DIGITALIS-LIKE COMPOUNDS OF TOAD BILE: SULFATION AND REDUCTION OF BUFADIENOLIDES DECREASE POTENCY OF Na^+,K^+-ATPase INHIBITION

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Abstract - The most abundant digitalis-like compound in the bile of the toad, *Bufo marinus*, was identified as 12ß-hydroxytetrahydroresibufogenin-3-sulfate. Other digitalis-like compounds in toad bile had the properties of bufadienolide-3-sulfates, the most abundant of which was found to be marinobufagin-3-sulfate, a compound previously described only as a trace component in toad skin. Both compounds were less effective than unconjugated marinobufagin in the inhibition of Na^+,K^+-ATPase enzymatic activity and of Na^+,K^+-ATPase ouabain binding. This study suggests that sulfation of the A ring and reduction of the lactone ring may be responsible for bufadienolide inactivation *in vivo*.

Dedicated to Professor Arnold Brossi on the occasion of his 70th birthday

Cardiotonic steroids, known as bufadienolides, and their more polar conjugates, called bufotoxins, are present in the skin and venom of toads of the genus *Bufo*.[1] Related compounds with similar chemical and pharmacological properties have been detected in the tissues and body fluids of toads,[2] but little is known about the structure or function of these related compounds. It has been suggested that, by virtue of their potency as digitalis-like inhibitors of Na^+,K^+-ATPase and therefore of active monovalent cation transport, bufadienolides and their derivatives may be important in sodium homeostasis in toads which migrate between fresh and salt water environments.[3]

It has also been suggested that bufadienolides and their derivatives may be prototypes for similar digitalis-like inhibitors of Na^+,K^+-ATPase in higher species.[4] Since little is known about vertebrate metabolism of endogenous cardiotonic steroids in any species, we have initiated studies of bufadienolide metabolism in the toad. The present report deals with the isolation, chemical identification and biochemical characterization of two major digitalis-like inhibitors of Na^+,K^+-ATPase in the bile of the toad, *Bufo marinus*. Both compounds are bufadienolide derivatives, but they differ in structure from the bufadienolides and bufotoxins of toad skin. Moreover, both biliary bufadienolide derivatives are considerably less potent than bufadienolides and bufotoxins as inhibitors of Na^+,K^+-ATPase, thus suggesting that they may be either prohormonal precursors or metabolic inactivation products of the active digitalis-like compounds of the toad.

MATERIALS

Toads, *Bufo marinus* (National Reagents, Bridgeport CT), were sacrificed by pithing according to a procedure approved by the Institutional Animal Care and Use Committee. Marinobufagin and marinobufotoxin were isolated from toad skin by methods described by Shimada *et al.*[1b,5] Sheep kidney Na^+,K^+-ATPase was a gift from Dr. Lois K. Lane, Department of Pharmacology and Cell Biophysics, University of Cincinnati.[6] A crystalline suspension of pyruvate kinase (700 units/ml) and lactic dehydrogenase (1,000 units/ml) in ammonium

sulfate was purchased from Sigma Chemical Company, St. Louis MO. [^{3}H(G)]ouabain (specific activity 20-27 Ci/mmole) was purchased from New England Nuclear, Billerica MA. Sephadex LH-20 was obtained from Pharmacia Fine Chemicals, Piscataway NJ.

METHODS

Droplet countercurrent chromatography (DCCC) was performed on an Eyela instrument (50 tubes, length 100 cm, inner diameter 0.35 cm). The lower phase of the solvent system, $CHCl_3$-CH_3OH-H_2O (2:2:3), was used for silica gel thin layer chromatography (tlc) as developing solvent; for DCCC the lower and upper phases were used, respectively, as the stationary and mobile phases. Tlc plates were sprayed with the Kedde reagent[7] or with antimony trichloride (5% in $CHCl_3$) and, with the latter reagent, examined under long uv after heating at 100 ° C for 5-10 min. Nmr spectra were measured on a 250 MHz Brücher WM-250 instrument using $CHCl_3$ (δ 7.24) or methanol (δ 3.30) as internal standards. Ir spectra were recorded on a Perkin-Elmer 983 Infrared spectrophotometer and uv spectra were measured on a Perkin-Elmer 320 spectrophotometer. Mass spectra were obtained from a Ribermag 10-10 or VG 70 EQ instrument.

