3. The Canadian standard is slightly stronger than the International standard but definitely less active than the U. S. P. XI digitalis standard.

4. Very comparable results were obtained by the one-hour and the twelve-hour frog methods for the activity relationship between any two of the several official standards examined.

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THE SIGNIFICANCE OF SUGAR COMPONENT IN THE MOLECULE OF CARDIAC GLYCOSIDES.*

BY K. K. CHEN, E. BROWN ROBBINS AND HAROLD WORTH.[1]

According to literature, the aglycone appears to have a lower activity than its parent glycoside. Thus, digitoxigenin in frogs is about one-half, and "cymarigenin" about one-third, as potent as digitoxin and cymarin, respectively, as shown by Straub (1). Although Rothlin (2) indicated that the ratio of activity between scillaren A and scillaridin A is 10:1, Stoll (3) recently admitted, "Scillaridin A is so sparingly water soluble that, up to the present time, it has not been possible to test its physiological activity." Digoxigenin is almost one-eleventh as active as digoxin, as reported by Smith (4). Oleandrigenin, however, is only slightly less effective than folinerin (25:24) according to Flury and Neumann (5).

The purpose of this paper is to present data obtained with five aglycones, strophanthidin, digoxigenin, digitoxigenin, scillaridin A and calotropagenin, and to compare them with those of their parent glycosides, cymarin, digoxin, digitoxin, scillaren A and calotropin, respectively. Our sample of strophanthidin, isolated from *Strophanthus Kombé*, was generously supplied by Dr. Walter A. Jacobs, the Rockefeller Institute for Medical Research, New York City; that of digoxigenin by Dr. Sidney Smith of London, England, through the kindness of Howard B. Fonda, Experimental Research Laboratories, the Burroughs Wellcome and Company, Tuckahoe, New York; that of digitoxigenin by Dr. Rudolf Tschesche, Berlin, Germany; that of scillaridin A by Dr. Arthur Stoll of Basel, Switzerland, through the kindness of E. W. Marti, Sandoz Chemical Works, Inc., New York City; and that of calotropagenin by Dr. Gerhard Hesse, München, Germany.

The experimental procedures were the same as those previously reported (6). Since success depends upon the solubility of these substances in an inert medium, considerable care was taken in making the solutions—needless to say, the aglycones are comparatively less soluble in water than the glycosides. For a 1:1000 concentration, strophanthidin and calotropagenin required 19 per cent ethyl alcohol by volume, digoxigenin 27.5 per cent, and digitoxigenin 38 per cent. It was necessary to increase the alcoholic content of a 1:500 concentration—38 per cent for digoxigenin and 47.5 per cent for digitoxigenin. Much difficulty was encountered with scillaridin A, for in absolute alcohol it settled out in the concentration of 1:1000. It was finally decided to employ a 1:2000 solution in methanol. For the determination of the cat unit, intravenous injections were

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* Scientific Section, A. PH. A., New York meeting, 1937.

[1] From the Lilly Research Laboratories, Indianapolis, Indiana.

made by means of a three-way glass stop-cock, as shown in Fig. 1. The drug was not introduced continuously as with other substances, but was administered intermittently from a burette, 0.1 cc. every 30 seconds, each time followed by 1 cc. of saline solution from another burette. Ether anesthesia was reduced as experiment progressed. By this method, scillaridin A produced a typical digitalis-like poisoning, which finally caused the death of the animal. Control experiments with the same or a larger volume of methanol injected in like manner showed no fatalities in a group

