Secondary Metabolites from the Marine Gastropod Molluscs of Antarctica, Southern Africa and South America

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Abstract. Despite their perceived inaccessibility, the marine intertidal and benthic environments of Antarctica, southern Africa and South America have continued to provide marine natural products for chemists with unique opportunities to study the secondary metabolite constituents and chemical ecology of a diverse array of marine gastropod molluscs. This review covers the literature up to 31 January 2005 and describes the structures and, where applicable, biological activities of 100 secondary metabolites isolated from 21 species of marine gastropod molluscs. Not unexpectedly, the chemistry of chemically defended shell-less opisthobranchs dominates the natural product studies of molluscs collected from these regions of the southern hemisphere.

6.1 Introduction

The majority of molluscan species are assigned to the class Gastropoda, the second most species-diverse class of animals after the class Insecta. For over 70 years, taxonomists have traditionally divided the class Gastropoda into three sub-classes: Prosobranchia, Opisthobranchia and Pulmonata. Unfortunately, not all gastropod molluscs are readily accommodated by this higher classification system and the trichotomy of gastropod sub-classes has recently been treated with circumspection by molluscan taxonomists (Kay et al. 1998). However, the general division of gastropods into prosobranchs, opisthobranchs and pulmonates is universally accepted within the marine natural products literature and this traditional taxonomic triad provides a useful framework for reviewing the secondary metabolite diversity reported from marine gastropod molluscs. The further classification of the gastropod molluscs described here to the level of order and family has been adopted from Beesly et al. (1998) and Cimino et al. (2001).

Marine natural products, including marine molluscan metabolites, are regularly reviewed (Faulkner 2002; Blunt et al. 2005). This review, which covers the molluscan chemistry literature up to 31 January 2005, focuses on the marine molluscs of the southern hemisphere and provides details of the chemical structures and, where reported, the ecological role or

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general bioactivity of the secondary metabolites isolated from molluscs collected from the oceans surrounding Antarctica, southern Africa and South America. Australian molluscan secondary metabolites are described elsewhere in this volume. Metabolites isolated from molluscs collected from islands near southern Africa, for e.g. the dolastatins, first obtained from Mauritian specimens of the sea hare *Dolabella auricularia* (Pettit 1997), will not be discussed here. Conversely, the chemistry of common molluscan species with a circumpolar distribution collected from Antarctic islands are included, e.g. *Austrodoris kerguelenensis* from the South Shetland Islands (Gavagnin et al. 2000). The rationale for the inclusion of the latter is based on the assumption by McClintock and Baker (1997) that the effective isolation of Antarctic benthic ecosystems from those of the surrounding oceans by the strong Antarctic circumpolar current results in the chemical defence strategies employed by Antarctic marine invertebrates remaining reasonably consistent over their geographic range.

6.2 Prosobranch Secondary Metabolites

The subclass Prosobranchia is the largest of the three traditionally recognised subclasses of gastropods; and prosobranch molluscs are renowned for their adaptive radiation and concomitant diversity of both morphological and physiological characteristics (Fretter et al. 1998). Marine prosobranchs are typically shelled snails and the physical defence provided by a robust external shell obviates an additional acquired chemical defence to protect their soft tissues. However, the presence of a chemical defence system, sequestered from other marine invertebrates, is occasionally observed in prosobranchs where the shell is reduced, e.g. the Antarctic prosobranch *Marseniopsis mollis* (Family Velutinidae, formerly Lamellariidae). *M. mollis* is similar to other lamellarian gastropods, in that, this species possesses a vestigial internal shell enveloped by a large fleshy mantle. There are no reports of the isolation of secondary metabolites from either southern African or South American prosobranch molluscs.

6.2.1 Antarctic Marine Prosobranchs

The conspicuous bright yellow colour of the mantle tissue of *M. mollis* probably provided the first indication of the possible utilization of a chemical defence system by this species. Seastars are the main marine invertebrate predators in Antarctic benthic communities (McClintock

1994) and feeding deterrent studies, mostly utilizing the large predatory Antarctic seastar *Odantaster validus*, have been regularly used to investigate the chemical defence strategies employed by Antarctic marine invertebrates. From the results of a series of *O. validus* feeding deterrent assays, McClintock et al. (1994a) proposed that the ubiquitous osmolyte, *N*-methyl picolinic acid or homarine (**1**), was the principal feeding deterrent utilised by *M. mollis* as a form of chemical defence. In common with most other chemically defended gastropod molluscs, *M. mollis* sequesters **1** from its diet. Paradoxically, McClintock et al. (1994a) discovered that homarine was not present, as expected, in the tunic of *M. mollis*' primary food source, the large solitary ascidian *Cnemidocarpa verrucosa*, but instead occurred in the small epizoites (predominantly hydroids and bryozoans) that reside on the exterior surface of *C. verrucosa*.

6.3 Opisthobranch Secondary Metabolites

Opisthobranch molluscs are almost exclusively marine and there are no terrestrial opisthobranch species. Only one or two opisthobranch species could be considered to be freshwater (Smith and Stansic 1998). Unlike shelled marine snails (prosobranchs), marine opisthobranch molluscs exhibit an evolutionary trend towards elimination of the shell (Faulkner and Ghiselin 1983; Gosliner 1987; Cimino and Ghiselin 1998, 1999). In shell-less opisthobranchs, the physical defence offered by a shell has generally been replaced with a chemical defence system incorporating bioactive metabolites either sequestered from the opisthobranch's diet or, less commonly, derived from de novo biosynthesis. The chemistry and biological activity of metabolites isolated from marine opisthobranch molluscs have been reviewed by Cimino and Ghiselin (1998), Cimino et al. (1999, 2001) and Gavagnin and Fontana (2000).

6.3.1 Antarctic Marine Opisthobranchs

McClintock and Baker (1997) summarised their contribution to chemical ecological studies of Antarctic opisthobranch molluscs in their comprehensive review of the chemical ecology of Antarctic marine invertebrates. Natural product and associated chemical ecology studies have been conducted on opisthobranch molluscs collected from several regions around the continent of Antarctica, including under the sea ice in McMurdo Sound (McClintock et al. 1994b, McClintock and Baker 1997), the adjacent Italian Antarctic base at Terra Nova Bay (Gavagnin et al.

