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Comparative Activity of Bufadienolides

K. K. CHEN and FRANCIS G. HENDERSON, *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Indiana*

Bufadienolides are cardiac aglycones with a six-membered lactone ring which has two double bonds of α-pyrone type.[1] When they are conjugated with sugar(s), as in plants, they become typical glycosides. Several species of the lily family are rich in bufadienolides and their corresponding glycosides. In the animal kingdom the toads of the *Bufo* genus produce cardiac substances of similar structure in the secretion of their 'parotid' glands. No glycosides of bufadienolides have been discovered in the toad poison.

The present report is concerned with the pharmacology of 29 bufadienolides and their derivatives, as listed in Table I. The first 12 compounds are the products of the bulbs of *Bowiea volubilis*, which is indigenous to South Africa and has caused poisoning and death in animals and man.[2] Active glycosides were isolated by Jaretzky,[3] and homogeneous bufadienolides were independently obtained by Katz,[4] and Tschesche and Sellhorn.[5] In previous publications from these laboratories the results on bovoside A and D, and bovogenin E were discussed.[6, 7] Tschesche and his associates[8] recently changed the names of bovoside D and bovogenin E to bovochrysoide and bovoeolotoxin, respectively. In our collaborative work with Professor Tschesche, we first shipped the bulbs of *B. volubilis* to his laboratory via Indianapolis, and later arranged for shipment to Hamburg from Cape Town, South Africa. Fig. 1 shows the plant under cultivation in a local garden.

For our pharmacological studies, Dr. A. Katz of Professor Reichstein's laboratory kindly furnished us with bovogenin A, bovosidol A, acetyl bovosidol A, and acetyl bovoside D, scilliglaucosidin-19-ol, 3-episcilliglaucosidin-19-ol, and 3-dehydroscilliglaucosidin; Professor Tschesche with glucobovoside A, bovocryptoside, bovoruboside, bovoerythrotoxin, bovopurpuroside, bovocyanotoxin, bovoxanthotoxin and kilimandscharogenin A; Professor

Table I. Potencies in cats

Compound name	Formula no.	No. of cats used	Mean LD ± S.E. (geometric), mg/kg
Bovogenin A	(I)	20	0·1317 ± 0·0083
Glucobovoside A	(III)	10	0·1460 ± 0·0067
Bovosidol A	(IV)	10	0·1157 ± 0·0040
19-Acetyl bovosidol A	(V)	6	1·338 ± 0·0460
Bovocryptoside	(VI)	10	0·0704 ± 0·0033
Bovoruboside	(VII)	10	0·0920 ± 0·0055
Acetyl bovoside D		10	0·1246 ± 0·0055
Bovoerythrotoxin		10	0·1119 ± 0·0053
Bovopurpuroside		10	0·1923 ± 0·0111
Bovocyanotoxin		10	0·1795 ± 0·0132
Bovoxanthotoxin		10	0·1538 ± 0·0115
Kilimandscharogenin A		10	0·1293 ± 0·0081
Scilliglaucoside	(VIII)	10	0·0709 ± 0·0031
Scilliglaucosidin	(IX)	10	0·0834 ± 0·0044
Scilliglaucosidin-19-ol	(X)	10	0·0951 ± 0·0059
3-Episcilliglaucosidin-19-ol	(XI)	8	0·3604 ± 0·0243
3-Dehydroscilliglaucosidin	(XII)	10	0·1981 ± 0·0165
Hellebrigenin glucoside	(XIV)	10	0·0980 ± 0·0054
Altoside		10	0·1254 ± 0·0056
Desacetyl bufotalin	(XVI)	10	0·2634 ± 0·0195
Acetyl cinobufotalin	(XVIII)	10	0·1786 ± 0·0129
Desacetyl cinobufotalin	(XIX)	3	two survived 0·7602 and 3·469 mg/kg; one died with 1·07 mg/kg atypically
Acetyl cinobufagin	(XXI)	10	0·5888 ± 0·0399
Desacetyl cinobufagin	(XXII)	2	two died with 4·415 and 4·565 mg/kg
Acetyl resibufogenin	(XXIV)	3	one survived 4·09 mg/kg; two died with 4·087 and 5·666 mg/kg atypically
Marinobufagin	(XXV)	20	1·520 ± 0·074
Acetyl marinobufagin	(XXVI)	10	0·9471 ± 0·0479
Bufotalinin	(XXVII)	6	0·6190 ± 0·1190
Arenobufagin	(XXVIII)	10	0·0767 ± 0·0067

