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Original Article

Preliminary investigation of the disposition of the molluscicidal saponin deltonin from *Balanites aegyptiaca* in a snail species (*Biomphalaria glabrata*) and in mice

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A mixture of deltonin and 25-isodeltonin (approx. 1 : 1) was found to be the molluscicidal principle in seeds of *Balanites aegyptiaca* (L.) Del. The disposition in the schistosomiasis vector *Biomphalaria glabrata* and in mice was studied. Snails were exposed to ^{3}H-labelled saponin in water. Administration to mice was oral (O) and intravenous (IV). Snails absorbed the compounds rapidly and showed a high degree of bioaccumulation. The highest concentration was found in the foot followed by the intestines. Snails could excrete the compounds from all organs investigated. Saponin given orally to mice was partly hydrolysed in the intestine, a fraction of genuine and hydrolysed compound being absorbed. Genuine saponin given IV was rapidly distributed to the liver, kidney (medulla), lung and spleen. Compounds did not pass the placenta nor enter the CNS. Excretion was mainly biliary, a minor fraction being excreted in the urine all as genuine compound. About 80% was excreted within 72 hr. While only two (more lipophilic) metabolites were formed in significant amounts by the mice, three such metabolites were seen in extracts of snails. The final toxic agent and the mode of action to target snails remains to be further investigated.

Keywords: molluscicide, deltonin, disposition, *Balanites aegyptiaca*, *Biomphalaria glabrata*, mouse.

Introduction

Schistosomiasis (=bilharzia) is the name of a complex of several vectorborn trematode infestations in man[1)] and animals.[2)] Human infestations are mainly due to three species of blood flukes, *i.e. Schistosoma haematobium* (endemic in Africa and the Middle East), *S. mansoni* (Egypt, northern and southern Africa, some West Indies islands, northern two thirds of South America) and *S. japonicum* (Japan, China, the Philippines, Celebes, Thailand, and Laos). *S. mansoni* is often seen in Puerto Ricans living in the United States.[3)]

The life history of schistosomes is made up of four components: Two free living but non-feeding larval stages which pass through hypotonic fresh water and two parasitic stages, the intermolluscan larval stage during which they reproduce asexually within snails, and adult worms which reproduce sexually in humans.[4)] A number of species of water dwelling snails are intermediate hosts for these diseases, for example, snails belonging to the genus *Bulinus* (host of *S. haematobium*) and snails of the genus *Biomphalaria* (host of *S. mansoni*). *Biomphalaria glabrata* is the most important host in the Western Hemisphere.[1)]

After asexual multiplication in the snail, the parasite leaves the snail as infective cercariae released into the water. These infect the human host by penetrating the skin.[4)]

Although the prices of effective drugs for treatment such as praziquantel have decreased dramatically since the lifting of the patent, *i.e.* from around US$ 3 to US$ 0.07 per tablet, and thus strengthened the role of control strategies based on treatment instead of eradication of vector snails,[5)] there still exists a need for effective, water-soluble and safe molluscicides for the latter purpose.

Natural molluscicidal organic compounds include a variety

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of substance classes such as coumarins,[6] diterpenes,[7] alkaloids[8] and saponins.[9,10] Several saponins were investigated as pesticides for the control of snails, which are vectors for *Schistosomiasis*.[11–13]

The desert tree *Balanites aegyptiaca* (L.) Del. was shown to contain molluscicidal constituents in its different plant parts,[14] followed by other species of *Balanites*.[15] This property goes hand in hand with high toxicity, also to fish,[16] as is characteristic of saponins. A number of saponins have already been isolated from the fruit mesocarp of *B. aegyptiaca*[17,18] and others from the bark and root.[19]

The aims of the present study were threefold: (1) to identify the major molluscicidal constituent of the seeds of *B. aegyptiaca*, (2) to study its uptake, distribution and excretion in a mammal species (mouse), and (3) to study its uptake, distribution and excretion in a pesticide target organism (*Biomphalaria glabrata*). It was considered of great interest to make two parallel disposition studies. Few studies are available of the fate of saponins in mammals. Such data are prerequisites for further toxicological studies,[20] if saponins from *B. aegyptiaca* are to be approved as a pesticide, as demonstrated for constituents of *Phytolacca dodecandra*.[21–27] Knowledge about their disposition in a water-dwelling snail (target organism) on the other hand, can help better understanding of the mechanisms behind—and treatment regimes needed—using saponins to combat of vector snails for bilharzia. A number of non-mammal freshwater living organisms, such as fish, are also vulnerable to the toxicity of many saponins.[16]

Materials and Methods

1. Instruments and chemicals

Spectra were recorded on the following instruments: NMR (Bruker AC 250 P), IR (Shimadzu FTIR-8100), FAB-MS (Jeol AX 505 W). Chemicals were analytical grade from Merck, Darmstadt, Germany. Thin-layer-chromatography (TLC) was carried out using aluminium sheets precoated with Silica Gel $60F_{254}$ (Merck no. 5554).