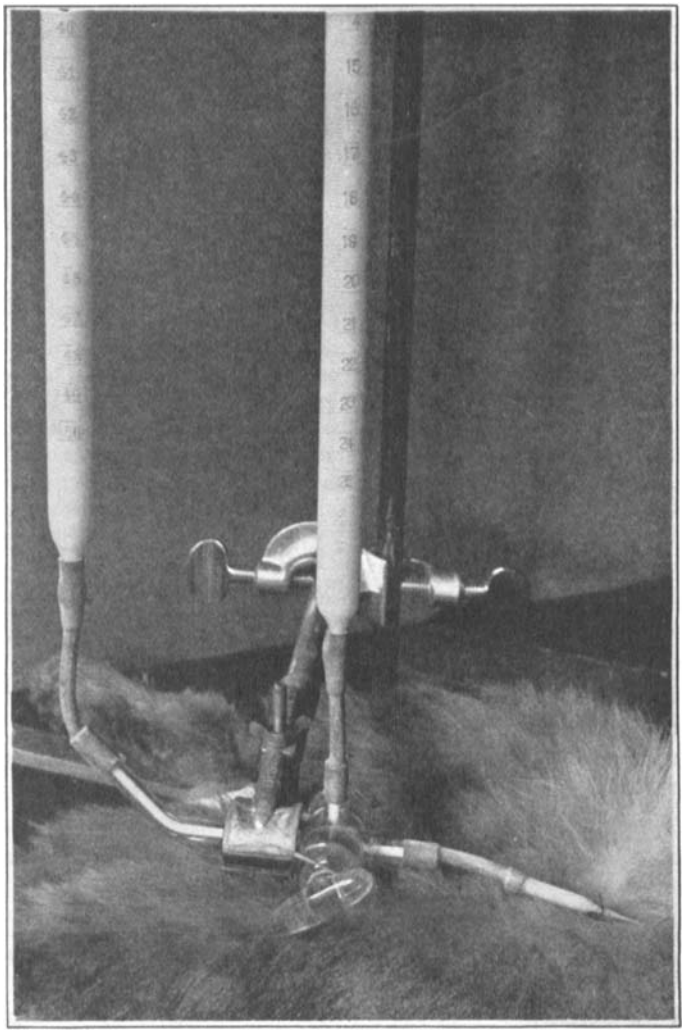

Fig. 1.—Arrangement for the intravenous injection of scillaridin A in cats. The 25-cc. burette contains a 0.05 per cent solution of scillaridin A in methanol, and the 50-cc. burette the saline solution.

of normal cats. However, a volume of 0.2 to 0.4 cc. of methanol per Kg. of body weight, rapidly injected at once, resulted in prompt deaths of the cats. It was thus not possible to determine the minimal emetic dose; nor the minimal systolic dose in frogs, for the same reason.

The results of the aglycones are summarized in Tables I, II and III, and are compared with those of their parent glycosides, which were published previously (6), (7), in Table IV. It should be noted (Table IV) that the splitting of sugar reduces the cardiac activity in every case. The

diminution of potency is much greater in frogs than in cats. Thus, the ratio of activity between cymarin and strophanthidin is approximately 3:1 in cats, but $4^1/_2$:1 in frogs. These figures are not identical with, but tend in the same direction as, those reported by Straub (1) and Dresbach (8). Similarly, the ratio between digoxin and digoxigenin is about 2:1 in cats but $11^1/_2$:1 in frogs,

TABLE I.—CAT UNITS OF STROPHANTHIDIN, DIGOXIGENIN, DIGITOXIGENIN, CALOTROPAGENIN AND SCILLARIDIN A.

Drug.	Cat Number.	Sex.	Body Weight, Kg.	Fatal Dose, Mg. per Kg.
Strophanthidin	1656	F	2.426	0.225
	1657	M	2.271	0.243
	1658	M	2.522	0.594
	1659	F	2.192	0.361
	1660	F	2.314	0.631
	1661	F	1.900	0.507
	1662	F	2.222	0.408
	1663	F	2.176	0.176
	1664	M	1.961	0.188
	1665	M	2.030	0.306
	1666	F	2.003	0.431
	1667	F	2.223	0.271
	1668	F	1.883	0.237
	1669	M	2.117	0.234
	1670	M	2.888	0.569
	1671	F	2.080	0.370
Digoxigenin	1729	M	1.837	0.775
	1730	F	1.846	0.550
	1731	F	1.832	0.783
	1735	F	1.894	0.419
	1736	F	2.760	0.313
	1737	M	2.500	0.867
	1738	M	1.913	0.431
	1739	F	1.937	0.244
	1740	M	2.147	0.354
	1741	M	2.472	0.354
	1742	F	1.865	0.317
	1743	M	1.992	0.409
	1744	F	2.024	0.314
	1745	M	1.943	0.626
	1746	F	1.630	0.481
	1747	M	1.614	0.337
Digitoxigenin	1706	M	2.073	0.442
	1707	F	2.035	0.433
	1708	F	2.407	0.254
	1709	F	2.673	0.609
	1710	F	1.965	0.480
	1711	M	2.465	0.432
	1712	F	2.330	0.572
	1713	F	2.449	0.497
	1714	M	2.565	0.409
	1715	M	1.907	0.579
Calotropagenin	1875	M	1.903	1.540
	1877	F	1.988	1.937
	1878	M	2.625	3.836
	1879	F	2.510	2.084
	1884	F	1.848	3.636
	1885	F	2.028	2.233
	1886	F	1.867	2.769
	1887	F	1.846	2.205
	1888	M	1.993	3.507
	1889	M	1.982	3.012
Scillaridin A	1760	F	2.150	0.814
	1761	F	1.751	2.627
	1762	M	1.722	1.509
	1763	F	1.892	1.771
	1865	F	1.893	1.778
	1866	F	1.817	1.853
	1867	M	2.780	2.014
	1868	M	2.003	1.781
	1869	M	2.358	1.399
	1874	F	2.416	1.656