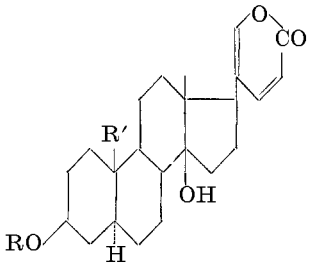
A total of 286 cats were used including the 8 for the tincture of *B. volubilis*. Their average weight was about 2 kg, ranging from 1·6 to 2·9 kg. Females outnumbered males. A few females were in their early pregnancy. The figures for 21 compounds in the fourth column of Table I were obtained from 10 cats each. The results of bovogenin A and marinobufagin from two different sources were pooled and thus the figures represent the means of 20 cats. Fewer animals were injected with the remaining substances, because of a limited amount of material, or because of low potency or complete lack of activity. The action of 5 toad products was explored on the frog heart following an injection into the ventral lymph sac. Whenever convulsions occurred they were recorded.

Results

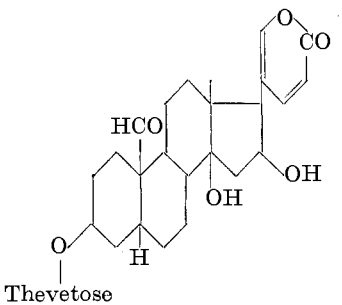
The bulbs of *B. volubilis* measured 3–4½ in. in diameter, weighed 400 g on the average, and were dull green in colour. The loss of weight upon drying at 60° amounted to 85·7 per cent. The mean (geometric) LD in 8 cats as determined from the tincture was 24·2 ± 1·35 mg/kg, representing the activity of total glycosides.

A survey of the data in Table I reveals that 19 out of 29 compounds had a cat mean LD of less than 0·2 mg, and 7 had values of less than 0·1 mg/kg; the smaller the figure, the more potent the compound. Two samples of the aglycone bovogenin A (I) were studied: that from Dr. Katz gave a cat mean LD of 0·1345 ± 0·0092, and that from Dr. Tschesche, 0·1290 ± 0·0140 mg/kg. Since the difference between the two is insignificant, they are combined to make 0·1317 ± 0·0083 mg/kg. Glucobovoside A (III),[20] a bioside, according to Elderfield's terminology,[21] has about the same potency as bovogenin A. Bovosidol A (IV)[22] differs from bovoside A by substitution of a carbinol group on C_{10} for an aldehyde group and is more active than the latter which assayed 0·1293 ± 0·0064 mg/kg.[6] When acetylation[22] takes place at C_{19} of bovosidol A, the resulting compound (V) loses more than 11 times the activity. The C_5 of bovocryptoside (VI) is in β-orientation in contrast to bovoside A. In addition, the former has an OH group at C_{16}. Its potency, which is greater than that

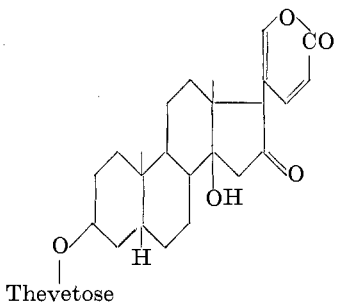
of bovoside A, suggests the favourable influence of β-orientation. Oxidation of the 16-OH of bovocryptoside to bovoruboside (VII)[20] slightly reduces the cardiac activity. The structures of bovoerythrotoxin, bovopurpuroside, bovocyanotoxin, bovoxanthotoxin, and kilimandscharogenin A have not been completely



(I); R = H, R′ = CHO. Bovogenin A
(II); R = Thevetose, R′ = CHO. Bovoside A
(III); R = Thevetose-glucose, R′ = CHO. Glucobovoside A
(IV); R = Thevetose, R′ = CH_2OH. Bovosidol A
(V); R = Thevetose, R′ = CH_2OAc. 19-Acetyl bovosidol A



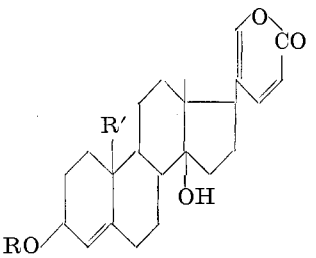
(VI) Bovocryptoside



(VII) Bovoruboside

established. Their cat mean LD's are all under 0·2 mg/kg, indicating their relatively high potency.