The activity of Na^+,K^+-ATPase in the presence and absence of inhibitors was measured at 37°C in a final reaction volume of 2.5 ml containing 25 mM histidine (pH 7.4), 5 mM Na_2ATP, 25 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 0.4 mM ß-NADH, 1 mM phosphoenolpyruvate and 20 µl pyruvate kinase/lactic dehydrogenase.[8] The reaction was initiated by the addition of enzyme (2 to 3 µg). After an initial equilibration period (5-10 min), the reaction was linear up to 30 min. Activity was estimated from the decrease in absorbance at 340 nm over this linear portion. Ouabain at a final concentration of 10^{-4} M completely inhibited the ATPase activity, so that ouabain-insensitive activity was essentially zero.

The inhibition of Na^+,K^+-ATPase binding of [^{3}H]ouabain was determined by a nitrocellulose membrane filtration method.[8] Experiments were carried out at 37°C in the presence of 5 mM $MgCl_2$, 50 mM Tris-Cl (pH 7.4), 25 nM [^{3}H]ouabain and varying concentrations of inhibitors in a final volume of 1.0 ml. The reaction was initiated with the addition of enzyme (0.9-1.0 µg) and allowed to proceed for 2 h. Nonspecific binding was measured in the presence of 10^{-4} M nonradioactive ouabain, and was only 2-3% of control binding (measured in the absence of added inhibitor).

The inhibition data from the enzymatic and binding assays were fit to a two compartment model (Equation 1) using NNLSQ, an interactive non-linear fitting program available from CET Research Groups Ltd., P.O. Box 2029, Norman, OK 73070, U.S.A.

$$A = B_0 + B_1/(1 + I/B_2) \qquad \text{(Equation 1)}$$

In Equation 1, A is enzyme activity or [^{3}H]ouabain binding in the presence of a known concentration (I) of inhibitor, B_0 is ouabain-insensitive activity or nonspecific binding, B_1 is enzyme activity or radioligand binding in the absence of inhibitor, and B_2 is the concentration of inhibitor exerting 50% inhibition of enzyme activity or of radioligand binding (I_{50}). All data were corrected for nonspecific activity or binding and normalized to control activity or binding (as measured in the absence of added inhibitor).

EXPERIMENTAL PROCEDURES AND RESULTS

The gall bladders of 200 toads were lyophilized to give 17.3 g of residue which was triturated with methanol (200 ml x 3) and filtered. The filtrate was evaporated under reduced pressure at <40 °C to give a total of 14 g of a green amorphous residue. The extract was dissolved in methanol at a concentration of 250 mg/ml and passed, in five 2.5 g portions, through a Sephadex LH-20 column (73 x 4.2 cm), eluted with methanol and

collected in 9.5 ml aliquots. The fractions showing similar tlc patterns and uv absorption spectra (with maxima at 296 nm) were combined, and the results are shown in Figure 1. The earlier, more hydrophobic, fractions did not contain significant amounts of bufadienolides or bufotoxins, as indicated by the absence of compounds which exhibited a λ_{max} of 296 nm or which were detected on tlc with the antimony trichloride staining reagent; in contrast, the later eluting, more hydrophilic, fractions V and VI contained compounds which had absorption maxima at 296 nm and which gave a positive reaction with antimony trichloride on tlc plates. In addition, fractions IV through VI also gave transient positive purple reactions on tlc plates with the Kedde reagent.

Marinobufagin-3-sulfate, (1a) - Fractions V and VI contained several compounds which had uv absorption maxima at 296 nm and which were effective inhibitors, both of Na^+,K^+-ATPase enzymatic activity and of the binding of [^{3}H]ouabain by Na^+,K^+-ATPase. On preliminary characterization, these compounds appeared to be similar in properties to the bufadienolide-3-sulfates described by Shimada *et al.* in toad skin.[1b] Fraction VI (320 mg) was further separated on DCCC and collected in 5 ml aliquots. Fractions 18 to 24 were combined to give 5.3 mg of marinobufagin-3-sulfate **(1a)**,[5] (Figure 2) *Rf* 0.4; negative chemical ionization-mass spectrometry (CI-ms) (methane) *m/z* 479 (M - NH_4), 399 (479 - SO_3), 382 (399 - OH), 364 (382 - H_2O); ^{1}H-nmr (CD_3OD) δ 7.90 (22-H, dd, *J* 9.7, 2.4 Hz), 7.44 (21-H, d, *J* 2.4 Hz), 6.26 (23-H, d, *J* 9.7 Hz), 4.75 (3-H, br s), 3.62 (15-H, s), 0.97 (19-Me, s) and 0.77 (18-Me, s); ^{13}C-nmr (CD_3OD) δ 151.8 (22-C), 149.5 (21-C), 124.5 (20-C), 115.3 (23-C), 77.6 (3-C), 75.8 (14-C), 74.9 (5-C), 61.3 (15-C), 17.2 (18-C) and 17.0 (19-C).

