solution freshly made from crystals.
	.000,000,80	1	5	
	.000,000,85*	0	4	
1912	.000,000,80	2	0	
July 24	.000,000,85*	0	2	Solution of June 27, 1910.
	.000,000,90	0	2	
July 25.....	.000,000,80	2	0	
	.000,000,85*	0	2	Solution of Sept. 25, 1911.
	.000,000,90	0	2	
July 26	.000,000,80	3	0	
	.000,000,85*	1	2	Solution freshly made up.
	.000,000,90	0	2	
December 20	.000,000,65	3	1	
	.000,000,70*	1	2	Solution of Sept. 25, 1911.
	.000,000,75	0	3	
December 20	.000,000,65	3	0	
	.000,000,70*	0	3	Freshly made up.
	.000,000,75	0	2	
December 27	.000,000,60	2	0	
	.000,000,65	2	2	Solution of June 27, 1910.
	.000,000,70*	0	5	
December 27	.000,000,60	2	0	Solution of December 20, 1912.
	.000,000,65*	1	3	
	.000,000,70	0	3	

The results of over two years show the really remarkable permanency of such a solution.

No systematic study of the heart action was undertaken. However, a few typical tracings showing the action upon the blood pressure and heart of dogs are given. Plate I shows the action of crystalline Kombe strophanthin, and II that of its amorphous acid modification. These are not given with any view to quan-