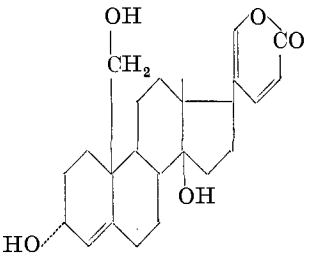
Scilliglaucoside (VIII) is slightly more active than its aglycone scilliglaucosidin (IX). Scilliglaucosidin, which has an aldehyde group at C_{10}, is more potent than the carbinol analogue at the same position (X). The 3-epimer (XI) of scilliglaucosidin-19-ol has about one quarter of the activity of the 3-β-isomer. Oxidation of the 3-OH of scilliglaucosidin results in 3-dehydroscilliglaucosidin



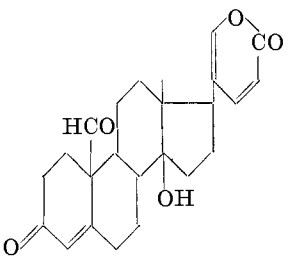
(VIII); R = glucose, R′ = CHO. Scilliglaucoside

(IX); R = H, R′ = CHO. Scilliglaucosidin

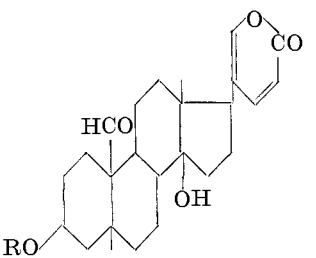
(X); R = H, R′ = CH_2OH. Scilliglaucosidin-19-ol



(XI) 3-Episcilliglaucosidin-19-ol

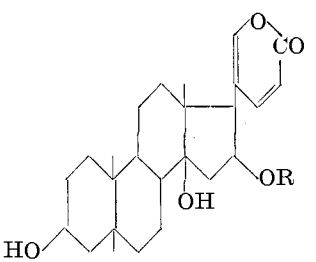


(XII) 3-Dehydroscilliglaucosidin



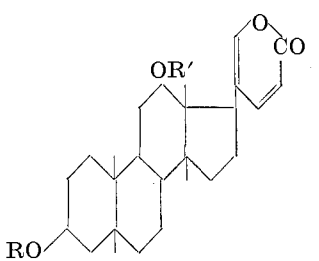
(XIII); R = H. Hellebrigenin

(XIV); R = glucose. Hellebrigenin glucoside



(XV); R = Ac. Bufotalin

(XVI); R = H. Desacetyl bufotalin



(XVII); R = H, R′ = Ac. Cinobufotalin

(XVIII); R = Ac, R′ = Ac. Acetyl cinobufotalin

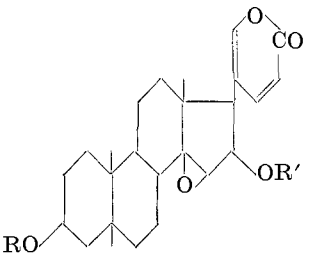
(XIX); R = H, R′ = H. Desacetyl cinobufotalin

(XII); it reduces the activity to less than one half. The glucoside (XIV) of hellebrigenin (XIII) is less active than the aglycone, which was reported to have a cat mean LD of 0·0769 ± 0·0055 mg/kg.[23] Altoside is an isomer of scilliglaucoside,[24] but it is definitely less active than the latter. It is also less soluble in aqueous alcohol.

Among the toad poison constituents, bufotalin (XV) is a 16-acetyl derivative.[13] When the acetyl group is removed, the activity of desacetyl bufotalin (XVI) drops to about one half (Table I) since the cat mean LD of bufotalin is 0·1317 ± 0·0069 mg/kg.[25] 3-Acetylation of cinobufotalin (XVII) to make acetyl cinobufotalin (XVIII)[13] has a slightly favourable influence on the potency as cinobufotalin has a cat mean LD of 0·1990 ± 0·0244 mg/kg.[26] When both acetyl groups are removed, the resulting compound (XIX) is devoid of action. Four frogs injected with doses from 25 to 56 mg/kg also showed negative results.