2003a,b), Tethys Bay (Gavagnin et al. 1995), the Weddell Sea (Avila et al. 2000) and the South Shetland Islands (Gavagnin et al. 1999a,b, 2000).

Pteropods (Order Gymnosomata)

The evolution of the pteropod foot into a pair of "wings", adapted for both flotation and swimming through the water column, enables pteropod molluscs to adopt a pelagic lifestyle (Rudman and Willan 1998). The wing-like structure of the foot also contributes to the common name of sea butterfly given to this group of gastropods. Only a few species of pteropods belong to the order Gymnosomata and one of these species, the yellow, shell-less pteropod *Clione antarctica*, loses its shell, mantle and mantle cavity in the adult stage of its life cycle (Cimino et al. 2001). *C. antarctica* is common in McMurdo Sound, Antarctica, near the under-surface of the sea ice and is rarely preyed upon by pelagic predators. McClintock and Janssen (1990) were the first to report the abduction and transport of *C. antarctica* on the dorsal surface of the amphipod *Hyperiella dilatata* as a novel form of physically acquired chemical defence, on the part of the amphipod, against fish predators. The feeding deterrent, polypropionate-derived pteroenone (**2**), responsible for the chemical defence of *C. antarctica*, was later isolated and identified by Yoshida et al. (1995) following a bioassay guided fractionation of an extract of *C. antarctica*, in which various zooplanktivorous Antarctic fish species, e.g. *Pagothenia borchgrevinki*, were used as the bioassay test organisms. The 6*S* configuration of **2**, established in the usual manner via the modified Mosher's method, provided the key to determining the configuration at C-5. Large (10.1 Hz) diaxial coupling constants between H-4 and H-5 and between H-6 and H-5, observed in the ^{1}H NMR spectrum of the *syn*-acetonide (**3**) prepared from one of the epimeric sodium borohydride reduction products of **2**, required the C-5 methyl group to be equatorial and thus secured the *R* absolute configuration at the homoallylic chiral centre.

Nudibranchs (Order Nudibranchia)

Three dorid nudibranch species (Suborder Doridina) viz. *Tritoniella belli*, *Bathydoris hodgsoni* and *A. kerguelenensis* have exclusively dominated the natural product and chemical ecology studies of Antarctic marine opisthobranch molluscs over the past decade. Dorid nudibranchs are carnivorous and prey on other marine invertebrates, including sponges, octocorals, bryozans and ascidians (Cimino et al. 2001), which act as a basic food source and in many instances provide the dorid nudibranchs with a source of bioactive metabolites for their chemical defence systems. Ironically, of the three Antarctic species of dorid nudibranchs studied thus far, two species, *B. hodgsoni* and *A. kergulensis*, have been reported

to obtain their chemical defence metabolites through de novo biosynthesis.

The feeding deterrent chimyl alcohol (**4**), ubiquitous in tropical and temperate molluscs, was found to be the major glycerol ether present in extracts of the dorid nudibranch, *T. belli* collected from Ross Island, McMurdo Sound (McClintock et al. 1994b). Chimyl alcohol was also isolated from the stoloniferan octocoral *Clavularia frankliniana*, which forms a major component of *T. belli*'s diet, suggesting that this octocoral was the source of **4** used in *T. belli*'s sequestered chemical defence system. The increasingly widely used and ecologically relevant seastar (*O. validus*) tube foot retraction assay confirmed the feeding deterrent properties of **4** (McClintock et al. 1994b).

B. hodgsoni (Eliot 1907) is a member of a primitive group of polar dorid nudibranchs belonging to the superfamily Bathydoridae. An extract of the mantle tissue of *B. hodgsoni* from animals dredged at depths >200 m in the eastern Weddell Sea yielded the first 2-substituted drimane sesquiterpene, hodgsonal (**5**) to be isolated from the marine environment (Iken et al. 1998, Gavagnin et al. 2000). The relative configurations of the four chiral centres in **5** were initially assigned from NOE data. Cyclization of deacetylated **5**, in the presence of manganese dioxide, afforded a tricyclic γ-lactone (**6**). The 2*S* configuration in **6**, established using Mosher's method, thus provided the absolute configuration of the remaining three chiral centres and unequivocally confirmed the drimane skeleton of the native hodgsonal, which proved to be repugnant to *O. valdivus* in a modified version of McClintock's predatory seastar feeding deterrent assay (Avila et al. 2000).

The absence of any detectable amounts of **5** in the gut of *B. hodgsoni* and the constancy of its concentration in the mantle tissues of a number of specimens collected from different depths and localities around Antarctica led Avila et al. (2000) to suggest that **5** was a product of de novo biosynthesis in *B. hodgsoni* and was not sequestered by this organism from its diet. The structures of compounds **1–6** are presented in Fig. 6.1.

A. kerguelenensis (Bergh 1884) is a large nudibranch varying in colour from white to yellow. *A. kerguelenensis* is not confined to Antarctica and is also reported from the southern tip of South America and some sub-Antarctic islands, e.g. the Kerguelen Islands, whence it takes its name. The apparent chemical defensive properties of the mantle tissue of *A. kerguelenensis* initially reported by McClintock et al. (1990) was followed by a study of the natural product chemistry by Davies-Coleman and Faulkner (1991) of specimens of this species collected in McMurdo Sound (note: the species name "*kerguelensis*" was incorrectly spelt in the paper by Davies-Coleman and Faulkner). Five *ent*-labdane diterpene glycerides (**7–11**) were isolated from the McMurdo Sound *A. kerguelenensis* extracts.

The assignment of diterpenes **7**–**11** to the *ent*-labdane series followed from comparison of the optical rotation of the methyl ester of saponified **7** ($[\alpha]_D$-49) with that reported for methyl (5*R*, 10*R*, 13*R*) labda-8-en-15-oate ($[\alpha]_D$-48). Biosynthetic arguments were used to extrapolate the absolute stereochemistry of the diterpene moiety in **7** to compounds **8** and **9**, while the diketone obtained from ozonolysis of **7** was found to be identical with **10** thus confirming the absolute stereochemistry of the latter compound and also, by further recourse to biosynthetic arguments, the absolute stereochemistry of **11** (Davies-Coleman and Faulkner 1991).