12ß-Hydroxytetrahydroresibufogenin-3-sulfate (2) - Fraction IV in Figure 1 was divided in four 505 mg aliquots. Each aliquot was fractionated on a Sephadex LH-20 column (31 x 3.0 cm), eluted with methanol-H_2O

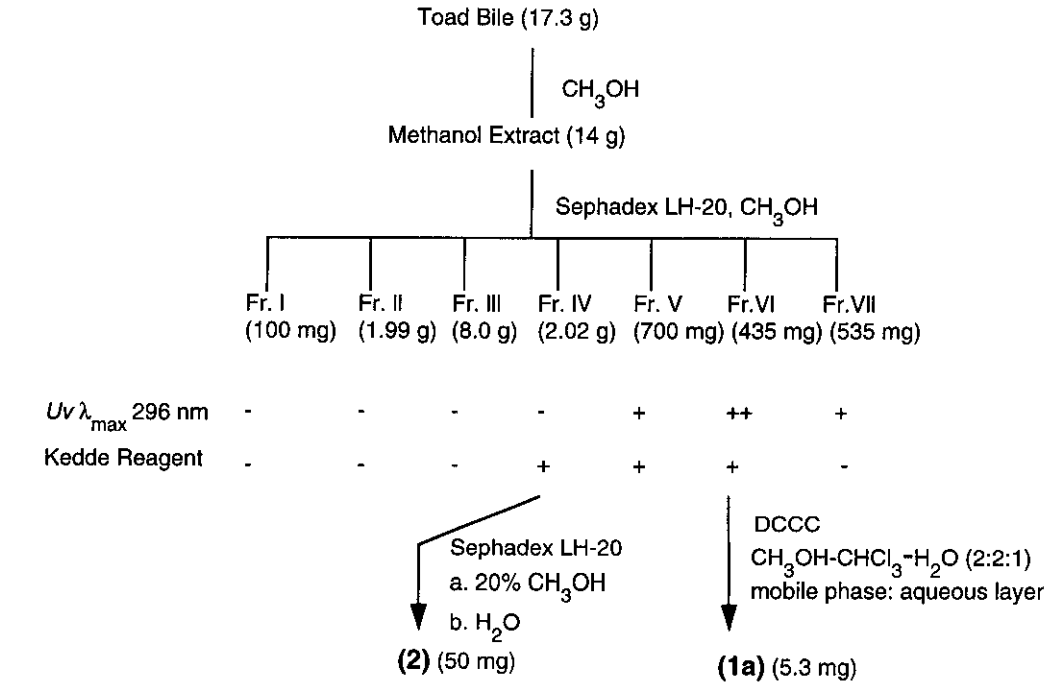

Figure 1. Fractionation and separation of cardiotonic agents from toad bile.

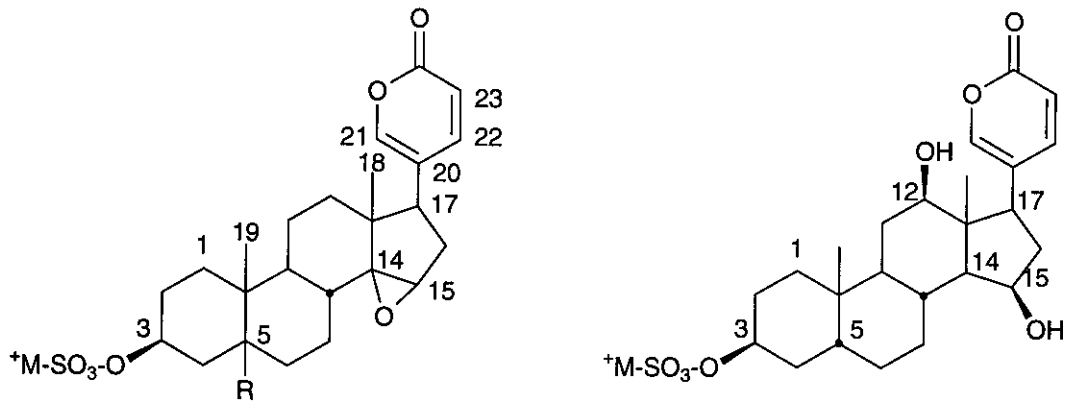

Figure 2. **1a** Marinobufagin-3-sulfate; **1b** Resibufogenin-3-sulfate;

2 12ß-Hydroxytetrahydroresibufogenin-3-sulfate.