2. Isolation and radiolabelling of molluscicidal saponin(s)

2.1. Plant material, extraction and purification

Whole ripe fruits of *Balanites aegyptiaca* (L.) Del. were sampled from trees in the western part of Sudan. Herbarium samples of leaves and fruits are deposited at the Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan. Ground seeds (7 kg) were defatted by mechanical pressing. The press cake was percolator extracted at approx. 30°C with ethanol–water (9 : 1, v/v). The extract was concentrated *in vacuo* at 50°C. Residual oil was extracted with petroleum ether to leave 400 g of dry "crude saponin." This was extracted several times with methanol–acetone (60 : 40, v/v). Pooled extracts were evaporated *in vacuo* at 40°C to give 141 g "partly purified crude saponin". Only one major molluscicidal fraction was obtained using four separate steps of bioassay guided[25] column chromatography (Si-gel; mobile phases): (1) $CHCl_3$/MeOH/H_2O (6 : 6 : 1), (2) $CHCl_3$/MeOH/H_2O (6 : 4 : 1), (3) $CHCl_3$/MeOH/H_2O (6 : 3 : 1; lower phase), and (4) EtOAc/$(CH_3)CO$/MeOH/ $CHCl_3$/H_2O (40 : 30 : 12 : 10 : 8).

2.2. Assay for molluscicidal effect of fractions

For each fraction/compound and concentration (see below) the assay was as follows: 200 ml of aqueous solution and five seemingly healthy snails (*Biomphalaria glabrata*) (6–10 mm) were used and the exposure time was 24 hr. Snails recovered from the test solution after 24 hr in 200 ml of water were inspected. Criteria of death were inactivity, discolouration and (in the case of doubt) foul odour upon crushing.[28] Each experiment was duplicated. A control was carried out using water with the same resulting concentration of dimethylsulfoxide (DMSO; see below). Each dry chromatographic fraction was dissolved in DMSO and diluted with water to give a 100 ppm solution (DMSO concentration less than 1%). The assay was carried out at 100 ppm. For molluscicidal pure compound(s) isolated LC_{50} and minimum LC_{100} were estimated using factor 2 serial dilutions of 100 ppm solution.

2.3. Compound identification

Identification was by means of ^{13}C NMR (including DEPT spectra at 90 and 135°), TLC analysis of the mixture obtained upon enzymatic hydrolysis using crude enzymes from *Helix pomatia* snail, enzymatic assay to prove the D-form of isolated glucose moieties and Fast Atom Bombardment Mass Spectrometry (FAB-MS) with dithiothreitol : dithioerythritol (5 : 1) as a matrix to give the molecular weight. The isolate was found to be a mixture of deltonin (25*R*) and isodeltonin (25*S*). ^{13}C NMR signals (pyridine-d_5) for the isolated compound (*R*/*S*) and deltonin[29,30] (in brackets): C_1–C_{27} δ 37.5 (37.6), 30.1 (30.0), 78.1 (78.5), 38.9 (39.0), 140.8 (141.0), 121.8 (121.8), 31.8 (32.3), 32.2 (31.9), 50.3 (50.5), 37.2 (37.3), 21.1 (21.2), 39.9 (40.0), 40.5 (40.5), 56.6 (56.8), 32.3 (32.4), 81.1/81.2 (81.2), 62.9/61.9 (62.8), 16.4 (16.3), 19.4 (19.2), 41.9/42.5 (42.1), 15.1/14.9 (15.0), 109.3/109.8 (109.3), 31.7/22.7 (31.9), 30.0/26.2 (29.4), 30.6/26.4 (30.7), 66.9/65.1 (67.0), 17.3/16.4 (17.3); glucose moieties G 1–6 δ 100.0 (100.0), 77.8 (78.0), 76.2 (76.0), 82.1 (82.0), 77.3 (77.5), 62.6 (62.0); G 1′–6′ δ 105.2 (105.0), 75.0 (75.0), 78.3 (78.0), 71.2 (71.3), 78.5 (77.5), 62.1 (62.5); rhamnose moiety R 1–6 δ 101.8 (101.5), 72.5 (72.0), 72.8 (72.5), 74.2 (74.0), 69.5 (69.0), 18.7 (18.5). FAB-MS *m*/*z*: 907 and 885 ($M+Na^+$ & $M+H^+$), 739 ($M+H^+-146$; deoxyhexose), 723 ($M+H^+-162$; hexose), 415 ($M+H^+-470$; 2 hexose+1 deoxyhexose). In accordance with the proposed structure, the mixture obtained after hydrolysis in 2N HCl/50% dioxane (1 : 1)[19] gave spots on TLC (Si-gel with 15% EtOAc in benzene as mobile phase) corresponding to both diosgenin and yamogenin, and at nearly the same intensity. The mean ratio of (*R*- and *S*) signal intensities of the ^{13}C NMR spectrum (C-16 to C-27) was 1.2,[29] while a 1 : 1 ratio was estimated from the signals at 900 and 920 cm^{-1} in the IR-spectrum.[29] In conclusion, deltonin/25-isodeltonin (approx. 1 : 1), both compounds de-

