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PLANT MOLLUSCICIDES

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Key Word Index—Angiospermae; schistosomiasis; bilharzia; molluscicides; natural products.

Abstract—A review on the application of plant molluscicides in the control of schistosomiasis is presented. Laboratory bioassays are discussed, together with criteria for activity. An attempt has been made to provide a comprehensive list of known molluscicidal natural products.

INTRODUCTION

Molluscicides are agents toxic to snails (molluscs). Although generally harmless to humans, some snails, most notably of the genera *Biomphalaria*, *Bulinus* or *Oncomelania*, are directly implicated in the transmission of schistosomiasis (bilharzia). This parasitic disease is endemic throughout South America, Africa and the Far East, affecting more than 200 million people in over 70 countries. Incidences of schistosomiasis are increasing as a result of the construction of dams and the introduction of irrigation schemes, which inadvertently provide ideal breeding sites for the snail vectors.

Eggs from infected individuals enter water and produce miracidia (Fig. 1), which locate snails of the appropriate species and multiply into thousands of cercariae. The cercariae, after leaving the snails, can penetrate the skin of humans who come into contact with the appropriate water sources. Once through the skin, they change gradually into the mature trematodes (or flukes), known as schistosomes. The schistosomes (three main species are known: *Schistosoma haematobium*, *S. mansoni* and *S. japonicum*) mate and lay eggs which are carried away with faeces or urine.

Destroying the snails which harbour the developing schistosome larvae is one way to interrupt the parasite's life cycle and prevent human infection. The best method of controlling the disease, however, is chemotherapy with orally-administered schistosomal drugs for individuals with moderate or severe levels of infection [1]. The disadvantages of this approach are that it does not eliminate the infection entirely, the cost of recurrent treatment may become prohibitive and drug resistance may become a problem. A combination of efforts to break the schistosome life cycle, including focal-point molluscicíding may therefore be essential.

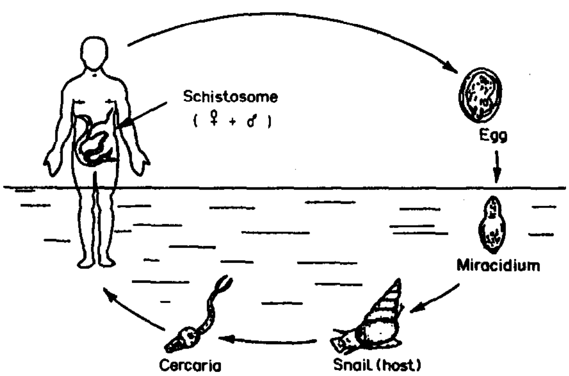


Fig. 1. Life cycle of *Schistosoma* species.

SYNTHETIC MOLLUSCICIDES

Considerable success has been achieved by the use of chemicals for the elimination of molluscs which transmit cercariae. Such molluscicides include copper sulphate, trifenmorph, Bayluscide[R] and sodium pentachlorophenate [2]. Extensive studies on their mode of application, their effectiveness, their toxicology and cost have been published [2].

PLANT MOLLUSCICIDES

When undertaking bilharzia control programmes using molluscicides, the deciding factor, especially for Third World countries, is the cost. Synthetic molluscicides are expensive and, in addition, may lead to problems of toxicity to non-target organisms and deleterious long-term effects in the environment. The possible development of resistance in schistosomiasis-transmitting snails is another important factor. The use of plants with molluscicidal properties is a simple, inexpensive and appropriate technology for focal control of the snail vector [3, 4]. Furthermore, investigation of plants used in traditional medicine or recorded in ethnopharmacological literature provides a ready means of increasing the diversity of available molluscicides and simplifying the choice of selective, ecologically-safe snail-killing compounds [5].

Since 1933, when control of schistosomiasis using fruits of *Balanites aegyptiaca* (Balanitaceae) was proposed [6], over 1000 plant species have been tested for molluscicidal activity [7]. Until about 5 years ago, relatively little was

Table 1. Major classes of natural products with recognized molluscicidal activity

Class of compound	Plant	Family	Reference
Triterpenoid saponins	*Phytolacca dodecandra*	Phytolaccaceae	[8], [9]
	Hedera helix	Araliaceae	[10]
	Lonicera nigra	Caprifoliaceae	[11]
Spirostanol saponins	*Cornus florida*	Cornaceae	[12]
	Balanites aegyptiaca	Balanitaceae	[13]
	Asparagus curillus	Liliaceae	[14]
Steroid glycoalkaloids	*Solanum mammosum*	Solanaceae	[15]
Diterpenes	*Wedelia scaberrima*	Compositae	[16]
	Baccharis trimera	Compositae	[17]
Sesquiterpenes	*Warburgia ugandensis*	Canellaceae	[18]
	Warburgia stuhlmannii	Canellaceae	[19]
	Ambrosia maritima	Compositae	[20]
	Podachaenium eminens	Compositae	[21]
Monoterpenes	Genus *Lippia*	Verbenaceae	[22]
Iridoids	*Olea europaea*	Oleaceae	[23]
Naphthoquinones	*Diospyros usambarensis*	Ebenaceae	[24]
Alkenyl phenols	*Anacardium occidentale*	Anacardiaceae	[25]
Chalcones	*Polygonum senegalense*	Polygonaceae	[26]
Flavonoids	*Baccharis trimera*	Compositae	[17]
	Polygonum senegalense	Polygonaceae	[27]
	Polygonum nodosum	Polygonaceae	[28]
Tannins	*Acacia nilotica*	Leguminosae	[29]
	Other species		[30]
Furanocoumarins	*Ruta chalepensis*	Rutaceae	[31]
Isobutylamides	*Heliopsis longipes*	Compositae	[32]
	Fagara macrophylla	Rutaceae	[33]
Alkaloids	*Culpurnia aurea*	Leguminosae	see text