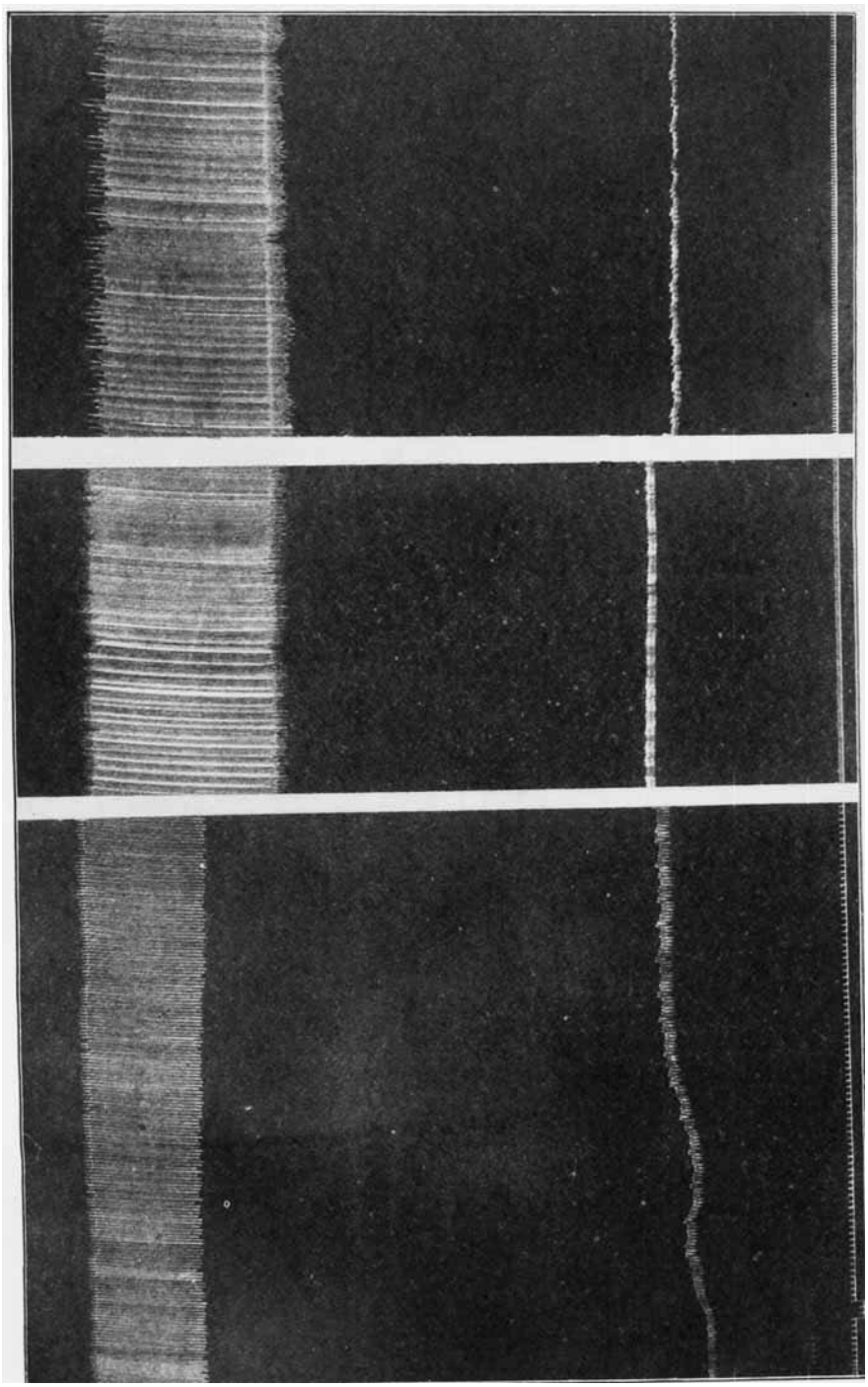

PLATE I.

Plate I shows the effect of the intravenous injection of 0.3 mg. of crystalline Kombe strophanthin on a 12 Kg. dog under chloretone anesthesia and artificial respiration.
Upper tracing is the ventricle beat by direct attachment to the apex and median groove of the heart. Down stroke is systole.
Middle tracing is the carotid blood pressure. Base line time in seconds and signal is at the bottom.
The second portion of the tracing is one-half hour later, and the third portion is one and one-half hour after the injection.