After Thiessen's suggestion[27] new structural formulas of several toad poison principles have been proposed. These substances have no free β-OH group at C_{14}, but a $14\beta:15\beta$ epoxide ring. This change does not necessarily deprive them of a digitalis-like action as exemplified by cinobufagin (XX)[26, 28] and other toad poison substances in the present study, and speaks against the belief that 14-β-OH is indispensable for the digitalis-like action. Acetylation of cinobufagin to make acetyl cinobufagin (XXI) in this case causes a depreciation of action. On the other hand, elimination of the 16-acetyl group actually nullifies the cardiac action. It is doubtful that desacetyl cinobufagin (XXII) is a cardio-active aglycone since the two cats used died after large doses. Like resibufogenin (XXIII),[7, 29] its acetyl derivative (XXIV) is inactive in cats (4·1–5·7 mg/kg) and in frogs (11–26 mg/kg). It is curious that this nuclear configuration confers no activity. Marinobufagin (XXV) occurs in the venom of both *Bufo marinus* and *B. bufo bufo*.[30] The cat mean LD of the sample from *B. marinus* was found to be 1·489 ± 0·091, and that from *B. bufo bufo*, 1·552 ± 0·123 mg/kg. Since there is no difference of significance, the data were combined, giving the mean LD of 1·520 ± 0·074 mg/kg. Acetyl marinobufagin (XXVI) is more potent than the parent substance by about one third, in spite of its larger molecular size. In frogs neither marinobufagin in doses of 22 to

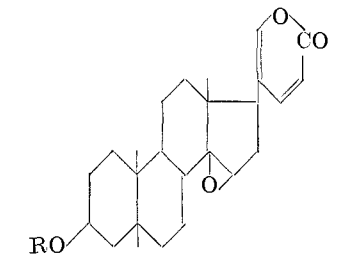
36 mg/kg, nor acetyl marinobufagin in doses of 13 to 41 mg/kg, caused systolic standstill, probably because absorption from the lymph sac is incomplete Bufotalinin (XXVII) is another bufadienolide of *B. bufo bufo*[27, 31] and also has a digitalis-like action.



(XX); R = H, R′ = Ac. Cinobufagin

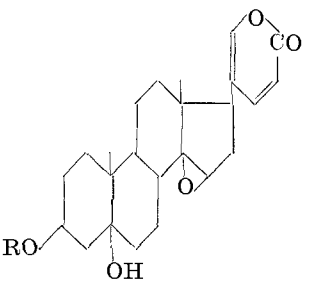
(XXI); R = Ac, R′ = Ac. Acetyl cinobufagin

(XXII); R = H, R′ = H. Desacetyl cinobufagin



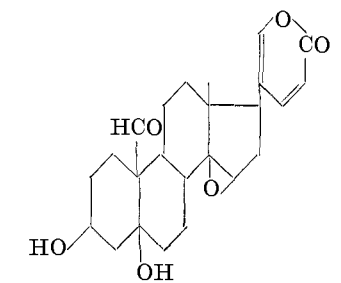
(XXIII); R = H. Resibufogenin

(XXIV); R = Ac. Acetyl resibufogenin

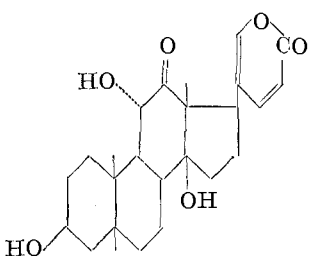


(XXV); R = H. Marinobufagin

(XXVI); R = Ac. Acetyl marinobufagin



(XXVII) Bufotalinin



(XXVIII) Arenobufagin

On the frog's heart, doses of 1–3 mg/kg gave negative results, but one of 17 mg/kg caused systolic standstill. Arenobufagin (XXVIII)[32] is a constituent of the venom of a South American toad, *B. arevarum*, and is highly potent, agreeing with the results of our early publication.[33]

Several toad-poison substances have a convulsive action. Acetyl cinobufagin caused convulsive movements in etherized cats. Marinobufagin, 0·4 mg/kg, produced violent clonic convulsions and rapid respiration in unanaesthetized cats. Both marinobufagin and acetyl marinobufagin brought about constant movements of all four legs and rapid respiration of etherized cats. Convulsions also occurred in frogs following the administration of marinobufagin and its acetyl derivative. Bufotalinin similarly induced movements of hindlegs and the tail of etherized cats, and clonic convulsions in unanaesthetized animals.