known about the natural products responsible for the molluscicidal activity of the plants concerned but in the intervening period a number of active compounds have been isolated (see Table 1). Isolation and identification of the active constituents is essential for the study of their toxicities and stabilities under field conditions, for dosage purposes, for structure–activity investigations and for effects on snail metabolism or physiology. Once the natural products have been identified, their content in different plant parts can be determined.

TEST SYSTEMS AND CRITERIA FOR EFFICIENT MOLLUSCICIDES

Snails of the genus *Biomphalaria* are the invertebrate hosts for *Schistosoma mansoni*, *Bulinus* for *S. haematobium* and *Oncomelania* for *S. japonicum*. Therefore, in test systems for molluscicidal activity, *Biomphalaria* or *Bulinus* species are most frequently used. *Oncomelania* snails are more amphibious in life style and bioassays with this genus are consequently more difficult to develop. Molluscicide screening is generally carried out according to W.H.O. specifications [35]. The W.H.O. quantitates toxicity by means of LC_{90} values but LC_{50} [36] and 100% snail kill [18] values, all in ppm are also currently used. There is an urgent need to standardise methods used for phytochemical screening and bioassay to permit a better comparison of results by different investigators.

The simplest bioassay [18] employs *Biomphalaria glabrata* snails and involves placing the snails in a distilled water solution or suspension of the compound under test. The snails are considered dead when there is no observable heart beat on microscopic investigation.

The conditions for a viable plant molluscicide are as follows:

(a) The molluscicidal activity should be high. The crude extract from which the compound is obtained should have an activity at concentrations lower than 100 ppm. The activity of the strongest synthetic molluscicides lies around 1 ppm; e.g. for trifenmorph, the LC_{100} for *B. glabrata* is 0.25 ppm after an exposure time of 24 hr [36]. Similar values are obtained for *Bulinus* snails [36]. Consequently, to be effective competitors for synthetic molluscicides, plant molluscicides must have LC_{100} values of this order of magnitude. It is advantageous if the molluscicide also kills snail eggs.

(b) The plant in question should grow abundantly in the endemic area. Either the plant should be of high natural abundance or, alternatively, easily cultivated. In addition, regenerating plant parts (fruits, leaves or flowers) should be used and, if possible, not the roots, since this leads to destruction of the plant.

(c) Extraction of the active constituents by water is an advantage. The cost of organic solvents and accompanying extraction apparatus could be prohibitive for schistosomiasis-control programmes in Third World countries.

(d) Application procedures should be simple and safe to the operator. In addition, formulations and storage must be straightforward.

(e) The plant extract or molluscicide should possess low toxicity to non-target organisms (including humans). The discovery of compounds more specifically toxic to snails would be a great advantage. Furthermore, isolation of the

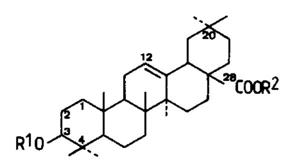
active compounds from those molluscicidal plants which are potentially applicable to field trials is important for toxicological and environmental impact studies.
(f) Costs should be low.

CLASSES OF PLANT MOLLUSCICIDES

Triterpenoid saponins

The molluscicidal properties of *Phytolacca dodecandra* (Phytolaccaceae) fruits were discovered by Lemma in 1964 [37] and this plant has subsequently become of great potential importance for the local control of schistosomiasis [38]. The dried berries of endod (*Phytolacca dodecandra*) are used as a soap substitute in Ethiopia. In rivers where people washed their clothes with these berries, Lemma noticed dead snails and came to the conclusion that *Phytolacca dodecandra* was responsible for this toxicity [37]. As a continuation of the investigation of the molluscicidal activity of this plant, a five-year pilot snail control programme in Ethiopia was found to significantly reduce *Schistosoma mansoni* infection rates [39]. Furthermore, the performance of endod was comparable to that of the synthetic molluscicide niclosamide. The compounds responsible for the activity are triterpenoid saponins (see Tables 2–4), with LC_{100} values as low as 2 ppm (compound **2**). Although it has been claimed that a pentacyclic triterpene of molecular formula $C_{30}H_{48}O_2$, isolated from *Hedychium gardnerianum* (Zingiberaceae) is active against *Biomphalaria alexandrina* snails [45], the aglycones oleanolic acid and hederagenin are inactive against *Biomphalaria glabrata* snails [11]. Compounds **1–13** are glycosides of oleanolic acid, **14–30** are glycosides of hederagenin, **31** and **32** are glycosides of bayogenin and **33** and **34** are glycosides of phytolaccagenin. The triterpene glycoside primulic acid is toxic to *Biomphalaria glabrata* at 8 ppm, whereas ginsenosides Rb_1 and Rf (dammarane glycosides) are inactive [40].