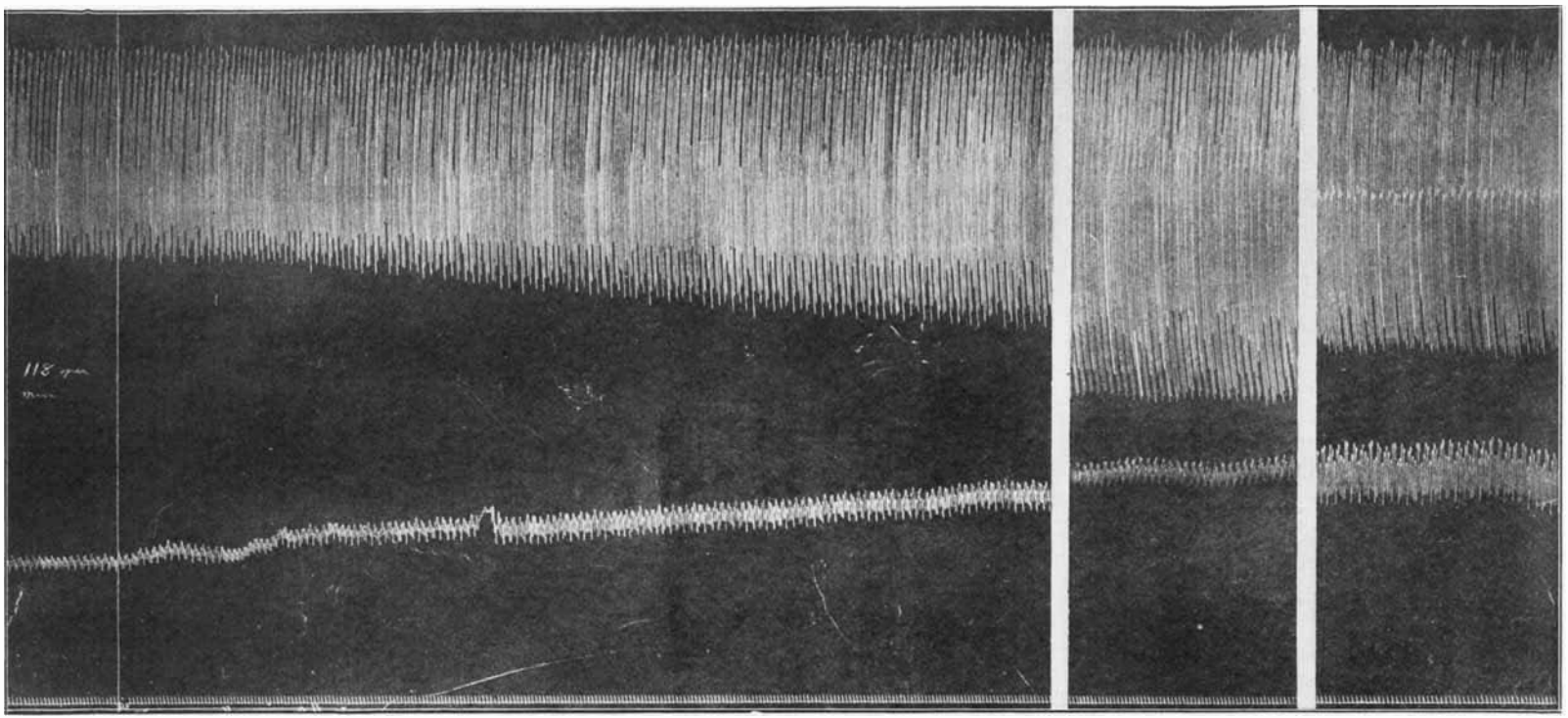

PLATE II.

Plate II shows the effect of the intravenous injection of 1 mg. of amorphous acid strophanthin (derived from crystalline Kombe strophanthin) on a 15 Kg. bitch under chloretone anesthesia and artificial respiration.
Upper tracing is the ventricle beat by direct attachment to the apex and median groove of the heart. Down stroke is systole.
Middle tracing is the carotid blood pressure.
Base line time in seconds and signal is at the bottom.
The second portion of the tracing is one-half hour later and the thirdportion is two and one-half hours after the injection.

titative comparison. Blood pressure effects and changes in heart action in warm-blooded animals give but an uncertain basis for comparing heart tonics on account of the long duration of the action, so that a subsequent injection may show a cumulative effect. Thus the response to a given preparation cannot be compared with that of a standard on the same animal, and on different animals the variation is too large for practical comparison.

Tests in connection with the preparation of crystalline Kombe strophanthin showed that strong alcohol precipitated from the tincture of Srophanthus, salts