TABLE II.—MINIMAL SYSTOLIC DOSES OF STROPHANTHIDIN, DIGOXIGENIN, DIGITOXIGENIN AND CALOTROPAGENIN IN FROGS.

Drug.	Solution.	Dose, Mg. per Kg.	No. in Systolic Standstill/No. of Frogs Used.
Strophanthidin	1:2000	0.00091	0/4
		0.00182	0/4
		0.00227	2/8
		0.00273	3/4
		0.00318	4/4
		0.00364	4/4
		0.00409	4/4
		0.00455	4/4

TABLE II.—*Continued from page 191.*

	1:2000	0.00250	0/4
		0.00333	0/4
		0.00417	0/4
		0.00500	0/4
Digoxigen	1:1000	0.01000	0/4
	1:500	0.02500	1/4
		0.02916	3/4
		0.03333	3/4
		0.04167	4/4
Digitoxigenin	1:500	0.05000	1/4
		0.05833	1/4
		0.06667	3/4
		0.07500	4/5
		0.08333	3/4
		0.09167	4/4
		0.10000	4/4
Calotropagenin	1:1000	0.01250	0/4
		0.01667	0/4
		0.02500	0/4

TABLE III.—EMESIS AND PERSISTENCE OF ACTION OF STROPHANTHIDIN, DIGOXIGENIN, DIGITOXIGENIN AND CALOTROPAGENIN IN CATS.

Drug.	Cat Number.	Sex.	Body Weight, Kg.	Initial Dose Injected Intravenously, Mg. per Kg.	Vomiting Occurred.	Final Fatal Dose (Exclusive of Initial Dose), Mg. per Kg.	Interval between Initial and Final Fatal Doses, Hours.
Strophanthidin	1697	M	1.880	0.070	0	*	
	1700	F	2.227	0.070	+	*	..
	1701	F	1.917	0.070	0	*	..
	1696	M	3.016	*0.080*	+	*	..
	1698	F	2.574	*0.080*	0	*	..
	1699	M	1.863	*0.080*	+	0.341	4.0
	1693	F	2.325	0.090	0	*	..
	1694	M	2.037	0.090	+	0.243	4.0
	1695	M	2.171	0.090	+	*	..
	1673	M	2.561	0.100	0	0.308	1.0
	1691	F	2.043	0.100	+	0.256	7.4
	1692	F	2.270	0.100	+	0.367	4.5
	1688	M	2.194	0.110	0	0.344	6.7
	1698	F	2.506	0.110	+	0.196	7.0
	1690	F	1.991	0.110	+	0.182	6.0
	1687	M	2.240	0.120	+	0.437	7.7
	1674	F	2.184	0.140	+	0.401	3.0
	1672	F	2.447	0.180	+	0.185	67.5
Digoxigenin	1749	F	2.167	0.030	0	*	..
	1750	M	2.531	0.040	0	*	..
	1751	M	2.838	0.040	0	*	..
	1748	M	2.691	*0.050*	+	1.290	1.0
	1752	M	3.080	*0.050*	+	0.683	4.5
	1733	F	2.465	0.100	+	0.340	16.0
	1734	F	1.752	0.150	+	0.589	16.5
	1732	M	2.326	0.200	+	0.459	21.0