It is interesting to note, from Tables 2–4, that all monodesmosidic saponins (glycoside chain in position 3) are active against *B. glabrata* snails, whereas bidesmosidic saponins (sugar chain in positions 3 and 28) are inactive. In this context, the extraction procedure plays an important rôle, extraction with water providing predominantly monodesmosidic saponins and extraction of the same plant with methanol providing larger quantities of bidesmosidic saponins [9]. During the water extraction procedure, a fermentation process is probably occurring, hydrolysing the more labile ester-linked glycosides, while leaving the ether-linked glycosides intact. The molluscicidal activities of saponins also vary with the nature of the sugar chains, the sequence of the sugars, the inter-

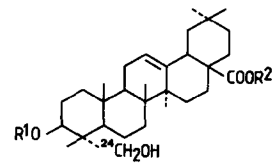


Oleanolic acid: $R^1=R^2=H$

Table 2. Molluscicidal activities of triterpene glycosides

Compound	R^1	R^2	Plant	Molluscicidal activity (ppm)	Reference
1	GlcA-	H	*Lonicera nigra*	2 (LC_{100})	[11]
2	Glc-^{2}Ara-	H	*Lonicera nigra*	2 (LC_{100})	[11], [40]
3	Rha-^{2}Ara-	H		80 (LC_{100})	[40]
4 (Lemmatoxin C)	Rha-^{2}Glc-^{2}Glc-	H	*Phytolacca dodecandra*	3 (LC_{90})	[41]
5	Xyl-^{3}Rha-^{2}Ara-	H		16 (LC_{100})	[40]
6	Rib-^{3}Rha-^{2}Ara-	H		32 (LC_{100})	[40]
7	Glc-^{4}Xyl-^{3}Rha-^{2}Ara-	H		32 (LC_{100})	[40]
8	Glc-^{4}Rib-^{3}Rha-^{2}Ara-	H		16 (LC_{100})	[40]
9 (Oleanoglycotoxin-A)	Glc-^{4}Glc (|^2 Glc)	H	*Phytolacca dodecandra*	6 (LC_{100}) 3 (LC_{90})	[9] [8]
10 (Lemmatoxin)	Glc-^{4}Glc- (|^3 Gal)	H	*Phytolacca dodecandra*	1,5 (LC_{90})	[42]
11	Glc-^{2}GlcA-	Glc-		—	[40]
12	Glc-^{2}Ara-	Glc-^{6}Glc-	*Lonicera nigra*	—	[11]
13	Glc-^{4}Glc- (|^2 Glc)	Glc-	*Phytolacca dodecandra*	—	[9]

—: inactive; LC_{100}: 100% lethal concentration to *Biomphalaria glabrata* snails; LC_{90}: 90% lethal concentration to *Biomphalaria glabrata* snails (LC values after 24 hr); Ara: α-L-arabinopyranosyl; Rha: α-L-rhamnopyranosyl; Xyl: β-D-xylopyranosyl; Rib: β-D-ribopyranosyl; Glc: β-D-glucopyranosyl; GlcA: β-D-glucuronopyranosyl; Gal: β-D-galactopyranosyl.

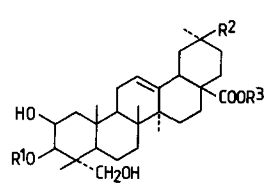


Hederagenin: $R^1=R^2=H$

Table 3. Molluscicidal activities of triterpene glycosides

Compound	R^1	R^2	Plant	Molluscicidal activity (ppm)	Reference
14	Glc-	H	*Hedera helix*	15 (LC_{100})	[10], [40]
15	Ara-	H	*Hedera helix*	3 (LC_{100})	[10], [40]
			Lonicera nigra		[11]
16	GlcA-	H	*Lonicera nigra*	16 (LC_{100})	[11]
17	Glc-²Glc-	H	*Hedera helix*	12 (LC_{100})	[10], [40]
18	Rha-²Ara-	H	*Hedera helix*	8 (LC_{100})	[10], [40]
19	Glc-²Ara-	H	*Lonicera nigra*	8 (LC_{100})	[11], [40]
20	Xyl-³Rha-²Ara-	H		8 (LC_{100})	[40]
21	Rib-³Rha-²Ara-	H		16 (LC_{100})	[40]
22	Glc-⁴Xyl-³Rha-²Ara-	H		8 (LC_{100})	[40]
23	Glc-⁴Rib-³Rha-²Ara-	H		8 (LC_{100})	[40]
24	Glc-⁴Glc- (²|Glc)	H	*Phytolacca dodecandra*	12 (LC_{100})	[9]
25	Ara-	Glc-⁶Glc-	*Lonicera nigra*	—	[11]
26	Glc-²Ara-	Glc-⁶Glc-	*Lonicera nigra*	—	[11]
27	Ara-	Glc-⁶Glc-⁴Rha-		—	[40]
28	Rha-²Ara-	Glc-⁶Glc-⁴Rha-		—	[40]
29	Glc-²Ara-	Glc-⁶Glc-⁴Rha-		—	[40]
30	Glc-⁴Glc- (²|Glc)	Glc-	*Phytolacca dodecandra*	—	[9]

Abbreviations: see Table 2.