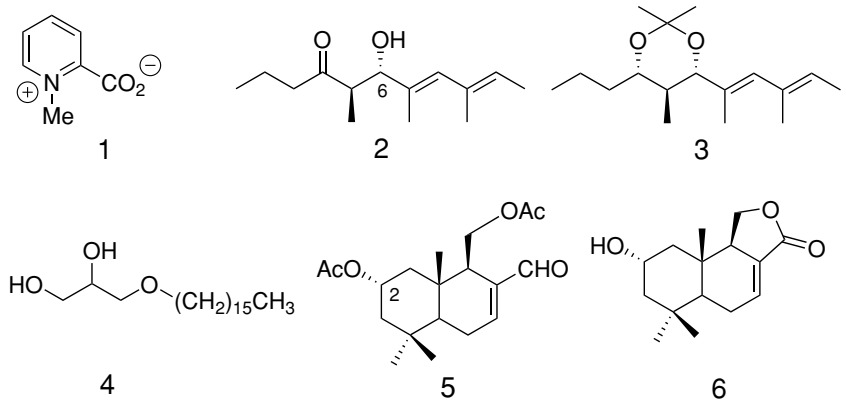


Fig. 6.1. Secondary metabolites isolated from the Antarctic prosobranch *Marseniopsis mollis*, the opisthobranchs *Tritoniella belli* and *Bathydoris hodgsoni* and two key derivatives (**3**, **6**) used to determine the absolute stereochemistry of **2** and **5**, respectively

From Dayton et al.'s (1974) field observations of the feeding habits of *A. kerguelenensis*, Davies-Coleman and Faulkner (1991) postulated that **7**–**11** were the products of de novo biosynthesis given the known paucity of organic biomass in the "glass sponges" (Order Hexactinellida) which predominate in *A. kergeulenensis*' diet. Davies-Coleman and Faulkner's initial de novo biosynthetic hypothesis was recently corroborated by Iken et al. (2002). In a meticulous chemical ecology study involving 117 specimens of *A. kerguelenensis* collected from 32 different locations in the Weddell Sea at depths of 65–1550 m, Iken et al. (2002) were able to clearly demonstrate that the diacylglycerides **7** and **8** were only present in the mantle tissue and not the viscera. The complete absence of these bioactive metabolites in the gut unequivocally confirmed that these compounds are not sequestered from the nudibranch's diet. Compounds **7** and **8** and a cohort of simple fatty acid monoacylglycerides co-occurring in the mantle tissue of *A. kerguelenensis* exhibited feeding deterrence activity towards the seastar *O. validus*. Iken et al. (2002) were also able to confirm that **7** and **8** were absent, as suspected by Davies-Coleman and Faulkner (1991), from the Antarctic hexactinellid sponges preyed upon by *A. kerguelenensis*.

The biogenesis of terpenoic acid glycerides in nudibranchs has been the subject of conjecture over the past two decades (e.g. Cimino et al. 1983; Gustafson and Andersen 1985; Graziani et al. 1996; Fontana et al. 1998). The early studies of Cimino et al. (1983) and Gustafson and Andersen (1985) showed low levels of incorporation of ^{14}C-labelled mevalonic acid into a sesquiterpene dialdehyde from the dorid nudibranch *Dendrodoris limbata* and diterpenoic acid glycerides from *Archidoris montereyensis* and *A. odheneri*, respectively. Frustratingly, evidence for the incorporation of ^{14}C-labelled mevalonic acid in the de novo biosynthesis of mantle metabolites from other dorid nudibranchs was elusive and a different approach to elucidating the biosynthesis of these metabolites was required (Graziani et al. 1996). Using NMR detection of stable isotopes, Graziani et al. (1996) were able to unequivocally elucidate the incorporation of [1,2-$^{13}C_2$]acetate into the diterpenoid skeleton of a diterpenoic acid glyceride biosynthesised by *A. montereyensis* and *A. odheneri*. More recently, Fontana et al. (1998) used [6-^{13}C]- and [5-^{13}C]glucose and [2-^{13}C]pyruvate to explore the de novo biosynthesis of a series of diterpenoic acid glycerides (verrucosins) by *Doris verrucosa*. Although incurporation levels into the diterpene skeleton were low, Fontana et al. (1998) were able to tentatively postulate an acetate/ mevalonate pathway for the biosynthesis of the diterpenoid moiety in the veruccosins. Conversely, they were able to unambiguously determine the biosynthetic origin of the *sn*-glyceride moiety in the verrucosins from D-glyceraldehyde 3-phosphate derived from the glycolysis of D-glucose.

The absolute stereochemistry at C-2 in the 1,3 diacylglyceride moiety of **8**, originally unassigned by Davies-Coleman and Faulkner, was later established as *R* by Gavagnin et al. (1999a) by applying Mosher's method to **8** isolated from the mantle tissue of two *A. kerguelenensis* specimens collected from the Weddell Sea. The 2*R* configuration was similarly assigned (Gavagnin et al. 1999a) to the 1,3 diacylglyceride moiety in the diterpene glyceride (**12**) isolated as a minor metabolite from two *A. kerguelenensis* specimens collected near the South Shetland Islands. The major metabolite in the South Shetland Island specimens of *A. kerguelenensis* was the 1,2-diacylglyceride (**13**; Gavagnin et al. 1999a). Both **12** and **13** are analogues of the halimane diterpene glyceride (**14**) first isolated as its diacetyl derivative (**15**) from *A. kerguelenensis* specimens from Tethys Bay (Gavagnin et al. 1995).