Digitoxigenin	1717	M	1.920	0.100	0	0.371	1.0
	1725	F	2.105	0.100	+	*	..
	1727	F	1.927	0.100	0	*	..
	1724	F	1.944	*0.110*	+	0.964	17.0
	1726	F	2.341	*0.110*	0	0.516	16.7
	1728	M	2.539	*0.110*	+	0.507	16.0
	1719	F	1.759	0.120	0	0.419	19.5
	1721	F	2.195	0.120	0	0.401	19.5
	1722	M	1.898	0.130	+	0.603	20.0
	1723	M	2.127	0.130	+	0.622	19.0
	1720	M	2.155	0.140	+	0.602	23.0
	1718	M	1.931	0.150	+	0.626	23.5
	1716	M	2.065	0.200	0	0.626	24.0
Calotropagenin	1880	F	2.271	0.100	0	*	..
	1896	F	2.208	0.150	0	*	..
	1897	F	2.352	0.175	0	*	..
	1898	M	2.671	0.175	0	*	..
	1882	F	2.512	*0.200*	0	*	..
	1892	M	2.265	*0.200*	+	*	..
	1895	F	2.131	*0.200*	+	*	..
	1891	F	1.750	0.225	+	*	..
	1890	F	1.970	0.250	+	*	..
	1883	F	2.005	0.300	+	*	..
	1881	M	2.225	0.400	+	**	..

* Not determined.

** Found dead two days after injection.

TABLE IV.—COMPARISON OF ACTION BETWEEN FIVE CARDIAC GLYCOSIDES AND THEIR AGLYCONES.

Drug.	Cat Unit ± Probable Error, Mg. per Kg.	Frog Minimal Systolic Dose, Mg. per Gm.	Cat Minimal Emetic Dose, Mg. per Kg.
Cymarin	0.126 ± 0.003	0.00060	0.08
Strophanthidin	0.359 ± 0.025	0.00273	0.08
Digoxin	0.225 ± 0.008	0.00250	0.07
Digoxigenin	0.473 ± 0.032	0.02916	0.05
Digitoxin	0.327 ± 0.008	0.00800	0.15
Digitoxigenin	0.471 ± 0.022	0.06667	0.11
Calotropin	0.119 ± 0.002	0.00050	0.06
Calotropagenin	2.676 ± 0.169	70.02500	0.20
Scillaren A	0.150 ± 0.007	0.00070	0.10
Scillaridin A	1.720 ± 0.099		..

and that between digitoxin and digitoxigenin $1^1/_2$:1 in cats but $8^1/_2$:1 in frogs. More marked differences existed between the remaining two pairs of compounds investigated. For example, calotropin is $22^1/_2$ times in cats, and more than 50 times in frogs, as potent as calotropagenin, and scillaren A in cats is $11^1/_2$ times as potent as scillaridin A. This remarkable loss in cardiac activity of calotropagenin and scillaridin A is not entirely due to the deletion of the sugar molecule, but rather, is due greatly to the alteration of the aglycone molecule during the process of hydrolysis, for scillaridin A, according to Stoll (9), must be considered as the anhydro-derivative of the true aglycone since it has lost one molecule of water with the formation of a double bond. A similar reaction apparently takes place with calotropagenin (10). Incidentally, it may be pointed out here that the chemical similarity between strophanthidin and calotropagenin, as suggested by Hesse and Reicheneder (10), is not borne out by our pharmacological data.

The aglycones obviously have a higher emetic action than their parent glycosides. For example, cymarin and strophanthidin have the same minimal emetic dose in cats (Table IV), and both digoxigenin and digitoxigenin have smaller minimal emetic doses than those of digoxin and digitoxin, respectively. The prominence of the emetic action of strophanthidin has already been emphasized by Dresbach and Waddell (11). In other words, strophanthidin, digoxigenin and digitoxigenin are more powerful in causing vomiting than cymarin, digoxin and digitoxin, molecule for molecule. One may also interpret that the emetic action of this class of compounds resides in the aglycone part of the molecule. Calotropagenin, which has 1/22.5 the cardiac activity of calotropin, is 1/3.3 as emetic as the latter, showing that the decrease in heart action is much greater than the reduction of emetic action following the modification of the structure of the aglycone during hydrolysis.