Bayogenin: $R^1=R^3=H$, $R^2=CH_3$
Phytolaccagenin: $R^1=R^3=H$, $R^2=COOCH_3$

Table 4. Molluscicidal activities of triterpene glycosides

Compound	R^1	R^2	R^3	Plant	Molluscicidal activity (ppm)	Reference
31	Glc-⁴Glc-	CH_3	H	*Phytolacca dodecandra*	12 (LC_{100})	[9]
32	Glc-⁴Glc-	CH_3	Glc-	*Phytolacca dodecandra*	—	[9]
33	Xyl-	$COOCH_3$	H	*Phytolacca americana*	60	[43], [44]
34	Glc-²Xyl-	$COOCH_3$	H	*Phytolacca americana*	80	[43], [44]

Abbreviations: see Table 2.

glycosidic linkages and the substitution patterns of the aglycone.

As water is the final medium in which the plant molluscicide has to play its snail-killing rôle, the solubility of saponins in water is an important point in favour of this class of compounds when considering snail control schemes.

The activities of other molluscicidal plants have been attributed to saponins, e.g. *Securidaca longipedunculata* (Polygalaceae) [46], *Swartzia madagascariensis* (Leguminosae) [47] and *Wedelia scaberrima* (Compositae), in which the saponin mixture is toxic at 8 ppm to *B. glabrata* snails [48]. *Securidaca longipedunculata* and *Swartzia madagascariensis* are at present under study in our laboratory, in order to characterize the individual saponins responsible for the activity.

Spirostanol saponins

The first report on the use of a plant for the control of schistosomiasis involved fruits of the desert palm *Balanites aegyptiaca* (Balanitaceae) [6]. This Sudanese medicinal plant, which is an effective fish poison, kills both molluscs and the cercariae of schistosomes. Even at the time of the first use of the berries, it was postulated that the activity was due to the presence of saponins [6]. However, it was not until 1982 that spirostanol saponins, with yamogenin as the aglycone (Table 6), were found to be the constituents responsible for the molluscicidal activity [13].

A number of other spirostanol glycosides are now known to be molluscicides (see Tables 5–7). As in the case of the non-spiro triterpene saponins, the bidesmosidic glycosides (Table 7) are not noticeably active against *Biomphalaria glabrata* snails.

Many monodesmosidic spirostanol saponins are to be found in monocotyledonous plants [5] and a great deal of work remains to be done in elucidating the structures of relevant molluscicidal saponins, for example from the family Agavaceae, which includes the sisal plant *Agave sisalana*. Waste from sisal production may provide a source of saponins to be applied locally in bilharzia infected regions.

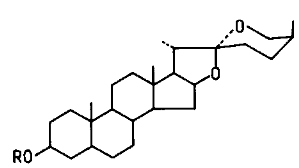
Steroid glycoalkaloids

Three azaspirostanol saponins, isolated from members of the family Solanaceae (which includes the tomato *Lycopersicon esculentum*) are toxic to snails (Table 8). A mixture of solasonine (**48**) and solamargine (**49**) was obtained from *Solanum mammosum* and this killed all *Lymnaea cubensis* snails at 10 ppm and all *Biomphalaria glabrata* snails at 25 ppm [15]. Commercially-available tomatine (**50**) was even more active. However, the aglycones solasodine and tomatidine were completely inactive [15].

It would appear that substitution of a nitrogen atom for an oxygen atom in the six-membered heterocyclic ring leads to very little change in molluscicidal activity (Tables 5, 6 and 8). However, a change in the aglycone from solasodine to solanidine (Table 8) gives rise to a complete loss of activity.

Diterpenes

Kaur-16-en-19-oic acid (**52**) from *Wedelia scaberrima* (Compositae) was found to be molluscicidal against

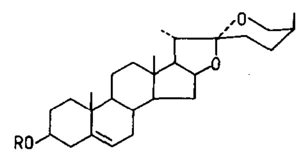


Sarsapogenin: R=H

Table 5. Molluscicidal activities of spirostanol saponins

Compound	R	Plant	Molluscicidal activity ppm (LC_{100})	Reference
35	Xyl-²Glc-	*Cornus florida*	6	[12]
36	Glc-²Glc-	*Cornus florida*	12	[12]
37	Rha-⁴Glc- (²Glc)	*Asparagus curillus*	20	[14]
38	Ara-⁴Glc- (²Rha)	*Asparagus curillus*	5	[14]
39	Ara (⁶) Rha-⁴Glc (²Glc)	*Asparagus curillus*	5	[14]

Abbreviations: see Table 2.

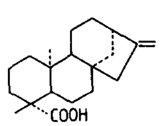


Yamogenin: R=H

Table 6. Molluscicidal activities of spirostanol saponins

<table>
<tr><th>Compound</th><th>R</th><th>Plant</th><th>Molluscicidal activity ppm (LC_{100})</th><th>Reference</th></tr>
<tr><td>40 (Balanitin-1)</td><td>Glc-^{4}Glc-
|^2 |^2
Rha Rha</td><td>Balanites aegyptiaca</td><td>5–10</td><td>[13]</td></tr>
<tr><td>41 (Balanitin-2)</td><td>Xyl-^{6}Glc-^{3}Glc-
|^2
Rha</td><td>Balanites aegyptiaca</td><td>5–10</td><td>[13]</td></tr>
<tr><td>42 (Balanitin-3)</td><td>Glc-^{4}Glc-
|^2
Rha</td><td>Balanites aegyptiaca</td><td>5–10</td><td>[13]</td></tr>
<tr><td>43</td><td>Rha-^{2}Glc-</td><td>Asparagus plumosus</td><td>25</td><td>[14]</td></tr>
<tr><td>44</td><td>Rha-^{3}Glc-
|^2
Rha</td><td>Asparagus plumosus</td><td>20</td><td>[14]</td></tr>
</table>

Abbreviations: see Table 2.