Surprisingly, the 2*R* configuration of **8** and **12** was at variance with the 2*S* stereochemistry reported for all other diacylglycerols previously isolated from dorid nudibranchs (Fontana et al. 1998; Gavagnin et al. 1999a). At a loss for an alternative explanation, Gavagnin et al. (1999a) tenuously suggested that the harsh Antarctic environmental conditions might be influencing de novo biosynthesis of diacylglycerols in *A. kerguelenensis* and resulting in this puzzling stereochemical anomaly. Gavagnin et al. (2003a) finally resolved the outstanding glyceride

stereochemical question four years later when they returned to the primary NMR spectroscopic data acquired for **7** and **13**, and unearthed a fundamental flaw in the structures originally proposed for these two compounds. Definitive heteronuclear multi-bond correlation (HMBC) connectivities, including those between the glyceride oxymethine proton and the diterpene ester carbonyl carbon in **7** and **13**, placed the diterpene moiety at C-2 and the acetate at C-1 in the glycerol moiety in these two compounds, thus confirming that they were indeed 1,2-*sn* diacylglycerides as expected. After revising the structures of **7** and **13** to **16** and **17**, respectively, Gavagnin et al. (2003a) deduced that the 1,3 diacylglycerides **8** and **12** were not natural products but rather artefacts of the extraction and chromatographic workup where the terpenoid acyl group in **16** and **17** undergoes facile migration from C-2 to C-3 in the glycerol moiety, resulting in the 2*R* configuration observed in **8** and **12**.

Careful analysis of the NMR data of an inseparable mixture of minor products from *A. kerguelenensis* specimens collected from Terra Nova Bay suggested the presence of clerodane diterpene (**18**) as the main component in this mixture (Gavagnin et al. 2003a). NMR resonances attributed to the diterpene residue of **18** were consistent with those assigned to the clerodane moiety in archidorin (**19**) previously isolated from the Atlantic nudibranch *A. tuberculata*. Further evidence for the structural affinity between **18** and archidorin was provided by methanolysis of the inseparable diterpene mixture followed by high performance liquid chromatography (HPLC) and NMR comparison of the methylated products with an authentic sample of methyl ester of archidorin (**20**) (Gavagnin et al. 2003a).

The propensity of *A. kerguelenensis* extracts to provide investigators with a steady stream of new terpenoid variants was further exemplified by the isolation of tricyclic isocopalane-type diterpenes austrodorins A (**21**) and B (**22**; Gavagnin et al. 1999b) and the *nor*-sesquiterpenes austrodoral (**23**) and austrodoric acid (**24**) (Gavagnin et al. 2003b) from *A. kerguelenensis* extracts. The absolute stereochemistry of **21** and **22** followed from comparison of the circular dichroism (CD) spectra of these compounds with the CD spectra of synthetic isocopalane and *ent*-isocopalane diterpenoids of known absolute configuration (Gavagnin et al. 1999b). The ^{13}C NMR data of **23** and **24** were compatible with the literature values quoted for a drimane skelton with a *trans*-A,B ring junction, while NOE data supported the assignment of a β-equatorial methyl substituent at C-8 in both these compounds. Interestingly, the concentrations of **23** and **24** in the *A. kerguelenensis* mantle tissue were significantly higher when these animals were kept in an aquarium for an extended period, suggesting that their biosynthesis may be induced by exposure to stress (Gavagnin et al. 2003b). The structures of the *A. kerguelenensis* metabolites **7**–**24** are presented in Fig. 6.2.

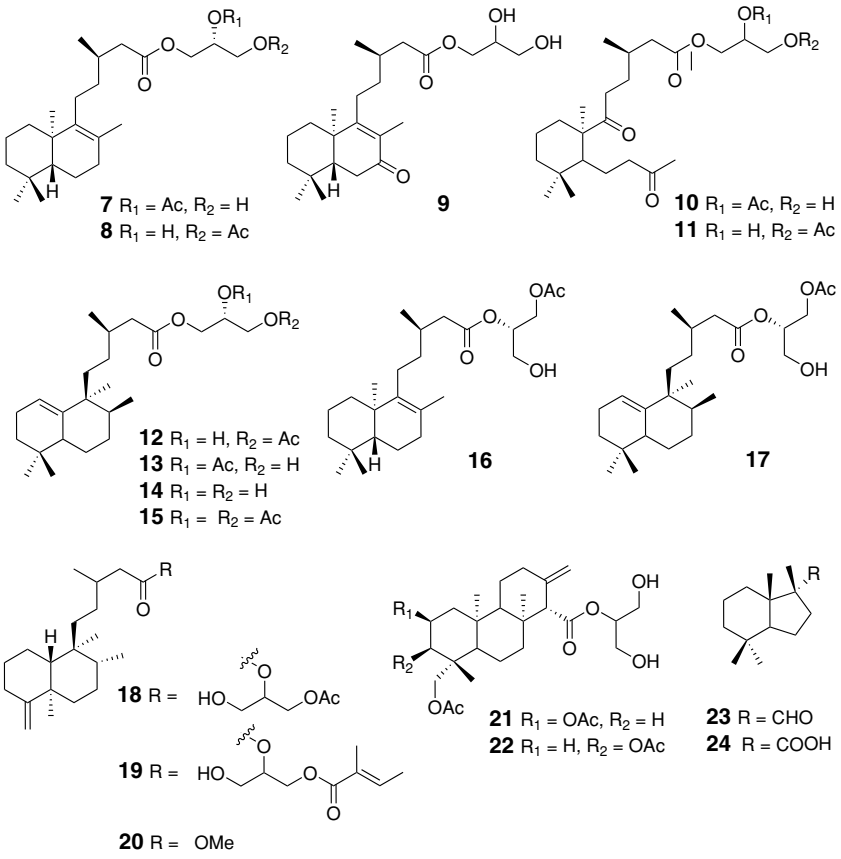


Fig. 6.2. Secondary metabolites isolated from the Antarctic opisthobranch *Austrodoris kerguelenensis* and the Atlantic opisthobranch *Archidoris tuberculata*

6.3.2 Southern African Marine Opisthobranchs

The southern African coastline, approximately 3,000 km long and stretching from Namibia in the west to southern Mozambique in the east, is broadly subdivided into three bio-geographical zones, the cool temperate west coast, the warm temperate south east coast and the subtropical east coast (Branch and Branch 1981). Each of the southern African bio-geographical zones sustains distinctive populations of marine flora and fauna and a large proportion of the over 10,000 species of marine organisms recorded off the southern African coast are reported to be endemic (Branch et al. 1994). The studies of the natural product chemistry of African marine molluscs have thus far been confined to southern African species collected predominantly on the warm temperate south east coast and the subtropical and tropical east coast of South

Africa and Mozambique. Gosliner (1987) estimates that more than 250 opisthobranch mollusc species occur off the southern African coast, of which many species remain undescribed.