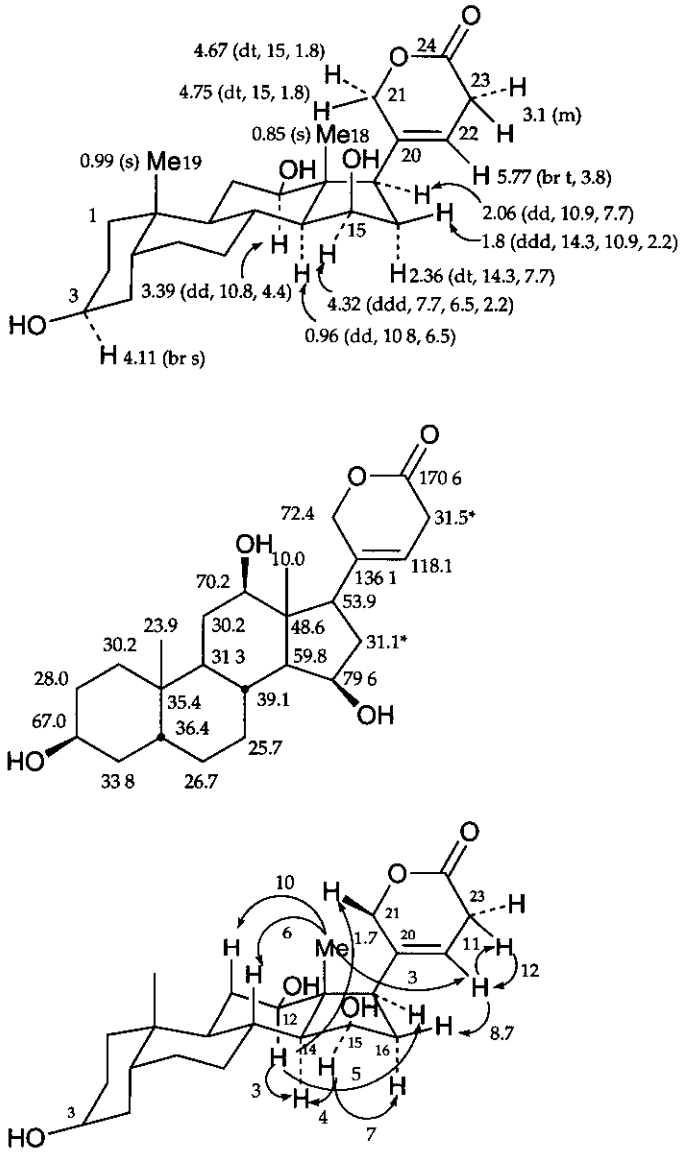

Figure 3. **a.** ^{1}H-Nmr assignment **b.** ^{13}C-Nmr assignment **c.** NOE difference studies of 12ß-hydroxytetrahydroresibufogenin **(3)**.

(1:4) and collected as 5 ml aliquots to give a total of 580 mg of a residue showing a faint reaction to the Kedde reagent. This residue was further purified by repeated chromatography on a Sephadex LH-20 column (55 g),

eluted with water, to give 50 mg of compound **(2)** as an amorphous solid, *Rf* 0.3; negative CI-ms (methane) *m/z* 483 (M-NH_4), 403 (483 - SO_3), 386 (403 -OH), 368 (386 - H_2O), 350 (368 - H_2O); ^{1}H-nmr (CD_3OD) δ 5.77 (22-H, t, *J* 3.7 Hz), 5.18 (21ß-H, br dd, *J* 14.8, 3.7 Hz), 4.69 (21α -H, br d, *J* 15.0 Hz), 4.70 (3-H, br s), 4.24 (15-H, m), 3.08 (23-CH_2, m), 2.29 (16α-H, dt, *J* 8.6, 7.5 Hz), 1.00 (19-Me, s) and 0.83 (18-Me, s); ^{13}C-nmr (CD_3OD) δ 137.7 (20-C), 118.3 (22-C), 80.2 (15-C), 76.9 (3-C), 70.5 (12-C), 24.2 (19-C) and 10.5 (18-C); no uv absorption at 296 nm was observed.

Solvolysis of compound (2) - A solution of compound **(2)** (22 mg) in pyridine-dioxane (1:1, 4 ml) was heated in a sealed tube at 120-125°C for 4 h.[9] After cooling, the brown reaction solution was partitioned between water (40 ml) and chloroform (40 ml x 2). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to give 15.3 mg of residue which was purified on a silica gel column (5 g) with methanol-$CHCl_3$ (2:98) as eluant. The desired fractions were combined to give 9.5 mg of compound **(3)**, mp 178-178.5°C (acetonitrile); ir ν_{max} (KBr) 3500, 3444, 3302 (OH), 1731 (C=O), 1648 (C=C); fast atom bombardment-high resolution mass spectrometry: MH^+ at *m/z* 405.2758 for $C_{24}H_{36}O_5$ + H (calcd 405.2638); ^{1}H-nmr ($CDCl_3$) see Figure 3a; ^{13}C-nmr ($CDCl_3$) see Figure 3b; no uv absorption was observed at 296 nm.