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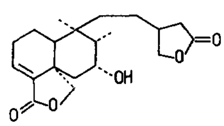

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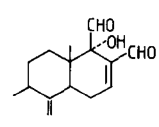

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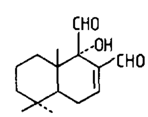

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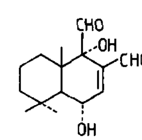

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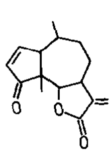

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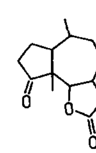

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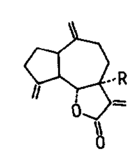


59 R=OH
60 R=H

Biomphalaria glabrata snails [16] but no details of activity were given. The *trans*-clerodane-type diterpene **53** from *Baccharis trimera* (Compositae) [17] only produced feeble toxicity (100 ppm for *Biomphalaria glabrata* snails).

Numerous species of Euphorbiaceae are piscicidal [49] and may possess molluscicidal properties [5]. The constituents of these plants responsible for the toxic effects are probably diterpenes but because they are also often skin irritants and tumour promoters [50], their possible future use in schistosomiasis-control programmes is not to be recommended.

Sesquiterpenes

The simple sesquiterpenes **54**–**56** and the sesquiterpene lactones **57**–**60** (Table 9) have quite potent molluscicide properties. Indeed, *Warburgia* species and *Ambrosia* species occur quite frequently in those parts of the world where schistosomiasis is endemic. *Ambrosia maritima* is molluscicidal, ovicidal and harmless to fish. Field trials in Egypt with the green plants and powdered material have met with moderate success [51].

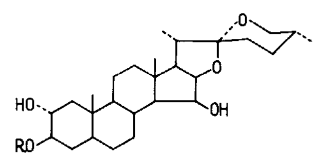
Unfortunately, caution should be exercised in the use of sesquiterpene-containing plants, especially those with sesquiterpene lactones, as they are known to be irritating and allergenic to humans [52]. A schistosomicidal heliangolide has been isolated from *Eremanthus goyazensis* [53] and may be worth investigating as a molluscicide.

Monoterpenes

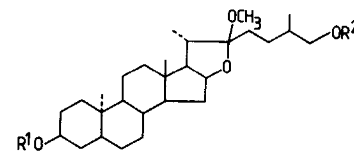
A brief report has appeared on the toxicity towards *Biomphalaria glabrata* snails of thymol (**61**), carvacrol (**62**) (not strictly terpenes but often included in this class of natural products) and limonene (**63**) from plants of the genus *Lippia* [22].

Iridoids

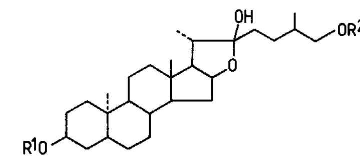
The iridoid glycosides ligstroside (**64**) and oleuropein (**65**) from the fruits of *Olea europaea* (Oleaceae) have been



A
Digitogenin: R=H



B
22-Methoxy (25*S*),5*β*-furostan-3*β*,26-diol: $R^1=R^2=H$

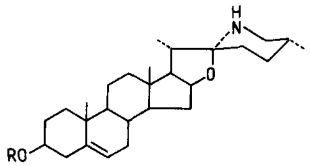


C
22-Hydroxy-(25*S*),5*β*-furostan-3*β*,26-diol: $R^1=R^2=H$

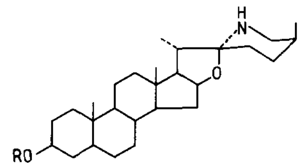
Table 7. Molluscicidal activities of spirostanol saponins

Compound	Aglycone	R	R^1	R^2	Plant	Molluscicidal activity: ppm (LC_{100})	Molluscicidal activity: Snails tested	Reference
45 (Digitonin)	A	Xyl-^{3}Glc-^{4}Gal- |^2 Glc-^{3}Gal			(*Digitalis purpurea*) (Scrophulariaceae)	10 25	*Biomphalaria glabrata* *Lymnaea cubensis*	[15] [15]
46	B		Ara |^6 Rha-^{4}Glc- |^2 Glc	Glc-	*Asparagus curillus*	—	*Biomphalaria glabrata*	[14]
47	C		Ara |^6 Rha-^{4}Glc- |^2 Glc	Glc-	*Asparagus curillus*	—	*Biomphalaria glabrata*	[14]

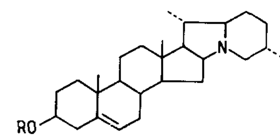
Abbreviations: see Table 2.