Nudibranchs (Order Nudibranchia)

The endemic southern African nudibranch *Leminda millecra* (Griffiths 1985) is the only representative of the family Charcotiidae (formerly Lemindidae) occurring off the coast of South Africa. Pika and Faulkner (1994) isolated four sesquiterpene metabolites, millecrone A (**25**), millecrone B (**26**), millecrol A (**27**) and millecrol B (**28**) from an extract of four *L. millecra* specimens collected off Coffee Bay on the Wild Coast of South Africa. The data provided from a series of 1D NOE difference experiments combined with an analysis of selective coupling constants provided the relative stereochemistry of compounds **25–28**. From an examination of the gut contents from the *L. millecra* specimens that provided **25–28**, Pika and Faulkner (1994) identified spicules from three soft coral species, *Alcyonium foliatum*, *A. valdivae* and *Capnella thyrsoidea*. From this evidence, they tentatively proposed that *L. millecra* sequesters **25–28** from one or more of these species. Of the four *L. millecra* metabolites, only **25** inhibited the growth of *Candida albicans*, while **26** was active against *Staphylococcus aureus* and *Bacillus subtilis* and **28** only exhibited antibiotic activity against the latter bacterium (Pika and Faulkner 1994).

Although initially purported to be rare off the coast of southern Africa (Gosliner 1987), *L. millecra* was found to be abundant in Algoa Bay, South Africa, ca. 500 km southwest of the Wild Coast (McPhail et al. 2001). From a combined extract of 32 specimens of *L. millecra* collected in Algoa Bay, McPhail et al. (2001) isolated **25**, **26**, isofuranodiene (**29**), (+)-8-hydroxycalamenene (**30**), algoafuran (**31**), cubebenone (**32**) and a cohort of seven triprenylquinones and hydroquinones (**33–39**). Standard spectroscopic techniques were used to determine the chemical structures of the *L. millecra* metabolites with NOESY data, providing the relative stereochemistry of **32**. In accordance with similar trends in the ^{13}C chemical shift data reported for analogous compounds, the relatively deshielded allylic methyl carbon (C-14′ δ_C 26) in the triprenyl side-chain of **36–38** suggested a *Z* configuration for the α,β unsaturated ketone in these three compounds. This latter assignment was corroborated by a NOESY correlation between the 3H-14′ methyl protons and the adjacent vinylic proton. There was no evidence to support the occurrence of millecrols A and B in the combined Algoa Bay *L. millecra* extract, suggesting some geographical variation in the sequestered chemistry of this species. While the bulk extraction of large numbers of a single nudibranch species provides an opportunity to rapidly survey the diversity of metabolites sequestered by that species in a particular area, it

does not provide details of the dietary selectivity, if any, of individual nudibranchs. Once isolated in sufficient quantities for structure elucidation studies, nudibranch metabolites can subsequently be used as analytical standards in gas chromatography (GC) or HPLC analyses of extracts of individual nudibranchs. Accordingly, GC analysis of extracts from eight individual specimens of *L. millecra* collected from a large reef in Algoa Bay revealed that each nudibranch contained **26** and **32** as minor and major metabolites, respectively. Extrapolation of the GC method to extracts of 21 octocorals collected in Algoa Bay confirmed that the sea fan *Leptogorgia palma,* on which *L. millecra* had often been observed feeding, was the source of **26** and **32** (McPhail et al. 2001). The structures of the structurally diverse metabolites isolated from *L. millecra* are presented in Fig. 6.3.

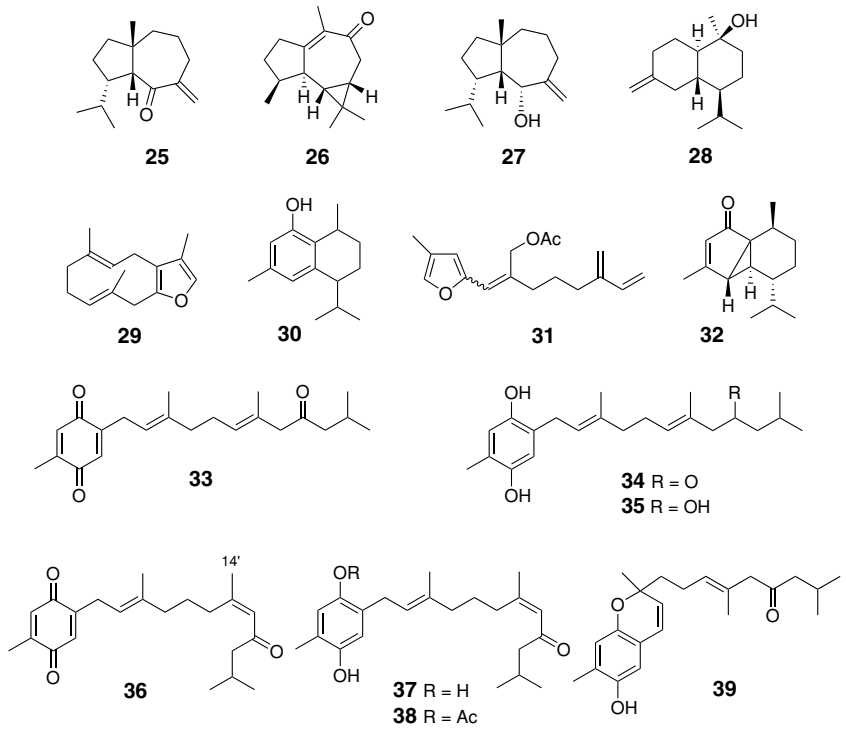


Fig. 6.3. Secondary metabolites isolated from the southern African opisthobranch *Leminda millecra*