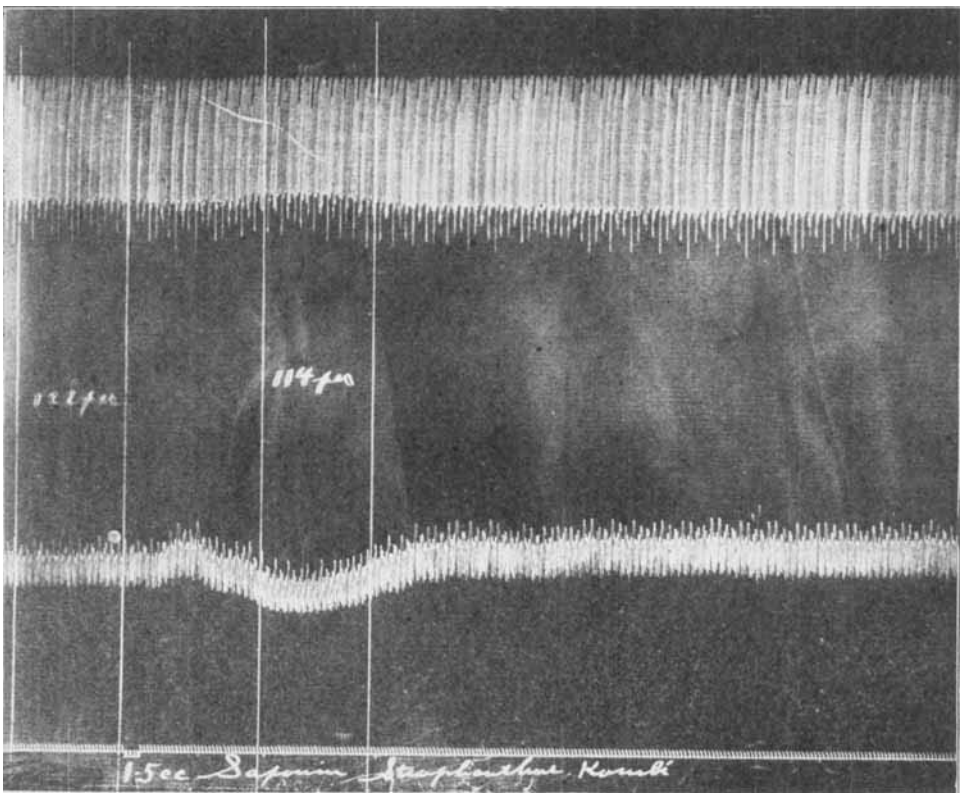

PLATE III.

Plate III shows the effect, on a 13 Kg. dog under chloretone anesthesia and artificial respiration, of the intravenous injection of 1.5 cc. of a solution (1 cc. of which contains 5 mg. of portion precipitated by strong alcohol from tincture of strophanthus and which shows a toxicity to frogs equivalent to .25 mg. per cc. of crystalline Kombe strophanthin.

Upper tracing is ventricle beat by direct attachment to apex and median groove of the heart. Down stroke is systole.

Middle tracing is the carotid blood pressure.

Base line, time in seconds and signal is at the bottom.

and saponin like bodies which carried down some substances very toxic to frogs and which generally produced a fall in blood pressure and weakened heart action. The dried precipitate killed frogs at .00002 gm. per gm., but in purifying by reprecipitation the toxicity and depressure action becomes less and less. A typical tracing of the effect on dogs is seen in Plate III.

The amount is so small in Strophanthus preparations that it is probably of no

therapeutic importance, although in preparations of other drugs[39] it may be of importance. Strophanthidin is 1/10 as toxic to frogs as the cryst. Kombe strophanthin from which it is derived, having an M. L. D. of 0.000009 gm. per gm. of frog when the standard killed at .0000009 gm. per gm. of frog.

The intravenous injection of strophanthidin has some difficulties on account of its slight solubility in water, but by quickly diluting a weak alcoholic solution a sufficiently fine suspension was obtained for injection. The effect upon the blood pressure and heart action is very similar to that of strophanthin.

DISCUSSION OF PHYSIOLOGICAL RESULTS.

In the hands of the authors, the 12-hour frog method of Houghton, Am. Journ. Pharmacy, Oct., 1909, gave the most accurate and reliable results, and for reasons previously stated seems the most logical one to use in standardizing the digitalis series of heart tonics. Equally logical is the method suggested (same reference) for giving tangible expression to the degree of activity of preparations of the series, namely, to use the M. L. D. of cryst. Kombe strophanthin as the basis for comparison; the ratio of the M. L. D. of each member of the series to that of strophanthin, thus determining the activity. Ten times this M. L. D. was chosen as the Heart Tonic Unit (see also Am. Journ. Pharmacy, March, 1912); therefore, the activity of any preparation is the reciprocal of ten times its M. L. D. adjusted to the average for that drug, by comparison with the M. L. D. of the standard (strophanthin). Whatever method is employed, however, it is necessary to compare the activity of an unknown preparation with that of a known or standard. This comparison can be expressed only in terms of a definite amount of the standard, and it is the logical procedure to speak of this quantity as a heart tonic unit (H. T. U.) What amount shall be adopted is unimportant. For our own work we have adopted the standard heart tonic unit (H. T. U.) as 0.00001 gm. of crystalline Kombe strophanthin as suggested above. This is ten times the minimum lethal dose for frogs (Rana Pipiens, 10 to 30 gm.)

The marked stability of alcoholic solutions of crystalline Kombe strophanthin is a very important point in connection with the use of this crystalline glucoside as a standard for determining the activity of galenical preparations, not only of strophanthus seed, but of other heart tonics.

The comparison of the physiological and chemical properties of crystalline and amorphous acid strophanthins shows that the molecular rearrangement brought about by the introduction of one molecule of water has markedly altered the physiological activity.

Other cases are known in which the addition of a molecule of water is accompanied by marked changes in physiological activity, as inactive ergotinin into active ergotoxin.