D
Solasodine: R=H



E
Tomatidine: R=H



F
Solanidine: R=H

Table 8. Molluscicidal activities of steroid glycoalkaloids

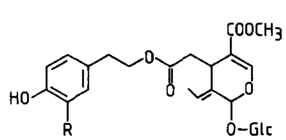
Compound	Aglycone	R	Plant	Molluscicidal activity: ppm (LC_{100})	Molluscicidal activity: Snails tested	Reference
48 (Solasonine)	D	Glc-^{3}Gal- |^{2} Rha	*Solanum mammosum* *Solanum nodiflorum*	see text		[15] [34]
49 (Solamargine)	D	2 × Rha, Glc	*Solanum mammosum*	see text		[15]
50 (Tomatine)	E	Xyl-^{3}Glc-^{4}Gal- |^{2} Glc	(*Lycopersicon esculentum*) (Solanaceae)	4 10	*Biomphalaria glabrata* *Lymnaea cubensis*	[40] [15]
51 (α-Solanine)	F	Glc-^{3}Gal- |^{2} Rha	(*Lycopersicon esculentum*) (Solanaceae)	—	*Biomphalaria glabrata*	[40]

Abbreviations: see Table 2.

Table 9. Molluscicidal activities of sesquiterpenes

Compound	Plant	Molluscicidal activity: ppm	Molluscicidal activity: Snails tested	Reference
54 (Muzigadial)	*Warburgia ugandensis*	5–10 (LC_{50})*	*Biomphalaria glabrata*	[18]
55 (Warburganal)	*Warburgia stuhlmannii*	2 (LC_{50})	*Biomphalaria glabrata*	[19]
56 (Mukaadial)	*Warburgia stuhlmannii*	20 (LC_{50})	*Biomphalaria glabrata*	[19]
57 (Ambrosin)	*Ambrosia maritima*	10–14 (LC_{100})	*Biomphalaria alexandrina* *Bulinus truncatus*	[20] [20]
58 (Damsin)	*Ambrosia maritima*	9–12 (LC_{100})	*Biomphalaria alexandrina* *Bulinus truncatus*	[20] [20]
59 (7α-Hydroxy-3-deoxyzaluzanin)	*Podachaenium eminens*	1 (LC_{100})	*Biomphalaria glabrata*	[21]
60 (3-Deoxyzaluzanin)	*Podachaenium eminens*	—	*Biomphalaria glabrata*	[21]

Abbreviations: see Table 2. LC_{50}: 50% lethal concentration.
*After 2 hr.

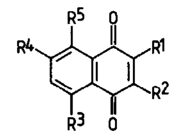


64 R=H
65 R=OH

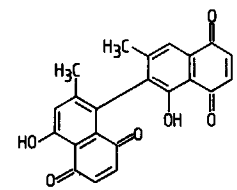
claimed to possess activity against *Biomphalaria glabrata* snails (100 ppm and 250 ppm, respectively) [23] but the activities of the pure compounds are so weak that they do not even meet the criteria for molluscicidal activity of plant extracts.

Naphthoquinones

The molluscicidal activity of this class of natural products has recently been reported for the first time [24]. Simple naphthoquinones are very effective at snail killing (Table 10), whereas prenylated (Vitamin K_1) and dimeric naphthoquinones (H, I) are inactive. Introduction of a hydroxy substituent into the quinonoid ring (isojuglone, lapachol) causes a significant decrease in activity, observed also for 3-methoxy-7-methyljuglone, an artefact obtained



G



H

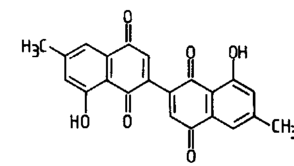


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Table 10. Molluscicidal activities of naphthoquinones

Compound	Skeleton	R^1	R^2	R^3	R^4	R^5	Plant	Molluscicidal activity ppm (LC_{100})	Reference
66 (Juglone)	G	H	H	OH	H	H	(*Juglans regia*) (Juglandaceae)	10	[24]
67 (Isojuglone)	G	OH	H	H	H	H	(*Lawsonia inermis*) (Lythraceae)	50	[24]
68 (7-Methyljuglone)	G	H	H	OH	CH_3	H	*Diospyros usambarensis*	5	[24]
69 (Plumbagin)	G	CH_3	H	OH	H	H	(*Drosera rotundifolia*) (Droseraceae)	2	[24]
70 (3-Methoxy-7-methyljuglone)	G	H	OCH_3	OH	CH_3	H		>50	
71 (Vitamin K_3; Menadione)	G	CH_3	H	H	H	H		3	[24]
72 (Naphthazarin)	G	H	H	OH	H	OH		50	
73 (Lapachol)	G							>50	[24]
74 (Vitamin K_1)	G							>50	
75 (Isodiospyrin)	H						*Diospyros usambarensis*	>50	[24]
76 (Mamegakinone)	I						*Diospyros usambarensis*	>50	[24]

Abbreviations: see Table 2.

during the extraction of *Diospyros usambarensis* with methanol [24].

The prothrombogenic drug menadione (Vitamin K_3) shows remarkable molluscicidal activity (Table 10).

Naphthoquinones are frequently found in species of African and Oriental plants [54] and may be utilisable for local control of bilharzia-transmitting snails. Furthermore, *Diospyros usambarensis* is used in Malaŵi traditional medicine as a schistosomiasis cure and further investigations are underway to evaluate naphthoquinones of this bush as dual molluscicidal and schistosomicidal agents. By this means, there is the possibility of controlling the transmission of schistosomiasis by a combination of snail control *and* chemotherapy [55].