Acetylation of compound (3) - A solution of **3** (7 mg) in 1 ml of pyridine-acetic anhydride (1:1) was kept at room temperature overnight. The reaction mixture was evaporated and the residue was purified on a silica gel column (3 g) using chloroform (30 ml) and 1% methanol in chloroform (70 ml) successively. The 1% methanolic fractions were combined and evaporated to give 3.8 mg of the triacetylated product **(4)**; chemical ionization-high resolution mass spectrometry: MH^+ at *m/z* 531.2926 for $C_{30}H_{42}O_8$ + H (calcd 531.2955); electron impact-mass spectrometry: *m/z* 530 (M^+), 470 (M^+-HOAc), 410 (M^+-2HOAc) and 350 (M^+-3HOAc); ^{1}H-nmr ($CDCl_3$) see Figure 4.

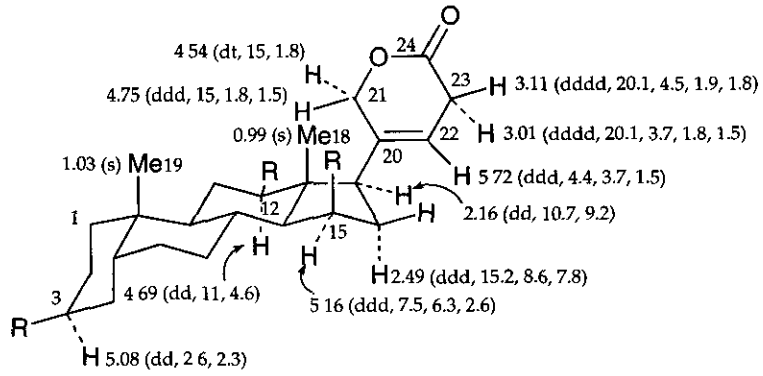

Figure 4. ^{1}H-Nmr assignment for 3,12,15-triacetoxytetrahydroresibufogenin **(4)**.

Structure determination of compound (2) - The structure of compound **(2)** characterized as its solvolysis product, compound **(3)**, was elucidated on the basis of spectral analyses. The molecular formula of compound **(3)**, $C_{24}H_{36}O_5$, suggested it to be a bufadienolide analog (C_{24}) instead of a cardenolide (C_{23}). The ^{1}H-nmr *spectrum and mass spectrum* of its acetate **(4)** indicated the presence of three hydroxy functions for compound **(3)**. The presence of a 3-sulfate function was supported by comparison with nmr data between the parent compound **(2)** *(3-H at δ 4.70, C-3 at δ 76.9) and the solvolysis product* **(3)** (3-H at δ 4.11 and 3-C at δ 67.0).[5] The other two hydroxy functions were assigned as 12ß and 15ß positions based on analyses of nmr spectra nuclear Overhauser effect (NOE) difference studies (Figure 3c). *12ß-Hydroxyl group was supported by the* coupling pattern of 12-H (δ 3.39, dd, *J* 10.8 and 4.4 Hz) and its γ *gauche* effect to C-18 which was shifted upfield by 6 ppm in ^{13}C-nmr spectra.[10] The *trans* nature of the C/D ring was supported by enhancement of 14-H upon irradiation of 12-H at δ 3.39; the 15ß-OH configuration was confirmed by enhancement of 14-H and 16α-H upon irradiation of 15-H at δ 4.32. In the lactone moiety, the double bond between 20-C and 22-C was established by irradiation of the olefinic proton at δ 5.77. This experiment enhanced 23-CH_2 by 11% and 16ß-H by 8.7%; reverse irradiation of 23-CH_2 at δ 3.10 again enhanced 22-H by 12%.

The conformation of the lactone moiety relative to the steroid ring system as shown in Figure 3 was also *suggested from* NOE difference studies, where irradiation of 12 α-H led to a 1.7% enhancement of the 21ß-H

signal. However, irradiation of the 21-H signals caused no NOE on the 16-H signals. Molecular models suggest that preference for this conformation may be due to the hydrogen bonding of the 12ß-OH to the lactone oxygen.