In the case of the conversion of crystalline Kombe strophanthin to the amorphous acid strophanthin there has been the change of a lactone group into

[39]In the case of ergot one of the bodies thrown down with strong alcohol is beta-imidazolethylamine (Histamine), which in this case is considered one of its therapeutically active principles.

Digitalis and very many other drugs show the presence of bodies with very similar properties and they become of importance when the therapeutically active bodies have poor solubility in water or weak alcohol, since infusions and such extracts may have quite different properties from those expected of the drug.

its acid and alcoholic portions, and accompanying this change a loss of two-thirds its toxicity to frogs.

The character of the blood pressure changes and heart action does not appear to be altered by the opening of one lacton ring formation, only a loss of activity is observed. It must be remarked that the loss of activity as observed with dogs was not as great as indicated by the toxicity tests on frogs and guinea-pigs, and also the toxicity to rats and mice show that the relation one to three is not true in all respects for the crystalline Kombe strophanthin and the amorphous acid Kombe strophanthin.

RESUME.

The seeds of Strophanthus Kombe Oliv. contain two strophanthins; a crystalline glucosid of the formula $C_{40}H_{56}O_{15}+3H_2O$ and a closely related amorphous strophanthin of apparently twice the molecular weight. By the action of water on crystalline Kombe Strophanthin there is formed a monobasic acid strophanthin or a mixture e. g. of a monobasic acid, a dibasic acid and the original crystalline strophanthin. These three strophanthins, crystalline, its acid derivative and amorphous Kombe Strophanthin, when split by dilute acids give strophanthidin of the formula $C_{27}H_{38}O_7+H_2O$. This strophanthidin is identical with the strophanthidin, described by Feist and by Heffter and Sachs.

Crystalline Kombe strophanthin contains neither a pentose nor a methyl pentose (rhamnose). Amorphous Kombe strophanthin apparently contains a pentose. The crystalline Kombe strophanthin prepared by Arnaud is doubtless identical with that prepared by us, but Arnaud was at fault in considering as a hydrate, a new chemical derivative, which we have spoken of as amorphous acid strophanthin.

The results of Kohn and Kulisch show a marked conformity with those of Arnaud and of our own upon amorphous acid strophanthin in everything except the data upon strophanthidin. It seems probable that the method of cleavage and purification accounts for the different strophanthidin.

Crystalline Kombe strophanthin apparently undergoes the following cleavage when heated with dilute acids:

$$C_{40}H_{56}O_{15}+4H_2O=C_{27}H_{38}O_7+C_{12}H_{22}O_{11}+CH_4O$$

Crys. K. strophanthin. strophanthidin disaccharide methyl-alcohol.

Notwithstanding the uncertainty as to the purity of amorphous substances we have shown that a strophanthin different from crystalline Kombe strophanthin is present in identified Kombe seed. Heffter and Sachs have shown that identified hispidus seeds do not contain a crystalline strophanthin, but an amorphous one which is identical or closely related to the amorphous strophanthin from Kombe.

Both crystalline Kombe strophanthin and amorphous acid strophanthin show the typical heart tonic response, diminished rate and increased amplitude of the heart beat, accompanied by a small rise in blood pressure.

The activity of the amorphous acid strophanthin is less than that of the crystalline strophanthin. By the frog method of Houghton, the activity of these strophanthins is in the ratio one to three.

It is very interesting to note that this loss of activity is associated with the loss of one lacton group.

We believe this crystalline Kombe strophanthin as the definite active constituent contained in Strophanthus Kombe Seed, U. S. P., should be adopted as the standard by which the value of the various preparations of the drug should be measured.

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DRUG PLANT CULTURE IN THE UNITED STATES.*

Within recent years considerable public interest has been manifest in the possible commercial growing of medicinal plants within the boundaries of the United States. This public interest usually expresses itself in the form of inquiries, verbal and in writing, directed to the U. S. Department of Agriculture, State Experiment Stations, Colleges of Agriculture, and to teachers of pharmacognosy in Colleges of Pharmacy, as to how to grow medicinal plants profitably. The more

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