The secondary metabolites of only two species of southern African dorid nudibranchs have been investigated. The first of these established that the brightly coloured *Chromodoris hamiltoni* (Rudman 1977) (Family Chromodorididae), collected from the Aliwal Shoal, a large sub-tropical

reef system off the coast of KwaZulu Natal, South Africa, was the source of four unusual chlorinated homoditerpenes, hamiltonins A–D (**40**–**43**), the sesterterpene hamiltonin E (**44**) and the relatively common sponge toxins latrunculin A and B (**45**, **46**; Pika and Faulkner 1995). The relative stereochemistry of **40** and **41**, established from coupling constants and NOE data, confirmed that both these compounds possessed the same unprecedented 3-homo-4,5-*seco*spongian skeleton. A paucity of **42** and **43** required structure elucidation of these two compounds, predominantly through comparison of their spectral data with those of the more abundant hamiltonins A and B. Compound **44** was also isolated as a minor metabolite from the *C. hamiltoni* extracts. The relative stereochemistry of the tricyclic ring system in **44** was proposed from comparison of the spectroscopic data of this compound with those of known compounds, while the magnitude of the negative Cotton effect observed at 218 nm in the CD spectrum of **44** compared favourably with the Cotton effects observed in the CD spectra of luffarin-I and 4*R*-manolalide, and led to the assignment of an *R* absolute stereochemistry to the asymmetric oxymethine carbon in **44**. Of these seven metabolites, only **46** was present in extracts of *C. hamiltoni* collected from the reefs off southern Mozambique (400 km north of the Aliwal Shoal), possibly reflecting geographical variation in the organisms that make up *C. hamiltoni*'s diet in this region of the southern African coast (McPhail and Davies-Coleman 1997). Although devoid of hamiltonins, the extract of the Mozambique specimens of *C. hamiltoni* yielded two new spongian diterpene lactones (**47**, **48**; McPhail and Davies-Coleman 1997). The relative stereochemistry of **47** and **48** followed from the NOESY and 1D NOE difference data acquired for these two compounds.

The genus *Hypselodoris* differs from the closely related genus *Chromodoris* both in its colouration and the structure of the radula, a chitinous ribbon of teeth used for feeding by most molluscs (Gosliner 1987). The endemic southern African species, *Hypselodoris capensis* (Barnard 1927), is a colourful member of the family Chromodorididae; and an investigation by McPhail et al. (1998) of the metabolites present in an extract of 16 specimens of *H. capensis*, collected in the Tsitsikamma Marine Reserve, situated on the warm temperate southeastern coast of South Africa, afforded the linear β-substituted sesterterpenes (18*R*)-variabilin (**49**), 22-deoxyvariabilin (**50**) and 22-deoxy-23-hydroxymethyl-variabilin (**51**), in addition to the known sesquiterpenes nakafurans 8 and 9 (**52**, **53**). The assignment of an 18*R* configuration to the variabilin isolated from *H. capensis* followed from comparison of the optical rotation obtained for **49** with published values for this ubiquitous bioactive metabolite. The absolute configuration at C-18 in **50** and **51** remains unassigned. McPhail et al. (1998, 2000) provided evidence for the sequestration of these compounds by *H. capensis* from a *Fasciospongia*

sponge (the source of **49–51**) and a *Dysidea* sponge (the source of **52, 53**). Field observations indicated that both sponges form part of *H. capensis*'s diet in the Tsitsikamma Marine Reserve.

The dark blue nudibranch, *Tambja capensis* (Bergh 1907) (Family Polyceridae), is endemic to the cool temperate coastal waters off the south-eastern coast of South Africa (Gosliner 1987). A recent comparative study by Rapson (2004) of the distribution of 4-methoxypyrrolic metabolites in three populations of this species collected from three well dispersed locations (False Bay, Algoa Bay, East London), revealed that all three populations contained the tetrapyrrole pigment (**54**) and the tambjamines A (**55**) and E (**56**) as major metabolites. In Algoa Bay, *T. capensis* has been observed feeding extensively on the blue bryozoan *Bugula dentata*. HPLC analysis of Algoa Bay specimens of *B. dentata* revealed that this species contained all three 4-methoxypyrrolic metabolites and thus suggested that *B. dentata* is the primary source of the sequestered chemistry of *T. capensis* off the coast of southern Africa. The structures of the metabolites isolated from *C. hamiltoni*, *H. capensis* and *T. capensis* are presented in Fig. 6.4.

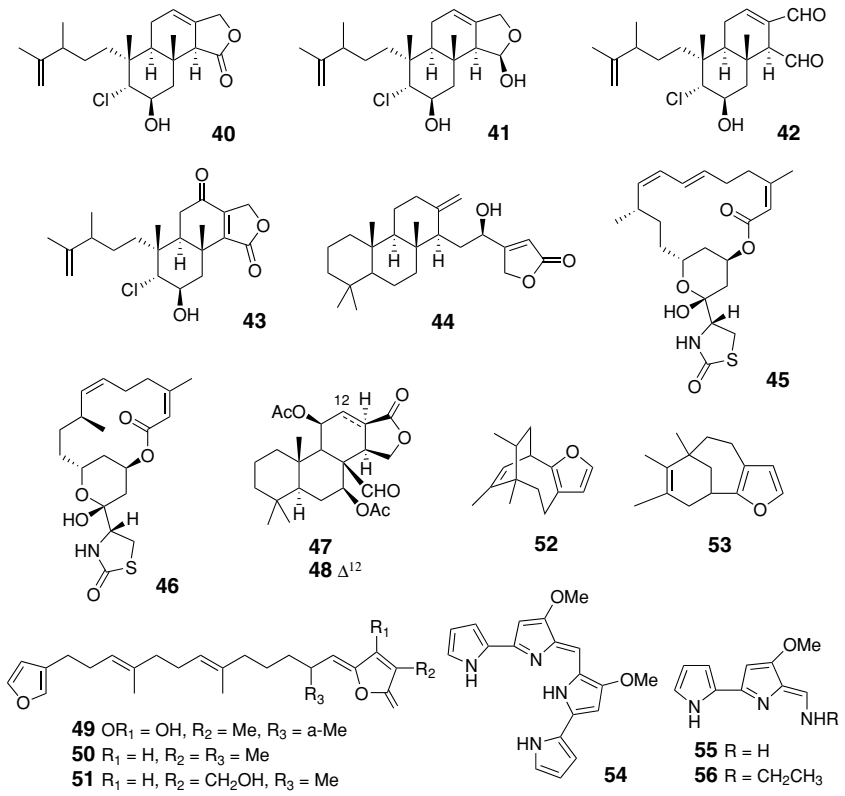


Fig. 6.4. Secondary metabolites isolated from the southern African opisthobranchs *Chromodoris hamiltoni*, *Hypselodoris capensis* and *Tambja capensis*

Sea Hares (Order Anaspidea)