^{13}C-Nmr of compound **(3)** (Figure 3b) was assigned mainly on comparison with digoxigenin[10] and steroids with *trans* C/D ring junctions.[11]

Na^+,K^+-ATPase studies - The abilities of marinobufagin-3-sulfate **(1a)** and of 12ß-hydroxytetrahydroresibufogenin-3-sulfate **(2)** to inhibit sheep kidney Na^+,K^+-ATPase were compared with the inhibitory potency of marinobufagin and of marinobufotoxin using an enzyme-linked spectrophotometric assay method. All four compounds inhibited enzymatic activity, with a relative potency of marinobufagin > marinobufotoxin > marinobufagin-3-sulfate > 12ß-hydroxytetrahydroresibufogenin-3-sulfate (Figure 5). As shown in Table I, marinobufagin (I_{50}=0.259 µM) was 2.5 times as potent as marinobufotoxin (I_{50}=0.646 µM), 10.8 times as potent as marinobufagin-3-sulfate (I_{50}=2.79 µM) and 1160 times as potent as 12ß-hydroxytetrahydroresibufogenin-3-sulfate (I_{50}=300 µM). The relatively good fit of the data (Figure 5) to the single site model is consistent with the presence in sheep kidney of a single (α_1) isoform of the catalytic subunit.[12]

All four compounds also inhibited the binding of [^{3}H]ouabain to its receptor with the same rank order of potencies (Figure 6). The relative potencies as inhibitors in the radioligand binding assay were similar to those in the enzymatic assay (Table I), except for the observation that the relative effectiveness of 12ß-hydroxytetrahydroresibufogenin-3-sulfate in the radioligand assay was somewhat greater (approximately 1/300 that of marinobufagin).

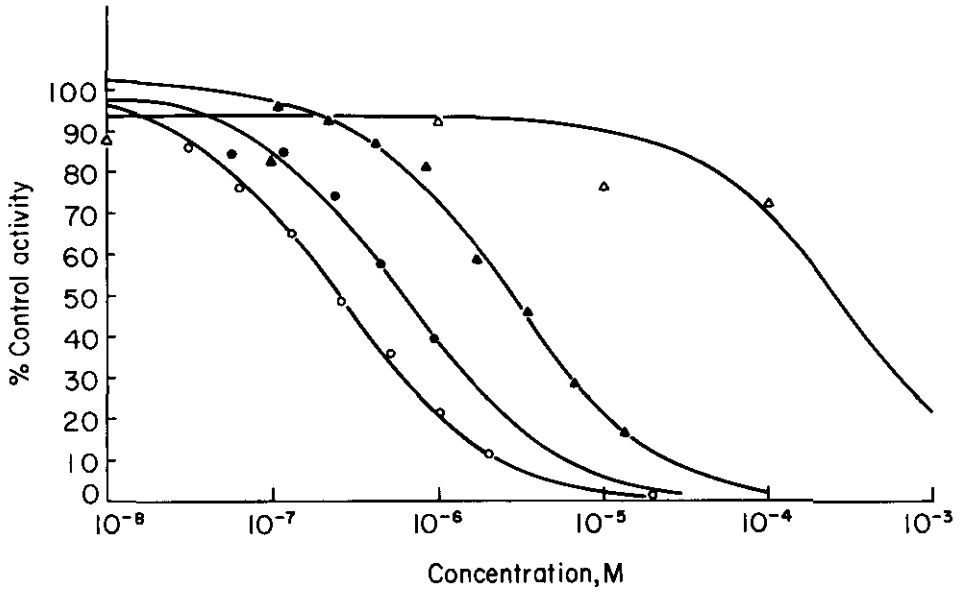

Figure 5. Inhibition of Na^+,K^+-ATPase enzymatic activity by marinobufagin (O), marinobufotoxin (●), marinobufagin-3-sulfate **(1a)** (▲) and, 12ß-hydroxytetrahydroresibufogenin-3-sulfate **(2)** (△).

Table I

Inhibition of Na^+,K^+-ATPase enzymatic activity and [^{3}H] ouabain binding

Inhibitor	Enzyme activity I_{50} (μM)	[^{3}H]ouabain binding I_{50} (μM)	K_I^* (μM)
Marinobufagin	0.259 ± 0.015	0.419 ± 0.016	0.032
Marinobufotoxin	0.646 ± 0.068	0.756 ± 0.078	0.059
Marinobufagin-3-sulfate **(1a)**	2.79 ± 0.18	4.17 ± 0.22	0.323
12ß-Hydroxytetrahydro-resibufogenin-3-sulfate **(2)**	300 ± 155	125 ± 7	9.7