scribed earlier,[29–32] was the molluscicidal agent isolated. ^{3}H-deltonin/25-isodeltonin was prepared by Amersham Labs., England, by oxidation with morpholine-*N*-oxide and tetrapropyl-ammonium-perruthenate followed by reduction using sodium-boro-(^{3}H)-hydride (personal information; Amersham Labs.). The reaction product was purified by preparative TLC (Si-gel with 1-butanol/H_2O/HOAc (12 : 5 : 3) as mobile phase), identified by FAB-MS and investigated for purity by TLC. Specific activity and radiochemical purity were 614 Gbq/mmol (16.6 Ci/mmol) and approx. 87%, respectively.

3. Animals

Pigmented NDF1 mice (F_1 hybrids between females of the outbreed stock NMRI and males of the inbred strain DBA/2J), were used. Male- as well as non-mated female mice (b.w. approximately 20 g), as well as mid-pregnancy (18 days) and late pregnancy (22 days) females were all obtained from Bomholdtgaard Breeding and Research Centre, Denmark. Mice were housed in plastic cages with a 12 hr dark cycle, fed a standard pellet diet and given water *ad libitum*. *Biomphalaria glabrata* snails were 10–20 mm in size, *i.e.* a little bigger than used for toxicity test during compound purification, and were obtained from the Danish Bilharziasis Laboratory (WHO Collaborating Centre), Charlottenlund, Denmark. The snails were maintained in distilled water with 0.104 g of $CaCl_2$ and 0.26 g of $MgSO_4 : H_2O$ per litre (snail water) at 23–25°C, with a light–dark cycle of 12 hr. Snails were fed dried lettuce.

4. Biological experiments

4.1. Compound administration and tissue preparation (mice)

Mice were given ^{3}H-labelled compound (0.15 μCi in 70% ethanol) as a single intravenous (IV) bolus injection of 0.2 ml in a tail vein (corresponding to approx. 400 μg/kg b.w.) or they were given the same dose orally by gastric intubation. Animals were placed separately in a normal plastic cage or a metabolic cage. After a predetermined time, the animal was either decapitated (metabolic cage studies) or anaesthetized with ether, rapidly frozen in a bath of acetone/dry ice and mounted in aqueous 2% carboxymethylcellulose. Freeze-sectioning: thickness 40 μm for autoradiography and 60 μm for sampling for liquid scintillation counting.

4.2. Autoradiography (mice)

Freeze-dried 40 μm sections on tape were pressed against X-ray films (Hyperfilm-3H, Amersham, England), exposure time of three months.

4.3. Metabolic cages studies (mice)

Mice dosed IV were sacrificed after 72 hr, while two dosed orally were allowed to live for 96 hr. Urine and faeces were collected at times (post admin.): 2, 4, 6, 24 and 72 hr (IV), and 96 hr (*per os*).

4.4. Uptake and excretion studies (snails)

Six groups of four snails were exposed to 3.75 μCi/ml (0.2 ppm) of labelled compound in snail water for 10, 30 min, 1, 4, 12 and 24 hr, respectively. The intestine, hepato-pancreas, kidney, reproductive tract, ovotestis and foot were dissected under a microscope. For each time and organ, pooled tissues were homogenized, and aliquots taken for liquid scintillation counting and chromatographic analysis, respectively. For studies of subsequent excretion, another batch of 28 snails was exposed to 1.88 μCi/ml for 24 hr. The lower dose was used to ensure that the concentration in the clean recovery (excretion) water did not rise significantly during the start of the excretion phase, and inhibited further excretion. After 24 hr the snails were subdivided into groups of four animals. Each group recovered in 200 ml of snail water for 0, 1, 4, 12, 24, 48 and 72 hr, after which they were treated as described above.

4.5. Liquid scintillation counting (mice and snails)

Mouse and snail organs, and faeces and urine from mice were analysed. Faecal samples were dried at 100°C and comminuted. To 20 mg of sample 100 μl of water was added for 30 min of rehydration. After 1 ml of Soluene 350 had been added, samples were incubated at 50°C for 48 hr. Following cooling, 0.5 ml of isopropanol and 0.2 ml of H_2O_2 (35%) were added to decolorize. Samples were counted (after 48 hr of equilibration) using 10 ml of a Hionic Flour scintillator (Packard). Weighed tissue samples of approx. 10 mg (from snail dissections or fresh 60 μm sections of mice) were added to 100 μl of water and 1 ml of Soluene 350, and further treated as faecal samples. To urine was added 10 ml of scintillator.