Anaspideans are large herbivorous molluscs with either a fragile, transparent external shell (Family Akeridae) or a reduced or absent internal shell (Family Aplysiidae; Cimino et al. 2001). The large circumtropical sea hare *Aplysia dactylomela* (Rang 1882) (Family Aplysiidae) typically stores a plethora of halogenated terpenes from its red algal diet in a large internal digestive gland. Although Algoa Bay is at the extreme southern end of *A. dactylomela*'s range off the east coast of southern Africa (Gosliner 1987), an uncommon prolonged influx of warm water into Algoa Bay during the late summer of 1998 resulted in a proliferation of this species on the reefs on the western edge of the bay. Six halogenated sesquiterpenes, algoane (**57**), 1-deacetoxyalgoane (**58**), 1-deacetoxy-8-deoxyalgoane (**59**), ibhayinol (**60**), nidificene (**61**) and prepacifenol epoxide (**62**) were isolated from extracts of the excised digestive glands of four specimens of *A. dactylomela* collected from Algoa Bay during this warm-water event (McPhail et al. 1999). An X-ray structure of **57** provided the initial entry into the structures and relative stereochemistry of **58** and **59**. A further X-ray analysis of ibhayinol (Copley et al. 2002) corrected an erroneous stereochemistry originally assigned to five of the seven chiral centres in this compound (McPhail et al. 1999) and necessitated a revision of the structure of ibhayinol to **63**. The revised stereochemistry of ibhayinol also enabled Copley et al. (2002) to postulate a putative biosynthetic link between **63** and compounds **55**–**57** through a hypothetical 8-hydroxy-1-deacetoxyalgoane precursor (**64**). Loss of the equatorial bromine substituent at C-10 in **64** followed by a 1,2-alkyl shift of the axial methyl group on C-11 facilitated ring closure at the resultant carbocation generated at C-11 via nucleophilic attack of the hydroxyl moiety resident on C-1 with *si* facial selectivity. The original red algal source of the halogenated sesquiterpenes isolated from *A. dactylomela* is unknown.

The cosmopolitan sea hare *A. parvula* (Mörch 1863) is the smallest of the *Aplysia* sea hares found off the coast of South Africa. McPhail and Davies-Coleman (2005) isolated (3*Z*)-bromofucin (**65**) from a combined extract of 49 specimens of *A. parvula* collected from the Tsitsikamma Marine Reserve on the temperate south coast of South Africa. Although (3*Z*)-bromofucin is analogous to several *Laurencia* algal metabolites, the algal source of **62** was not established. A combination of NOESY data and molecular modelling studies provided the relative stereochemistry of **65**. The chemical structures of the southern African *Aplysia* secondary metabolites are presented in Fig. 6.5.

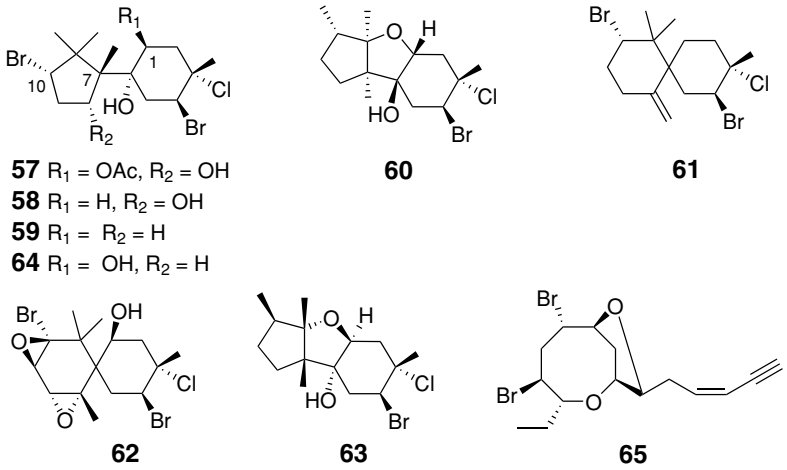


Fig. 6.5. Secondary metabolites isolated from the southern African opisthobranchs *Aplysia dactylomela* and *A. parvula* and a hypothetical biosynthetic precursor (**64**) of ibhayinol (**63**)

6.3.3 South American Marine Opisthobranchs

The natural product chemistry of Brazilian marine organisms, including molluscs, has been recently reviewed (Berlinck et al. 2004). The natural product studies of southern South American opisthobranch molluscs appear to have been confined to two Brazilian nudibranch species, *Doris* aff. *verrucosa* and *Tambja eliora*, and the sea hare *A. dactylomela* and two dorid nudibranchs, *Tyrinna nobilis* and *Anisodoris fontaini* collected off the Patagonian coast.

Nudibranchs (Order Nudibranchia)

Granato et al. (2000) isolated a plethora of common sterols and 9-[5′-(methylthio)-D-xylofuranosyl]adenine (xylosyl-MTA; **66**) from the mantle tissue of the dorid nudibranch *D.* aff. *verrucosa* (Family Chromodorididae) collected near São Paulo, Brazil. Xylosyl-MTA was previously isolated from a related Mediterranean nudibranch species (Cimino and Sodano 1993). Although *D.* aff. *verrucosa* preys extensively on the sponge *Hymeniacidon* aff. *heliophila*, the absence of **66** in this sponge's tissue led Granato et al. (2000) to conclude that *D.* aff. *verrucosa* does not sequester **66** from this sponge species. An extract of nine specimens of *Tambja eliora* (Family Polyceridae) also collected off the coast of Brazil afforded tambjamine A (**55**) and tambjamine D (**67**) as their imino salts, e.g. **67**

(Berlinck et al. 2004). Both **55** and **67** were originally isolated by Carte and Faulkner (1983) from *Tambja eliora* collected in the Gulf of Mexico.