*Calculated, using Equation 2 (see text for details)

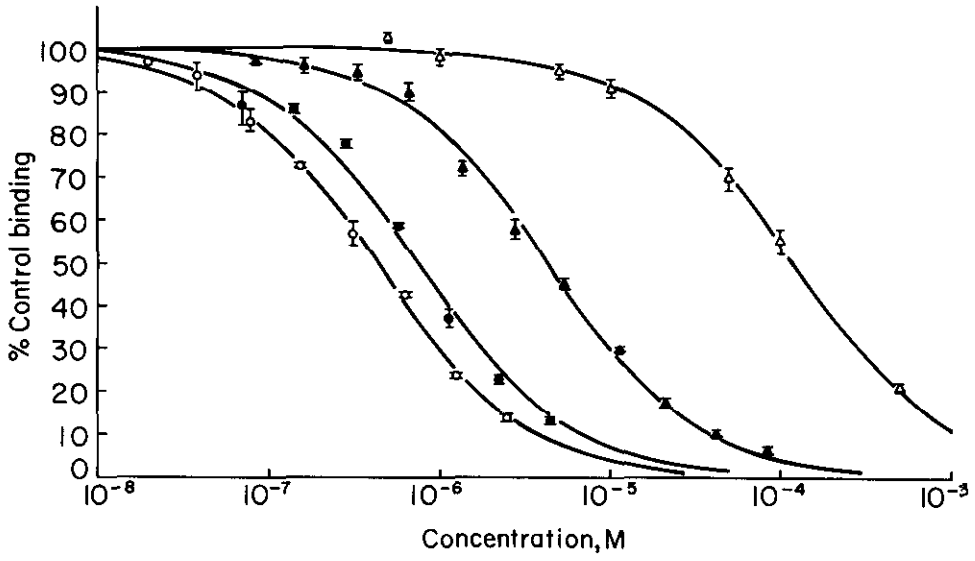

Figure 6. Inhibition of Na^+,K^+-ATPase binding of 25 nM [^{3}H]ouabain by marinobufagin (O), marinobufotoxin (●), marinobufagin-3-sulfate (**1a**) (▲) and, 12ß-hydroxy-tetrahydroresibufogenin-3-sulfate (**2**) (Δ). Binding of [^{3}H]ouabain was measured as described in Materials and Methods. Values are averages ± SD (or range) for 2-3 duplicate measurements at each concentration.

The I_{50} values for inhibition of radioligand binding (Table I) can be related to the dissociation constant (K_I) of each inhibitor by the Cheng and Prusoff equation[13] under certain conditions.

$$K_I = I_{50}/(1 + L/K_L) \qquad \text{(Equation 2)}$$

Equation 2 may be applied since: (a) the concentration of Na^+,K^+-ATPase receptor (2-4 nM) is low compared to L, the concentration of the [^{3}H]ouabain radioligand (25 nM); (b) K_L, the dissociation constant for the radioligand (2.1 nM) is known;[8] (c) there is a single homogeneous population of receptors; and, (d) the reaction time of 2 h is sufficient for equilibrium to be achieved (the $t_{1/2}$ for approach to equilibrium is *ca.* 10 min[8]). As shown in Table I, the K_I values for binding (measured in the absence of K^+) were

calculated, using Equation 2, and found to be lower than the K_I values for enzymatic activity (measured in the presence of K^+). For example, the affinity of the enzyme for marinobufagin and for marinobufotoxin was 8- to 11-fold greater under the conditions used in the binding assay. This higher affinity for bufadienolides in the absence of potassium is consistent with earlier studies[8] showing that [^{3}H]ouabain had an 8-fold higher affinity for the same sheep kidney enzyme (K_L=2.1 nM) in the presence of MgATPNa than in the presence of MgATPNaK (K_L=16 nM).

DISCUSSION

This study provides evidence that there are digitalis-like compounds in the bile of the toad, *Bufo marinus*. These biliary digitalis-like compounds are not the well-characterized bufadienolides and bufotoxins of toad skin and venom, but rather are a group of polar bufadienolide derivatives. The two most abundant of these compounds have been identified as the 3-sulfate derivatives of marinobufagin and of 12ß-hydroxy-tetrahydroresibufogenin, respectively. These compounds differ from the principal bufadienolides and bufotoxins of toad venom and skin not only in their chemical structure but also in their effectiveness as inhibitors of Na^+,K^+-ATPase.

