TABLE II. Effect of Thyroprotein on Plasma Protein.

Animals		Diet		Time on treatment, weeks	Plasma protein	
Group No.	Total No.	No.	Thyroprotein level, %		Mean, %	S.E. of mean
1	6	4	0	16	7.26	.25
2	5	1	0	4	6.08	.02
3	5	1	0	10	5.15	.15
4	4	2	.1	2	5.89	.06
5	4	2	.1	3	5.92	.14
6	4	2	.1	4	5.32	.16
7	3	2	.1	6	4.94	.38
8	4	3	.15	4	4.46	.21
9*	4	1†	0	1	7.12	.46

* These were depleted animals from Group No. 5.

† For a repletion period of 1 wk they were fed, in addition to Diet 1, enough casein to supply .24 g N/day.

tion of plasma protein occurred equally as rapidly as in other experiments in which rats were similarly repleted after bring depleted without thyroactive protein.

This modification of the Cannon technic has been applied to mixed feeds containing both animal and vegetable proteins. In this assay trial, nitrogen intake was kept constant. Commercial casein and blood fibrin, as standards representing good quality protein, raised the plasma protein 1.10 g and 1.29 g respectively. A mixed feed which had given questionable results with other animal species raised the plasma protein only .59 g. So far as the method was concerned the only effect of iodinated casein appeared to be to speed up reduction of body weight and plasma protein.

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Comparison of Cardiac Action of Bufalin, Cinobufotalin, and Telocinobufagin with Cinobufagin. (18493)

K. K. CHEN, ROBERT C. ANDERSON, AND FRANCIS G. HENDERSON.

From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

Ch'an Su is a commercial preparation of the Chinese toad venom employed in medicine for centuries. Previous investigations in this laboratory(1-3) resulted in the isolation of two digitalis-like principles, cinobufagin and cinobufotoxin. The source of Ch'an Su was proved to be the parotoid secretion of *Bufo bufo gargarizans*(4). The chemical structure of cinobufagin was elucidated by Tschesche and Offe(6) and Jensen(6). From a supply of Ch'an Su from this laboratory Meyer(7) in T. Reichstein's laboratory, succeeded in isolating 6 well-characterized substances, all having a digitalis-like action. They are cinobufagin, bufalin, bufotalin, cinobufotalin, gamabufotalin (gamabufagin), and telocinobufagin. Bufalin, cinobufotalin, and gamabufotalin were previously isolated by Kotake and Kuwada(8). Bufotalin is identical with

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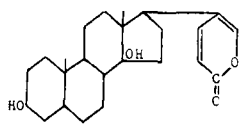
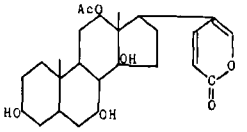
6. Jensen, H., *J. Am. Chem. Soc.*, 1937, v59, 767.

7. Meyer, K., *Experientia*, 1948, v4, 385; *Pharm. Acta Helv.*, 1949, v24, 222; *Helv. Chim. Acta*, 1949, v32, 1238; 1949, v32, 1593; 1949, v32, 1599; 1949, v32, 1993.

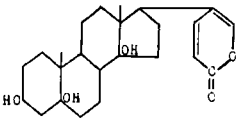
the principle which occurs in the venom of the common European toad, *Bufo vulgaris*. Telocinobufagin is a new substance. Through the cooperation of Prof. T. Reichstein of the Pharmaceutical Institute of the University of Basel, we had access to cinobufagin, bufalin, cinobufotalin, and telocinobufagin for a pharmacological study. The structural relationship of the four substances, based on the work of Meyer(7) and Kuwada and Kotake (9), is as follows:



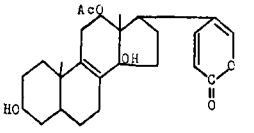
Bufalin



Cinobufotalin



Telocinobufagin



Cinobufagin

The formulas of cinobufotalin and cinobufagin are tentative. It must be stated that the cinobufagin originally reported on from this laboratory(1-3) was a molecular compound of constant composition and melting point. It contained 2 parts of cinobufotalin and 1 part of a second substance. Kotake and Kuwada (8) retained the term cinobufagin for the latter. It was only by chromatography that the two could be separated(7). The sample of cinobufagin in this investigation was therefore a single substance in contrast with the molecular compound which we previously studied.

Our principal interest was to compare their activity on the heart. All 4 substances required ethanol for solution. The stock solution of each was 0.1% with 47.5% of ethanol. Dilutions of this stock solution in water or saline were clear—suitable for animal experiments.

When a dose of 0.25 to 0.5 mg (total) was

TABLE I. Potency in Cats.