Discussion

The mean values in Table I are biological measurements rather than fixed numbers, such as melting points or optical rotations. They vary according to uncontrollable circumstances that make individual differences in the response of the animals to chemicals. The average or mean determined from 10 to 20 cats in this work is trustworthy only to the extent that a small group of animals from a large population has been examined. It is therefore necessary that each mean be qualified by its S.E. in order to know its reliability. For comparison of two samples or two compounds, one can add 1 S.E. to 1 mean and subtract 1 S.E. from the other to see if the resulting numbers overlap. If they do, there is no significant difference, by inspection. A t test can be applied by computation if one wishes to detect more definitely the significance of difference of two means.[17] The mean LD's in cats can be expressed in their reciprocals, as indicated in the last column of Table II, namely, the number of LD's in 1 mg. This avoids the inverse relationship and shows a direct proportion of activity. The S.E.'s can be computed proportionately from their percentages of the means. When considering the structure–activity relationship, one is struck by the fact that the observations can only elucidate certain trends, any attempt at generalization being

met with exceptions. Repetition of results on several compounds is given in Table II for the sake of convenient comparison.

The aglycone bovogenin A is about as active as bovoside A and glucobovoside A. Although scilliglaucosidin appears less potent than scilliglaucoside, the difference is near the borderline of significance. Hellebrigenin is superior not only to its glucoside, but also to hellebrin and desgluco-hellebrin.[23] We have previously reported[7] that scillirosidin[35] far surpasses the activity of scillirosside. In a recent communication, von Wartburg and Renz[36] show the relationship between scillaren A and scillarenin A. The identity of transvaalin to scillarin A has been demonstrated by Zoller and Tamm.[37] The reciprocal of their combined mean LD[7, 38] is indistinguishable from that of scillarenin A (Table II), and is greater than the desgluco derivative (proscillaridin A or desgluco-transvaalin). These results indicate very clearly that the conjugation of one molecule of sugar with OH at C_3 has little influence on cardiac activity, and conjugation of two molecules of sugar usually effects a decrease of activity. Among the glycosides of five-membered lactones (cardenolides), the monosides have several times the activity of the aglycone, and the biosides and triosides are usually more potent than the aglycone.[6] The larger lactone ring and the higher unsaturation of bufadienolides thus inhibit the enhancing effect of the glycosidic linkage.

The inherent higher potency of bufadienolides compared to cardenolides with an identical steroid skeleton is further contrasted by corotoxigenin[6] and bovogenin A; digitoxigenin and bufalin;[26] periplogenin[38] and telocinobufagin;[26] strophanthidin and hellebrigenin; sarmentogenin[6] and gamabufagin;[38] oleandrigenin and bufotalin;[38] and gitoxigenin and desacetyl bufotalin (Table II). On the other hand, desacetyl cinobufotalin is inactive in the doses studied, whereas digoxigenin[38] kills cats without fail. This exception is contingent upon the correctness of the formula of desacetyl cinobufotalin.

The angular methyl group on C_{10} makes a weaker compound than the aldehyde analogue as shown by marinobufagin and bufotalinin. A similar relationship exists between bufalin and hellebrigenin. The higher potency of bovosidol A than of bovoside A seems to place 19-OH above the aldehyde derivative. However, the order of activity between scilliglaucosidin-19-ol and