Marinobufagin-3-sulfate (**1a**) is one of the bufadienolide-3-sulfates originally described by Shimada *et al.*, but it has previously been found only in trace amounts in toad skin.[1b,5] In contrast with toad skin, however, we have found that bufadienolide-3-sulfates appear to be the major digitalis-like compounds present in toad bile. As previously reported by Shimada *et al.*,[14] we have found that marinobufagin-3-sulfate is about 10% as effective as marinobufagin in the inhibition of Na^+,K^+-ATPase activity.

Neither 12ß-hydroxytetrahydroresibufogenin nor its 3-sulfate derivative (**2**) has previously been described; these compounds differ from other naturally occurring bufadienolide derivatives in that their lactone rings are reduced. In the current study, 12ß-hydroxytetrahydroresibufogenin-3-sulfate was found to be less than 1% as effective as marinobufagin as an inhibitor of Na^+,K^+-ATPase. The presence of a reduced lactone ring is known to cause significant reductions in the potency of other cardiotonic steroids,[15] and it seems probable that the presence of the reduced lactone ring and the presence of the 3-sulfate group both contribute to the diminished effectiveness of 12ß-hydroxytetrahydroresibufogenin-3-sulfate as an Na^+,K^+-ATPase inhibitor. Because of the reduction of its lactone ring, 12ß-hydroxytetrahydroresibufogenin does not exhibit a λ_{max} at 296 nm, and was detected by a positive Kedde color reaction. Thus, in addition to being a mechanism for the *in vivo* inactivation of cardiotonic steroids, reduction of the lactone ring interferes with the detection of toad digitalis-like compounds by the standard methods of 296 nm uv absorption and of Na^+,K^+-ATPase inhibition.

The precise physiological role of 3-sulfate derivatives in toad bufadienolide metabolism is not clear at this time. Presumably, the 3-sulfates of marinobufagin and of other active bufadienolides may be either precursors or products of unconjugated bufadienolides; studies with radiolabeled bufadienolides will be required to determine the metabolic relationship. In the case of the 3-sulfate of 12ß-hydroxytetrahydroresibufogenin (**2**), resibufogenin or its 3-sulfate (**1b**) is probably a precursor. It is known that plant and intestinal microbes are capable both of 12ß-hydroxylation of resibufogenin[16] and of reduction of the lactone rings of other cardiotonic steroids.[17] Thus, it is possible that the 12ß-hydroxytetrahydroresibufogenin derivative reflects bufadienolide which has been metabolized by gut flora while undergoing enterohepatic circulation.

Toads of the genus *Bufo* are remarkably resistant to the toxic effects of cardiotonic steroids,[18] thus enabling them to tolerate the presence, in their tissues and body fluids, of high concentrations of potent

bufadienolides and bufotoxins.[1-3] However, this study presents evidence that *Bufo marinus* possesses at least two effective pathways, *viz.* 3-sulfation of the steroid A ring and reduction of the lactone ring, for rendering bufadienolide derivatives less active than the potent bufotoxins and unconjugated bufadienolides of venom and skin. If inactivation of digitalis-like compounds is physiologically advantageous or necessary in an extremely digitalis-resistant species such as the toad, analogous pathways of inactivation may be essential in any digitalis-sensitive species capable of producing endogenous digitalis-like compounds. Such pathways, particularly those involving modification of the lactone ring, could interfere with the identification of putative mammalian digitalis-like compounds both by chemical methods and by biochemical methods employing the inhibition of Na^+,K^+-ATPase activity.

In connection with mammalian metabolism of digitalis-like compounds, it has been noted that humans, who metabolize digitalis glycosides rather slowly,[19] metabolize some bufadienolide derivatives quite rapidly. Proscillaridin, a cardiac glycoside with a bufadienolide aglycone moiety, undergoes extensive first-pass inactivation, much of it by glucuronidation and sulfation, in the human intestinal wall,[20] with less than 5% of plasma proscillaridin and less than 1% of biliary proscillaridin being present in active, unconjugated form.[20,21] It has recently been reported that rats rapidly convert bufalin and another toad bufadienolide, cinobufagin, to relatively inactive derivatives.[22] These observations in humans and in rats are suggestive of the existence of mammalian enzyme systems potentially capable of preventing the accumulation of toxic concentrations of endogenous cardiotonic steroids. If such enzyme systems exist, inactive products and/or precursors of cardiotonic steroids may be present in mammalian body fluids in excess of the active compounds, thus accounting for the fact that the levels of specific Na^+,K^+-ATPase inhibitory activity found in human serum and urine are normally quite low.[23] Efforts are therefore being made to extend our studies of bufadienolide metabolism in the toad, in the hope that identification of inactivated bufadienolide products may provide useful clues for the detection of presumptive metabolites of mammalian digitalis-like compounds.

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