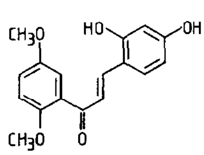
Alkenyl phenols

The unsaturated anacardic acids isolated by Sullivan *et al.* [25] from cashew nut shells are among some of the most potent naturally-occurring molluscicides (Table 11). In view of the high activity of shell extracts, field trials with the shell oil (a by-product of the cashew nut industry) have been carried out in Mozambique. It should be noted, however, that the constituents cause strong dermatitis in humans [56].

Salicylic acid and decarboxylated anacardic acid were both non-molluscicidal [25].

Chalcones

Investigation of the seeds and leaves of *Polygonum senegalense* (Polygonaceae) has led to the isolation of 2,4-dihydroxy-3′,6′-dimethoxychalcone (**81**), which is active within 6 hr at 40 ppm against snails of the species *Biomphalaria pfeifferi* and *B. sudanica* [26].



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Flavonoids and rotenoids

Some flavonoids investigated so far have mollusc-killing activity (Table 12). Eupatorin (**82**) is marginally active at 100 ppm but the flavone glycoside **83** from *Asparagus plumosus* is completely inactive. In addition to the molluscicidal chalcone found in *Polygonum senegalense* [26], the flavonol glycoside quercetin 3-(2″-galloylglucoside) **84** is toxic to snails [27]. Attempts in our laboratory to repeat this experiment with the closely-related compound quercetin 3-(2″-galloylgalactoside) have proved negative, even up to 70 ppm.

Following reports of the activity of *Tephrosia vogelii* (Leguminosae) leaves against snails [57], we investigated the possibility that insecticidal rotenones might be the causative agents [58]. Although a petroleum ether extract of *T. vogelii* leaves gave weak molluscicidal activity (400 ppm), the major rotenones deguelin and tephrosin proved to be inactive. Similarly, rotenone was inactive against *Biomphalaria glabrata* snails up to a concentration of 100 ppm [Marston, A. and Hostettmann, K., unpublished results].

Tannins

It seems very likely that the molluscicidal effects of the fruits from the Sudanese tree *Acacia nilotica* (Leguminosae) are due to the tannins present. Indeed, a spray-dried powder ('TAN'), containing 56% condensed and hydrolysable tannins is active against *Bulinus truncatus* snails at 50 ppm and against *Biomphalaria pfeifferi* snails at 75 ppm [29, 59, 60]. The active constituents have not yet been identified and there is still very little information on the molluscicidal activity of pure tannins, even though it is known that commercial tannic acid (tanninum) is active against *Biomphalaria glabrata* snails at 50 ppm [30]. Gallic acid, ellagic acid and (+)-catechin are, however, inactive up to 100 ppm [30].

A number of other plants rich in tannins have strong snail-killing activity [30]. Typical sources of tannins, such as *Krameria triandra*, *Hamamelis virginiana* and *Quercus*, give extracts which are active at 50, 100 and 200 ppm, respectively. Even a methanol extract of Japanese green tea leaves (*Camellia* spec.) kills *Biomphalaria glabrata* snails at 200 ppm [30]. As tannins may prove to be less toxic to non-target organisms than saponins, they might

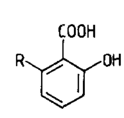
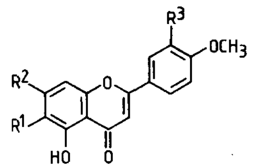


Table 11. Molluscicidal activities of alkenyl phenols

Compound	R	Plant	Molluscicidal activity ppm (LC_{90})	Reference
77	Triene (C_{15}:3)	*Anacardium occidentale*	0.7	[25]
78	Diene (C_{15}:2)	*Anacardium occidentale*	1.4	[25]
79	Monoene (C_{15}:1)	*Anacardium occidentale*	1.9	[25]
80	Saturated (C_{15}:0)	*Anacardium occidentale*	>5	[25]

Abbreviations: see Table 2.



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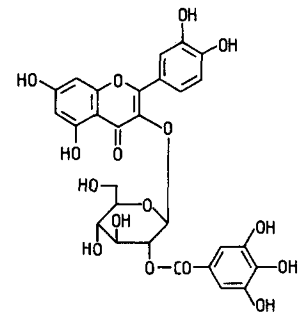

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Table 12. Molluscicidal activities of flavonoids

Compound	Skeleton	R^1	R^2	R^3	Plant	Molluscicidal activity		Reference
						ppm (LC_{100})	Snails tested	
82 (Eupatorin)	J	OCH_3	OCH_3	OH	*Baccharis trimera*	100	*Biomphalaria glabrata*	[17]
83	J	H	Rha-²Glc-	H	*Asparagus plumosus*	—	*Biomphalaria glabrata*	[14]
84					*Polygonum senegalense*	10	*Biomphalaria glabrata*	[27]
						10	*Biomphalaria pfeifferi*	[27]
						10	*Lymnaea natalensis*	[27]
					Polygonum nodosum			[28]

Abbreviations: see Table 2.

be a class of natural products worthy of further investigation.

Furanocoumarins

This class of compounds, commonly found in the Rutaceae, shows strong snail-killing properties (Table 13) but will probably be of no practical use because of phototoxicity phenomena. Furthermore, aflatoxin G_1 (**89**), isolated from mouldy peanuts, is one of the most powerful naturally-occurring molluscicides but at the same time a well-known carcinogen.

Isobutylamides

Three isobutylamides have been shown to kill snails but at rather high concentrations in water (Table 14).