Table II. Comparison of potencies by changes of functional groups

Functional group involved	Name of compound	Reciprocal of mean cat LD. No. of mean LD ± S.E. in 1 mg
Glycosidic linkage	Bovogenin A	7·6 ± 0·48
	Bovoside A	7·7 ± 0·38
	Glucobovoside A	6·9 ± 0·31
	Scilliglaucosidin	12·0 ± 0·63
	Scilliglaucoside	14·1 ± 0·62
	Hellebrigenin	13·0 ± 0·93
	Hellebrigenin glucoside	10·2 ± 0·56
	Desgluco-hellebrin	11·6 ± 0·50
	Hellebrin	9·6 ± 0·29
	Scillirosidin	14·2 ± 0·68
	Scilliroside	7·7 ± 0·33
	Scillarenin A	6·4 ± 0·27
	Scillaren A (transvaalin)	6·4 ± 0·40
	Desgluco-transvaalin (Proscillaridin A)	5·4 ± 0·27
Cardenolide *vs.* bufadienolides	Corotoxigenin	0·9 ± 0·08
	Bovogenin A	7·6 ± 0·48
	Digitoxigenin	2·2 ± 0·17
	Bufalin	7·3 ± 0·55
	Periplogenin	1·4 ± 0·16
	Telocinobufagin	9·8 ± 0·64
	Strophanthidin	3·9 ± 0·23[a]
	Hellebrigenin	13·0 ± 0·93
	Sarmentogenin	2·2 ± 0·15
	Gamabufagin	9·9 ± 0·50
	Oleandrigenin (unpublished)	4·6 ± 0·27
	Bufotalin	7·6 ± 0·40
	Gitoxigenin (unpublished)	0·3 ± 0·03
	Desacetyl bufotalin	3·8 ± 0·28
	Digoxigenin	2·3 ± 0·21
	Desacetyl cinobufotalin	inactive
10-CH_3 *vs.* 10-CHO or 10-CH_2OH	Marinobufagin	0·7 ± 0·03
	Bufotalinin	1·6 ± 0·31
	Bufalin	7·3 ± 0·55
	Hellebrigenin	13·0 ± 0·93
	Bovosidol A	8·6 ± 0·30
	Bovoside A	7·7 ± 0·38
	Scilliglaucosidin-19-ol	10·5 ± 0·65
	Scilliglaucosidin	12·0 ± 0·63
	3-Acetyl hellebrigenol	13·0 ± 0·59
	3-Acetyl hellebrigenin	15·6 ± 0·70
	Desgluco-hellebrol	10·8 ± 0·48
	Desgluco-hellebrin	11·6 ± 0·50
	19-Acetyl bovosidol A	0·7 ± 0·02

Table II—*continued*

Functional group involved	Name of compound	Reciprocal of mean cat LD. No. of mean LD ± S.E. in 1 mg
3-Acetylation	Cinobufotalin	5·0 ± 0·62
	Acetyl cinobufotalin	5·6 ± 0·40
	Marinobufagin	0·7 ± 0·03
	Acetyl marinobufagin	1·1 ± 0·05
	Cinobufagin	5·0 ± 0·44
	Acetyl cinobufagin	1·7 ± 0·11
	Hellebrigenin	13·0 ± 0·93
	Acetyl hellebrigenin	15·6 ± 0·70
	Scillirosidin	14·1 ± 0·68
	Acetyl scillirosidin	5·0 ± 0·21
	Resibufogenin	inactive
	Acetyl resibufogenin	inactive
16-Acetylation	Bovoside D	8·9 ± 0·66
	Acetyl bovoside D	8·0 ± 0·35
12- or 16-Deacetylation	Bufotalin	7·6 ± 0·40
	Desacetyl bufotalin	3·8 ± 0·28
	Cinobufotalin	5·0 ± 0·62
	Desacetyl cinobufotalin	inactive
	Cinobufagin	5·0 ± 0·44
	Desacetyl cinobufagin	inactive
Configuration	Scilliglaucosidin-19-ol	10·5 ± 0·65
	3-Episcilliglaucosidin-19-ol	2·8 ± 0·19
	Bovoside A	7·7 ± 0·38
	Bovocryptoside	14·2 ± 0·67
	Bovoruboside	10·9 ± 0·65

[a] This is the reciprocal of 0·2570 ± 0·0154, the mean of 20 cats with a homogeneous sample of strophanthidin. It supersedes the figure previously reported.[34]

scilliglaucosidin is in the reverse direction. Previously published data[7, 23, 39] on two other pairs—desgluco-hellebrol and desgluco-hellebrin, and 3-acetyl hellebrigenol and 3-acetyl hellebrigenin—also showed a greater reciprocal of mean LD of the aldehydes (Table II). Acetylation of 19-OH of bovosidol A reduces the cardiac activity to less than 10 per cent of the parent glycoside.