4.6. Chromatographic analysis

TLC studies were done on mouse urine as well as on extracts of stools and the following snail organs: intestine, hepato-pancreas, foot and reproductive tract. Extracts were prepared by adding 5 ml of a mixture of water and EtOAc (1 : 1) per gram of material followed by shaking for 1 hr and centrifugation—to separate phases. Both the water and organic phase were analysed on pre-coated silica plates using ethyl-acetate: acetone: methanol: chloroform: H_2O (40 : 30 : 12 : 10 : 8) as mobile phase.[33] The labelled genuine compound was applied as standard at 0.6 and 3.0 μCi. Chromatograms were visualized based on the radioactivity of spots, using the same technique as for sections from autoradiography.

Results

Deltonin/25-isodeltonin (approx. 1:1) was identified as the major molluscicidal principle in the seeds of *Balanites aegyptiaca* (L.) Del. collected in Sudan. The compounds are steroidal spirostanol glycosides (oligospirostanosides).

1. Snails

The LC_{50} was approx. 2 ppm and the minimum LC_{100} was found to be between 5 and 10 ppm (curve not shown). Subsequent disposition studies used a concentration of 0.2 ppm or lower. The result of a 24-hr uptake study, from a 0.2 ppm

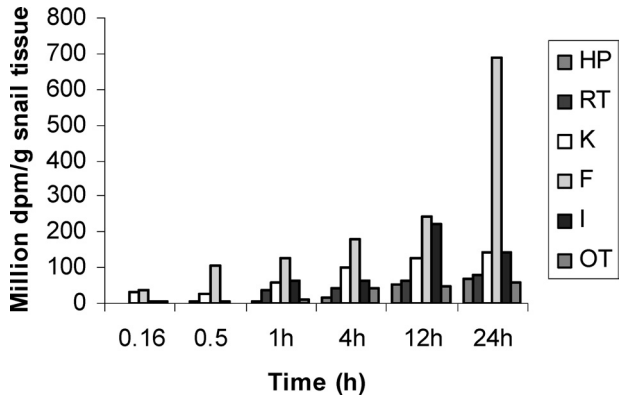

Fig. 1. Radioactivity in snail tissues after different times of incubation in 0.2 ppm of labelled deltonin/25-isodeltonin (uptake study). Symbols denote: HP (hepato-pancreas), RT (reproductive tract), K (kidney), F (foot), I (intestines), OT (ova-testis).

aqueous solution of ^{3}H-labelled compound, in *Biomphalaria glabrata* is shown in Fig. 1. An identical experiment using 0.1 ppm gave rise to very similar relative tissue distribution, the resulting activities being around half of those obtained at the 0.2 ppm level (results not shown). In both experiments, overall radioactivity in the body rose throughout the whole exposure period. This was also the case for each of the organs separately analysed, *i.e.* the hepato-pancreas, reproductive tract, kidney, foot, intestines and ova-testis; however, organs showed very different affinities to the agent (Fig. 1). Thus, activity in the foot was constantly the highest, and at most times close to double the activities seen in any other organs. Gradually, the activities in the kidney and then also the intestines increased, at times reaching levels between 50 and 90% of that seen in the foot; however, at 24 hr, the activity in the foot dominated the distribution picture (Fig. 1). The strong dominance of the activity in the foot was less pronounced in the 0.1 ppm experiment (Fig. 3, 0 hr). In tissues from snails exposed for 24 hr (at 0.2 ppm), about 50% of the labelling was due to the parent compound, the rest of the activity being found as three chromatographically well separated (R_f: 0.47, 0.49 and 0.53; parent compound 0.35) slightly more lipophilic metabolites, as envisaged from TLC analysis (Fig. 2).

When snails exposed to the low-dose 0.1 ppm level for 24 hr were transferred to fresh water, excretion started immediately and continued up to at least 12 hr. Quite rapidly during the excretion process, the relative activities found in the different investigated organs reached more or less the same level. Under the batch conditions used (no water change), a steady state was reached in the organs investigated after about 24 hr (Fig. 3).