The splitting of sugar from the glycoside molecule also exerts an influence upon the persistence of action. Thus, a part of 38 per cent of the cat unit of digitoxin may remain in circulation for more than 5 days in cats (12), but digitoxigenin is very rapidly eliminated as shown in Table III. Although there is no quantitative method yet available for the estimation of persistence of action, it may be stated, qualitatively, that strophanthidin and digoxigenin are also less persistent than cymarin and digoxin, respectively. Dresbach and Waddell (11) and Lenz (13) reported similar impressions on strophanthidin and digitoxigenin.

In confirmation with Straub's observations (1), it was noted that digitoxigenin caused convulsions in both cats and frogs, followed immediately by prostration. The most effective dose for cats was 0.12 mg. per Kg. and that for frogs was around 0.06 mg. per Gm. or more. Digitoxin, on the other hand, is devoid of such action in corresponding doses. One cat developed convulsions with digoxigenin in the dosage of 0.05 mg. per Kg., while the remaining animals with the same drug, as shown in Table III, were all on their feet during the period of observation (1 hour) after injection. Previously, it was pointed out that cino-bufagin (14) and cassaine (15) also induced convulsions, but with them depression and paralysis were much less prominent. It seems that the presence of sugar in the molecule of cardiac glycosides, digitoxin at any rate, tends to inhibit the initial stimulating and secondary depressing action of the aglycones upon the central nervous system.

SUMMARY.

The potency of 5 cardiac aglycones—strophanthidin, digoxigenin, digitoxigenin, calotropagenin and scillaridin A—has been carefully determined.

Each aglycone is less powerful on the heart than its parent glycoside—more pronounced in frogs than in cats. If the aglycone undergoes chemical changes during hydrolysis, as in the case of scillaridin A and calotropagenin, the cardiac action is reduced much further.

The emetic action of strophanthidin, digoxigenin and digitoxigenin, on the other hand, is greater than that of cymarin, digoxin and digitoxin, respectively, molecule for molecule. When the structure of the aglycone is modified during hydrolysis, such as calotropagenin, the emetic action is diminished, but not to the same extent as the cardiac action.

The persistence of action among the aglycones is slight; that is, they are all rapidly eliminated from the circulation. This is particularly true with digitoxigenin in contrast with digitoxin.

Digitoxigenin caused a brief initial stimulation as manifested by convulsions, followed by marked depression of the central nervous system in cats and frogs. Digitoxin has no such action in corresponding doses.

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A STUDY OF THE LEAVES OF IPOMŒA PES-CAPRÆ.*,[1]

BY B. V. CHRISTENSEN[2] AND J. A. REESE.[3]

Ipomœa Pes-Caprœ (*Convolvulaceœ*) is a denizen of nearly every tropical beach (1), the world over. Harshberger (2) regards it as the character plant of the low beaches. It is a perennial plant with a tough woody root as thick as a finger and many feet in length (3). From the enlarged crown of the root grow a number of creeping stems, fleshy and purplish when young, but becoming woody as they mature. The leaves are thick, and the entire plant is mucilaginous. The shape of the leaf is indicated by the name of the plant.

The plant has several scientific names and many colloquial names. Small (4) describes it under *Ipomœa Pes-Caprœ* (L) Sweet. The Index Kewensis (5) gives *Ipomœa Pes-Caprœ*, *Ipomœa biloba*, *Ipomœa maritima*, *Ipomœa bilobata* and *Convolvulus brasiliensis* as synonymous names. Gerth van Wijk (6) lists a number of colloquial names under *Ipomœa biloba*. Bailey (7) considers *Ipomœa Pes-Caprœ*, Roth as synonymous with *Ipomœa maritima*, R. Br. Wehmer (8) gives *Convolvulus brasiliensis* (L) as a synonym for *Ipomœa maritima* R. Br.

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