Alkaloids

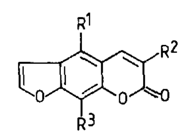
The only example of a molluscicidal alkaloid with any activity whatsoever is the quinolizine alkaloid 2,3-dehydro-*O*-(2-pyrrolylcarbonyl)virgiline (**93**), from the leaves of *Calpurnia aurea* (Leguminosae) [Kubo, I., personal communication]. This compound kills *Biomphalaria glabrata* snails at 130 ppm within 48 hr but will have no practical use.

CONCLUSION

A very large number of plant extracts has been investigated for molluscicidal activity and guidelines for further screening of numerous species have been outlined [5]. Despite all the data available for plant sources, very little is known about the active principles themselves; only about 70 natural products with recognised molluscicidal activity have been isolated. When it is considered that more than 20 000 compounds were screened in order to discover the synthetic molluscicide Bayluscide ᴿ [63], the immensity of the effort required in the search for natural, highly active molluscicides can be appreciated. However, the problem is somewhat simplified by the information already available from the testing of plant material, where the activity is known but the structures of the active components remain to be elucidated.

Of the natural products with the most potential in the fight against schistosomiasis, the triterpene glycosides appear to be in the forefront at the moment, especially as some plant parts can contain as much as 30% saponin. *Phytolacca dodecandra* is a very promising saponin-containing plant for snail control, since *Phytolacca* saponins can kill *Biomphalaria glabrata* snails at concentrations as low as 2 ppm (time of exposure 24 hr). More work needs to be done on varying the saponin aglycone and on structure–activity relationships of the sugar chain, i.e. length of sugar chain and branching positions. Care must be taken to investigate the mammalian toxicity of these natural products before successful snail eradication schemes can be considered.

A constant search for new classes of molluscicidal natural products is essential, so that problems of selectivity and low activity can be overcome. In addition, as wide a range as possible of structurally-related compounds should be isolated (or synthesized) for structure–activity and mode-of-action studies. Only by



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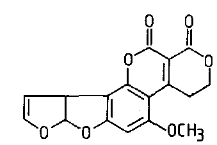

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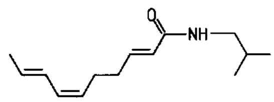
Table 13. Molluscicidal activity of furanocoumarins

Compound	Skeleton	R^1	R^2	R^3	Plant	Molluscicidal activity: ppm	Molluscicidal activity: Snails tested	Reference
85 (Xanthotoxin) (8-Methoxypsoralen)	K	H	H	OCH_3	(*Ammi majus*) (Apiaceae)	50 (LC_{100})	*Biomphalaria boissi*	[31]
86 (Bergapten)	K	OCH_3	H	H	(*Citrus bergamia*) (Rutaceae)	5 (LC_{100})	*Biomphalaria boissi*	[31]
87 (Isopimpinellin)	K	OCH_3	H	OCH_3		5 (LC_{69})	*Biomphalaria boissi*	[31]
88 (Chalepensin)	K	H	$-C(CH_3)_2-CH{=}CH_2$	H	*Ruta chalepensis*	2 (LC_{100})	*Australorbis glabratus*	[61]
89 (Aflatoxin G_1)					(*Aspergillus parasiticus*)	0.5 (LC_{100})	*Biomphalaria tenagophila*	[62]

Abbreviations: see Table 2. LC_{69}: 69% lethal concentration.



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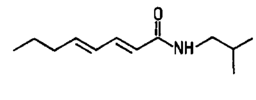

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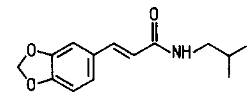

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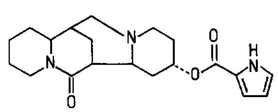
Table 14. Molluscicidal activities of isobutylamides

Compound	Plant	Molluscicidal activity		Reference
		ppm	Snails tested	
90 (Affinin) (*N*-Isobutyl-2,6,8-decatrienamide)	*Heliopsis longipes* *Spilanthes oleraceae* (Compositae) *Wedelia parviceps* (Compositae)	50 (LC_{100})	*Physa occidentalis*	[32]
91 (*N*-Isobutyl-2*E*,4*E*-octadienamide)	*Fagara macrophylla*	200 (LC_{50})	*Biomphalaria glabrata*	[33]
92 (Fagaramide)	*Fagara macrophylla*	200 (LC_{50})	*Biomphalaria glabrata*	[33]

Abbreviations: see Tables 2 and 9.



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understanding the mechanisms of action can newer, more efficient molluscicides be designed.

Despite the high activities of some plant molluscicides, the LC_{100} values of which compare very favorably with the most active synthetic molluscicides, the problems of application mean that plant mollusciciding will never provide a universal solution for the control of schistosomiasis. Only an integrated programme involving improvements in standards of hygiene, together with chemotherapy and mollusciciding will help reduce the transmission of the disease over large areas. However, plant molluscicides may prove of great importance in the focal control of pockets of infection, complementing other methods of treatment.

Practitioners of traditional medicine in Africa and elsewhere employ numerous plants to eliminate schistosomes in persons infected with bilharzia. These plants represent a source of schistosomicidal compounds, the structure elucidation of which has so far not been attempted and which could provide many new chemotherapeutic agents. The characterization of these natural products is currently being undertaken in our laboratory.

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