2. Mice

The results of distribution studies after IV administration to mice (female: non-mated, early pregnant and late pregnant; male) are summarized in Table 1, while Figs. 4 and 5 show selected autoradiograms of a non-mated female mouse 4 hr after dosing by IV and orally, respectively. Being a preliminary study to guide further detailed risk assessment studies in mammals, a single animal set-up per time/administration was used. Comparing the organ distribution seen in IV- administered animals 20 min (when immediate distribution has oc-

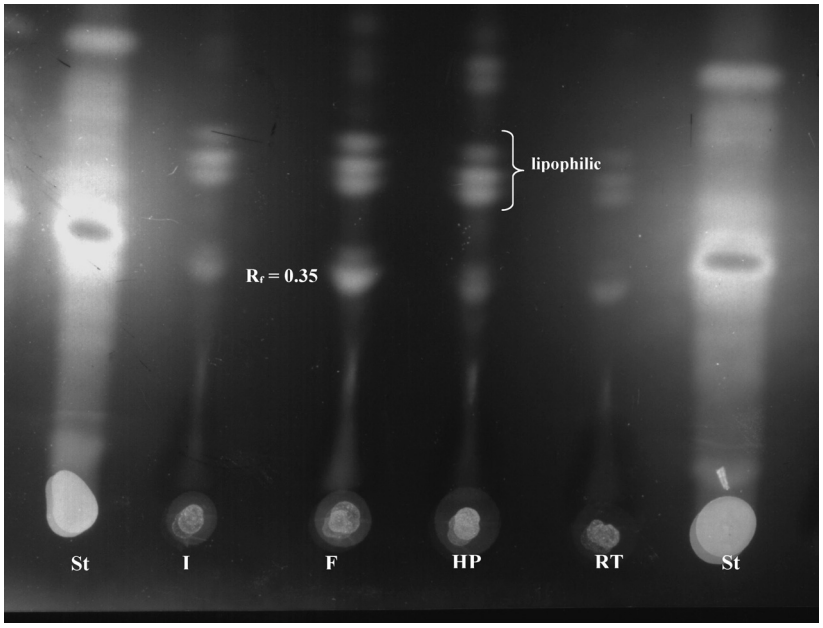

Fig. 2. Thin-layer chromatogram of extracts (water phase) of different tissues of snails exposed to ^{3}H-deltonin/25-isodeltonin. Symbols denote: St (standard; major spot at R_f=0.35 equal to the component deltonin/25-isodeltonin), I (intestine), F (foot), HP (hepato-pancreas), RT (reproductive tract).

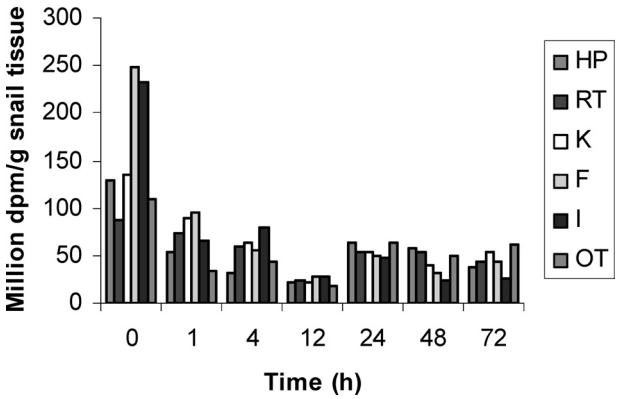

Fig. 3. Remaining radioactivity in snail tissues after different times of recovery in pure water (excretion study). Symbols denote: HP (hepato-pancreas), RT (reproductive tract), K (kidney), F (foot), I (intestines), OT (ova-testis).

curred) and 4 hr after dosing, respectively, *i.e.* ignoring sex and mating differences, the overall relative distribution of activity between central organs such as the liver, kidneys, lung, heart, brain and spleen are close to identical; however, after 20 min there is an overall tendency of significant but varying labelling in the lung, stomach and intestines, the rest of the organs showing more constant relative distribution of liver≥kidney≥heart≥brain≈spleen (Table 1). At 4 hr, labelling in the lungs in general had decreased significantly while the activity found in the stomach and the intestines still are significant but varying, the distribution between the rest of the organs is as follows: liver≥kidney≥lung≈heart≥brain≈spleen (Table 1). Even at 4 days after IV administration, significant labelling is seen in the intestines, liver, kidneys, lung, heart and stomach (Table 1). Autoradiograms of orally dosed females indicated a very high activity in the stomach and intestines (20 min, 4 hr) but after 4 hr also showed considerable labelling of the liver and kidneys, some genuine or transformed compound clearly being absorbed (Fig. 5).

The results of the quantitative excretory studies are illustrated in Fig. 6. Within the first 6 hr about 10% of the activity injected IV as ^{3}H-deltonin/25-isodeltonin was excreted in the faeces and a similar amount in the urine. The mice excreted approx. 80% (78.4) of the total activity within 72 hr, the main proportion (67%) by the faecal route and 11.4% in the urine (Fig. 6).