Cinobufagin			Telocinobufagin			Cinobufotalin			Bufalin		
Sex	Wt, kg	Dose to kill, μg per kg	Sex	Wt, kg	Dose to kill, μg per kg	Sex	Wt, kg	Dose to kill, μg per kg	Sex	Wt, kg	Dose to kill, μg per kg
M	2.513	264.6	F	2.336	102.7	M	2.374	155.0	M	2.060	183.5
F	2.819	119.9	F	2.460	100.0	F	2.798	120.4	M	2.227	148.6
M	2.710	260.9	M	1.668	99.5	F	2.750	133.1	F	2.062	177.5
F	2.043	243.3	F	2.139	102.4	M	2.542	329.7	F	1.938	96.0
M	2.448	257.4	M	2.482	110.0	F	1.864	279.0	F	2.293	145.7
F	2.192	158.8	M	2.887	122.6	M	2.628	188.7	F	2.054	158.7
M	1.624	142.2	M	2.646	70.7	M	2.112	276.5	F	2.585	91.3
F	1.612	212.8	F	2.114	151.4	F	2.148	185.3	F	2.335	118.6
M	1.646	243.0	M	2.242	82.1	M	2.480	135.1	F	2.414	137.1
M	1.868	183.1	M	2.639	94.7	F	2.076	326.6	M	2.448	145.4
Mean (geo.)					101.6 ± 6.6			199.0 ± 24.4			137.0 ± 10.2
L.D. ± S.E. 201.6 ± 18.1											

8. Kotake, M., and Kuwada, K., *Sci. Papers Inst. Phys. Chem. Res.*, 1937, v32, 1; 1937, v32, 79; 1939, v36, 106.

9. Kuwada, K., and Kotake, M., *Sci. Papers Inst. Phys. Chem. Res.*, 1939, v35, 419.

injected into the lymph sac of the common frog, *Rana pipiens,* weighing between 25 and 30 g, systolic standstill resulted as observed in an hour. In etherized cats a 1:100,000 solution injected intravenously at the rate of 1 cc per minute produced slowing of heart rate, followed by irregular pulse, secondary tachycardia, and finally ventricular fibrillation which was the cause of death. Electrocardiograms recorded from the barbitalized dog during the injection of a 1:50,000 solution at the rate of 1 cc per minute revealed changes typical of digitalis administration. Bradycardia, P-R prolongation, inversion of T-wave, ectopic rhythm, ventricular tachycardia, bundle branch block, and ventricular fibrillation, occurred in order. The results were similar with all 4 substances.

Each substance was then assayed in etherized cats by the intravenous infusion of a 1:100,000 dilution at the rate of 1 cc per minute until death occurred—10 cats for each substance. The mean (geometric) lethal dose of the present sample of cinobufagin in 10 cats was found to be 0.2016 $\pm$ standard error of 0.0181 mg per kg. When compared with the mean (geometric) lethal dose of the sample of cinobufagin previously isolated in this laboratory(10), which was 0.2191 $\pm$ 0.0115 mg per kg in 44 cats, the difference was statistically not significant. In other words, cinobufagin as a single substance has approximately the same potency as its molecular compound of cinobufotalin and itself.

Protocols are summarized in Table I. The potency of cinobufotalin, as judged by the mean lethal dose, is very close to that of cinobufagin. Both bufalin and telocinobufagin are decidedly more potent than cinobufagin. Telocinobufagin is the most potent of all four substances studied in this investigation.

It is obvious that the presence of the acetoxy group at C_{12} diminishes the cardiac activity since both cinobufotalin and cinobufagin are weaker than bufalin and telocinobufagin (see formulas above). The occurrence of an OH group at C_5 definitely confers a higher potency on telocinobufagin as compared with bufalin. No significant difference in activity occurs when a double bond appears between C_8 and C_9 in the molecule of cinobufagin, and an OH group replaces the H-atom at C_7 of cinobufotalin. All four substances have a higher activity on the cat's heart than many digitalis-like glycosides(10). This may be attributed to the 6-membered lactone ring in their structure, just as hellebrigenin exceeds its glycosides in activity(11). Conjugation with sugar molecules at the OH group of C_3 will very likely render bufalin, telocinobufagin, cinobufagin, or cinobufotalin less potent.

Like cinobufagin which was previously reported(3), bufalin, cinobufotalin, and telocinobufagin injected intravenously, induced vomiting in non-anesthetized cats, in the dose of one-half of the median lethal dose as determined in etherized animals. Sublethal doses of either substance rapidly injected by vein raised the carotid blood pressure. Contraction of isolated rabbit intestine immersed in Tyrode's solution took place when a small amount of either of the three substances was added to the bath.

Summary. The cardiac activity of bufalin, cinobufotalin, telocinobufagin and cinobufagin has been compared. They all have a digitalis-like action. In etherized cats the order of activity from high to low is telecinobufagin > bufalin > cinobufotalin = cinobufagin.

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