Among the bufadienolides, acetylation of the secondary OH group on C_3 may increase the cardiac potency as shown by acetyl cinobufotalin and acetyl marinobufagin. These observations agree with another example; namely, acetyl hellebrigenin has a higher activity than hellebrigenin.[23] In our early paper,[40] we

reported a lower potency of acetyl marinobufagin than of marinobufagin, the material being prepared by a similar conventional procedure. Further, our marinobufagin was more active than the present specimen.[41] Admittedly our isolation consisted of fractional precipitation and crystallization, while chromatography employed by the Basle group[14] gave rise to other bufadienolides that are more potent than marinobufagin. The possibility of a molecular mixture is not ruled out in our previous work. Meyer's acetyl cinobufagin is weaker than cinobufagin (Table II), confirming our previous observation.[40] This is supported by a similar relationship between acetyl scillirosidin and scillirosidin.[6] Also 3-acetylation does not activate resibufogenin. The oxidation of the 3-OH group of scilliglaucosidin to a ketone reduces the activity to less than 50 per cent. Katz[42] suggested in an earlier paper that bovoside D was 16-hydroxybovoside A. His acetyl bovoside D is slightly less potent than the parent compound. The structure of bovoside D as proposed by Katz was apparently accepted by Tschesche *et al.*[8]

Removal of the naturally-occurring acetyl group at C_{16} decreases the cardiac potency, as in the case of desacetyl bufotalin. In other instances deacetylation at C_{16} or C_{12} abolishes the digitalis-like effect as exemplified by desacetyl cinobufagin and desacetyl cinobufotalin.

As with cardenolides, optimal spatial arrangement of the molecule plays an important part in sustaining the cardiotonic action of bufadienolides. 3-Epimerization (XI) brings about 73 per cent loss of potency to scilliglaucosidin-19-ol. The relatively higher activity of bovocryptoside (VI) and bovoruboside (VII) than of bovoside A (II) may be due to their β-orientation of C_5.[20]

While the frog's heart frequently follows the cat's heart in its response to many digitalis-like drugs, it sometimes deviates from the latter. In the present study, bufotalinin is active and desacetyl cinobufagin and desacetyl resibufogenin are inactive in both cats and frogs. Marinobufagin and its acetyl derivative have determinable mean LD's in cats, but they do not cause ventricular systole in frogs. Difficulty of absorption from the lymph sac may account for the negative results.

Convulsive action occurs with acetyl cinobufagin, marinobufagin and its acetyl derivative, and bufotalinin. It may be

manifested in etherized cats as incessant movements of limbs and rapid respiration, or in non-anaesthetized animals as clonic or tonic convulsions. The effect is not related to the cardiotonic action, and is not attributable to the change of any special functional group.

Summary. 1. The bulbs of *Bowiea volubilis* have a high content of cardiotonic glycosides. A tincture prepared from the dried material has a mean cat LD of $24 \cdot 2 \pm 1 \cdot 35$ mg/kg.

2. Twenty-nine bufadienolides and their derivatives from various sources have been investigated, principally in cats. Using their mean (geometric) LD's as measurements, one can compare the potencies among themselves and with those which have appeared in previous papers. Certain trends of structure–activity relationship can be observed.

3. With one exception, the bufadienolides are inherently more potent than the corresponding cardenolides. The glycosidic linkage seldom enhances the activity of aglycones.

4. There is no unequivocal evidence that 3- or 16-acetylation increases the potency although deacetylation of a naturally-occurring acetyl group at C_{16} or C_{12} results in loss of activity or reduction of potency.

5. Reduction of the aldehyde group at C_{10} to carbinol analogues is rarely attended by an improvement of potency. The results on three out of four pairs indicate that the aldehyde form is slightly superior to the carbinol form. The methyl analogues on C_{10} are usually less active than the aldehyde derivatives.

6. 3-Epimerization and 5-α-configuration seem to reduce the cardiac potency.

7. While marinobufagin and acetyl marinobufagin are active on the cat's heart, they are inactive on the frog's heart following injection into the lymph sac.

8. In addition to their cardiotonic action, acetyl cinobufagin, marinobufagin, acetyl marinobufagin, and bufotalinin have a convulsive action.

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