Chromatograms of the water and EtOAc phases, respectively, from the extraction of faeces of mice after IV administration, proved about 50% of the labelling to be excreted in faeces as the parent compound, the other approx. 50% being assigned to two slightly more lipophilic substances (R_f-values: 0.50 and 0.60; parent compound 0.35, TLCs not shown). The less important urinary excretion consisted of the parent compound only, since no other spots were detected on the chromatograms made from the EtOAc phase (TLC not shown) or the water phase (Fig. 7). For oral administration, the result for faeces was about the same as for IV; however, a different pattern were seen for the urine, where both the parent compound and at least two (R_f-values: 0.56 and 0.63; parent compound 0.35) slightly more lipophilic metabolites were also found (TLCs not shown). Both parent saponin and metabolites formed in the gastrointestinal tract were thus ab-

Table 1. Tissue distribution of radioactivity at different time intervals after intravenous injection of a single dose of 8 μg (0.15 μCi) of ^{3}H-deltonin/25-isodeltonin in mice[a),b)]

Tissue	Exposure time[c)]/	Female mice (non-pregnant)					Male mice		Early pregnancy		Late pregnancy	
	5 min	20 min	1 hr	4 hr	24 hr	4 days	20 min	4 hr	20 min	4 hr	20 min	4 hr
Intestine	440	2289	6449	19984	3816	539	1513	410	454	2159	643	3381
Liver #	1986	3796	4541	9040	17139	992	—	3280	1301	2386	1387	4575
Kidney	1052	1900	1693	3434	33965	1317	1482	2426	938	1950	752	2387
Lung#	532	13534	763	2213	23411	349	62869	1248	562	—	—	—
Brain	283	413	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
Heart	420	510	1368	376	1272	369	916	709	183	1285	776	304
Stomach	247	10895	1356	4231	1583	1175	549	820	4058	5212	1785	1363
Spleen#	1107	2185	—	—	—	—	—	—	—	—	—	—
Muscle#	16042	—	—	—	—	—	—	—	—	—	—	—
Foetus									<100	<100	<100	<100
Placenta									182	<100	422	141

[a)] dpm/mg of tissues. [b)] (—) means no activity above background detected. [c)] As defined by period from administration to death.

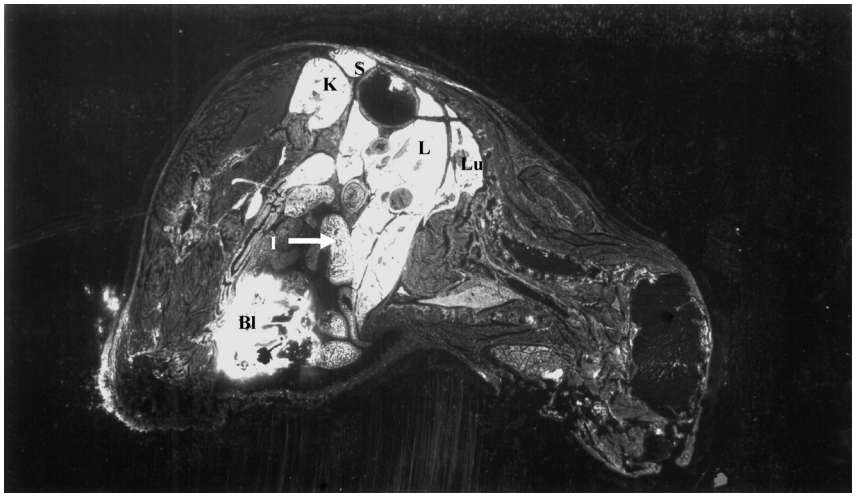

Fig. 4. Whole-body autoradiogram of a non-mated female mouse 4 hr after intravenous injection (IV) of ^{3}H-deltonin/25-isodeltonin. Symbols denote: K (kidney), S (spleen), L (liver), Lu (lung), Bl (bladder), I (intestines).

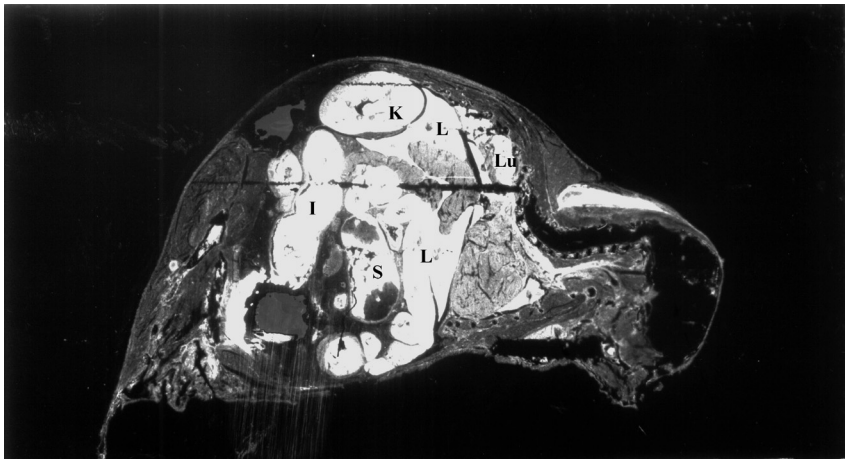

Fig. 5. Whole-body autoradiogram of a non-mated female mouse 4 hr after oral administration of ^{3}H-deltonin/25-isodeltonin. Symbols denote: K (kidney), L (liver), Lu (lung), I (intestines), S (stomach).

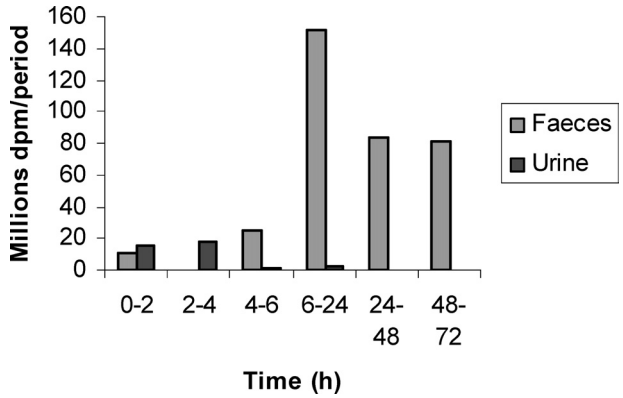

Fig. 6. Mean excretion of radioactivity from labelled deltonin/25-isodeltonin and its metabolites in faeces and urine of four female mice during different time intervals after intravenous administration.

sorbed and excreted in the urine.

Discussion

Deltonin/25-isodeltonin was originally isolated and its structure elucidated from *Dioscorea deltoidea* and has also been shown as present in cell suspensions of this species.[31,32] It was found in the stem bark of *Balanites roxburghii* in a search for insect antifeedant compounds.[29] It has not peviously been shown to possess molluscicidal activity, as have on the other hand a number of structurally closely related saponins.[17–19]

1. Snails

Most molluscicidal compounds have been subjected only to very limited investigations concerning their disposition, their more detailed effect(s), and their mode of action in the target organism(s).

Many glycosides with a steroidal or a pentacyclic triterpene

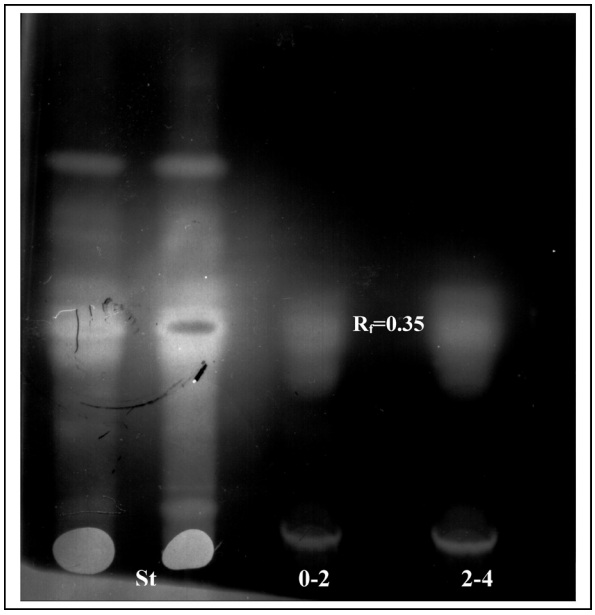

Fig. 7. Thin-layer chromatogram of extracts (water phase) of urine samples from mice given ^{3}H-deltonin/25-isodeltonin by the intravenous route. Symbols denote: St (standard; major spot at R_f=0.35 equal to the component deltonin/25-isodeltonin), 0–2 (urine pooled over the period 0–2 hr after administration), and 2–4 (urine pooled over the period 2–4 hr after administration).

genin are molluscicidal at low concentrations. Within this group of saponins, saponin-like compounds and steroidal alkaloids some, although restricted, knowledge has been gained concerning the structural demands for their activity, as also for their effects on cell membranes.[13,34] Nevertheless, Francis *et al.* recently concluded that "the precise details of the interactions between saponins and membranes need more elucidation so that the molecular mechanisms involved could be better understood."[13]

Concerning their disposition in target vector snails and in mammals, only a study on aridanin[20] exists and can be used for comparison. This study also uses the vector snail *B. glabrata*.[20]

Comparing the uptake and distribution of deltonin/25-isodeltonin with the patterns seen for aradanin,[20] both differences and similarities are seen. In the present study, the concentration in the foot was constantly the highest and rose throughout the 24 hr of incubation (Fig. 1). In the aridanin study, the concentration in the foot also showed the relative highest concentration among the organs at most measurement points during the 24 hr; however, it peaked after 1 hr of exposure after which it found a constant level at around half that seen at 1 hr. After 24 hr of constant exposure (0.2 ppm in both studies) the relative distribution of compound concentration between the different organs was as follows in the two studies [deltonin (this study)/aridanin[20]]: F/F≥I/I≥RT/HP≥HP/RT≥OT/OT (abbreviations as explained in Fig. 1). The excretion process closely follows that of aridanin. Both studies show a minimum overall content of around 1/10 of the start concentration in the snails after 12 hr, all organs showing a considerable decrease in radioactivity (ref. 20 and Fig. 2). The snail metabolises the compound(s) by the action of surface-linked enzymes or after uptake. The identity of the metabolites remains to be established. Hydrolytic cleavage of sugar moieties to give more lipophilic compounds seems to be a reasonable explanation. It seems logical that partial or total hydrolysis of the genuine saponin(s) may mean a reduction of molluscicidal activity.[13] In the study on aridanin, the snail converted the glycoside to more lipophilic substances.[20]

2. Mice

In general, the results from counting on tissues, analysis by autoradiography and TLC analysis of excretions support each other concerning the conclusion on the disposition of deltonin-25-isodeltonin in the mouse. After 4 hr, the picture seen for IV administration was entirely dominated by biliary excretion as evidenced by the very high labelling of the intestines/intestinal contents (Table 1). This is in agreement with the fact that the majority of labelling after IV administration was excreted through the faecal route (Fig. 6). The fact that considerable labelling was still seen in all major organs except the brain and spleen 4 days after IV administration (Table 1) further points to the possibility of considerable enterohepatic circulation, maybe mostly of the more lipophilic metabolites found in the faeces. Dominating biliary excretion is in accordance with earlier findings for aridanin[20]; however, it seems that higher oral uptake occurs with deltonin/25-isodeltonin than seen for aridanin. Another difference is seen in that only more hydrophilic metabolites (than genuine aridanin) were detected in both the urine and the faeces after IV administration of the compound,[20] while this study in general showed the formation of more lipophilic metabolites.

No radioactivity was generally detected in the CNS throughout the total study period, as also seen for aridanin.[29] Importantly, this observation also holds for oral administration with its uptake of both genuine compound and partially hydrolysed more lipophilic constituents. Labelling did not show any affinity to melanin, *i.e.* neither the eyes nor skin showed any significant labelling. Also, the present study gave no results to indicate any substantial differences between the two sexes.

For pregnant mice, no (or very low) radioactivity was found in the foetus although faint labelling was seen in the placenta (Table 1). These results were confirmed by autoradiograms of pregnant mice after 4 hr of exposure (not shown). Again, the results are in accordance with those earlier reported for aridanin.[20]

Although the formation of more lipophilic metabolites was seen for mice as well as snails, clearly nothing more specific can be said about their identity. The two metabolites detected in the faeces of both IV and orally administrated mice may be identical to the urine metabolites of orally fed mice. Theoreti-

cally, two of the three snail metabolites may be identical to the compounds formed in mice.

Conclusion

In conclusion, the compound and other lipophilic metabolites are accumulated by the snail *Biomphalaria glabrata* when exposed in water. If exposed to sublethal concentration-time combinations the snail is able to subsequently excrete the compound with a concentration decrease in all investigated organs. The highest concentration is always found in the foot, which may be used in studies of resulting exposure in the field.

The mouse absorbs a fraction of the parent compound together with some lipophilic metabolites formed in the gastrointestinal tract. None of these compounds pass the blood-brain barrier or the barrier to the foetus. Excretion is relatively slow and dominated by the faecal route.

The high toxicity of certain saponins to fish has traditionally been ascribed to the demonstrated damage to the respiratory epithelia although also damage to the intestinal mucosa has been described. When it comes to snails and molluscs many, especially monodesmosidic, saponins are toxic at mg per litre concentrations; however, concerning the mode of action, only general speculations about the detergent effect on the soft body membranes have been presented.[13] Experiments with isolated (natural or artificial) membranes have disclosed a very complex structure–activity relationship. A majority of investigations points to monodesmosidic compounds as the most damaging, the composition of the target membrane, the structure of the aglycone and the saccharide moiety being important.[13] The present study used a mixture of deltonin with 25-isodeltonin, monodesmosidic saponins with a branched trisaccharide moiety. Further studies will have to disclose whether there are differences between the two isomers concerning the biological properties observed here, if the metabolites formed in the intestines of mice are identical to those found in snails, and whether the high toxicity to snails is due to the genuine compound(s) or to one or more of its more lipophilic metabolites.

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