The dorid nudibranch *Tyrinna nobilis*, collected of the cost of Patagonia, yielded a novel *seco*-11,12-spongiane diterpene, tyrinnal (**68**) and the known compounds dendrolasin (**69**), pallescensin A (**70**) and dehydropallescensin-2 (**71**) (Fontana et al. 1998). NOE data provided the relative stereochemistry of **68**. The probable sponge source of the sequestered chemistry of *T. nobilis* is unknown (Fontana et al. 1998). A second Patagonian nudibranch *Anisodoris fontaini* yielded a series of five new minor metabolites, anisodorins 1–5 (**72–76**), and two known metabolites (**77**, **78**) as the major mantle metabolites in this dorid nudibranch (Gavagnin et al. 1999c). The structure elucidation of **72–78** proved to be relatively straightforward, given the similarities in the isocopalane skeleton common to all seven metabolites isolated from *A. fontaini* and the already well established structures of **77** and **78**. Semi-syntheses of both *ent*-anisodorin 1 (Gavagnin et al. 1999c) and *ent*-anisodorin 5 (Ungur et al. 1999) from the commercially available terrestrial plant diterpene (–)-sclareol unequivocally confirmed the structures and absolute stereochemistry of **72** and **76**. The absolute stereochemistry of **76** is opposite to that of similar isocopalane diterpenoids typically isolated from sponges of the genus *Spongia*, adding credence to the hypothesis that all the compounds **72–78** are the products of de novo biosynthesis and not sequestered by *A. fontaini* from its diet. Circular dichroism, supported by NOE data, suggested that anisiodorin 3 (**74**) shares the same absolute stereochemistry as **72** and **73**.

Sea Hares (Order Anaspidea)

An examination of extracts of the mantle tissue and viscera of specimens of *A. dactylomela* collected from the intertidal zone on the coast of Rio de Janeiro State, Brazil, yielded four known halogenated chamigrane sesquiterpenes: prepacifinol epoxide (**62**), johnstonol (**79**), pacifidiene (**80**) and the diol (**81**; Pitombo et al. 1996). Although the former two compounds were originally isolated from the red marine algae *Laurencia nidifida* and *L. johnstoni* collected from Hawaii and the Gulf of California, respectively, the Brazilian algal source of **62** and **79–81** was not established (Pitombo et al. 1996). In the original reports describing the structures of **62** and **79–81**, the NMR data had been unassigned and the isolation of these compounds from the Brazilian specimens of *A. dactylomela* provided an opportunity for these spectral assignments to be established and the preferred conformation of chamigrane sesquiterpenes in solution to be explored (Kaiser et al. 1998, 2000, 2001). The chemical structures of secondary metabolites isolated from South American opisthobranch molluscs are presented in Fig. 6.6.

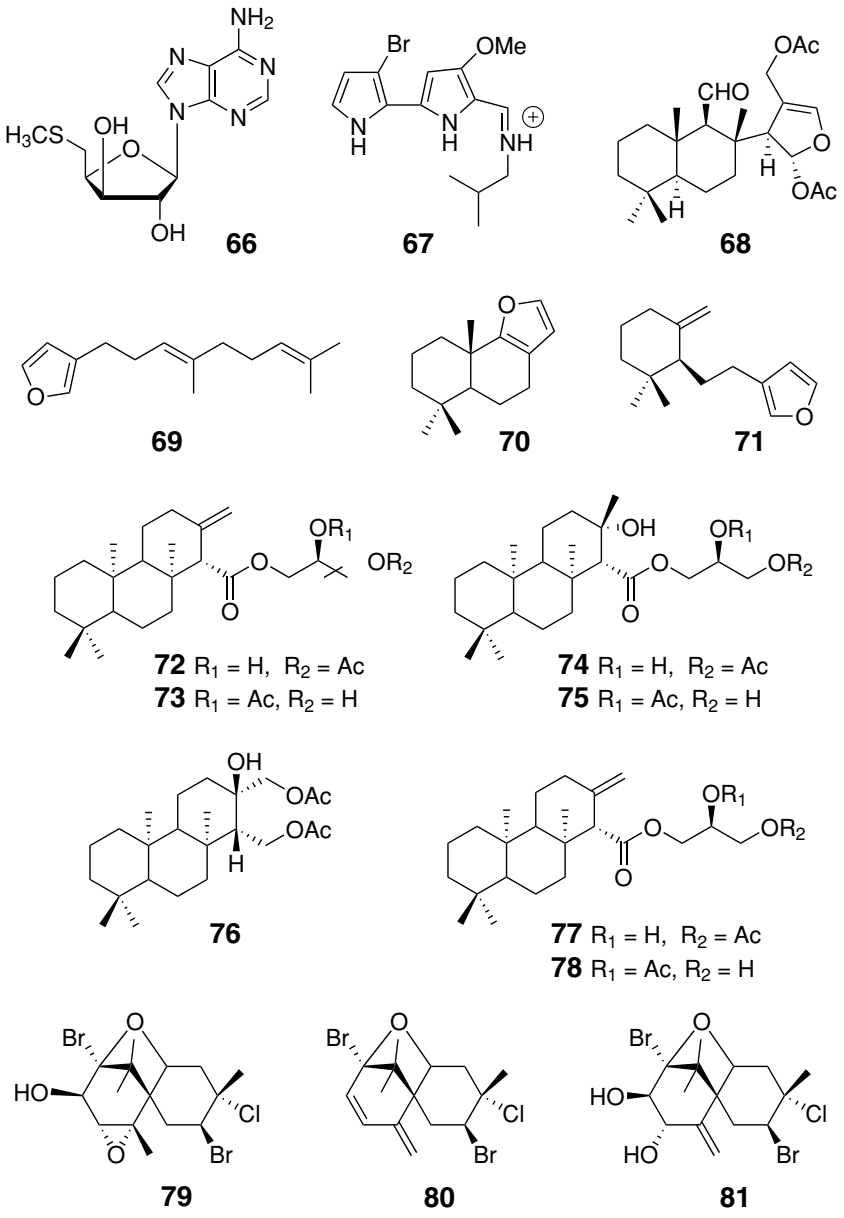


Fig. 6.6. Secondary metabolites isolated from the South American opisthobranchs *Doris* aff. *verrucosa*, *Tambja eliora*, *Tyrinna nobilis*, *Anisodoris fontaini* and *Aplysia dactylomela*

6.4
Pulmonate Secondary Metabolites

In contrast to other gastropods, the sub-class Pulmonata is dominated by terrestrial and to a lesser extent freshwater species. As a requirement for their terrestrial existence, pulmonates do not use gills to breathe but instead possess a pulmonary cavity within their mantle tissue which functions as a primitive lung. The roof of the pulmonary cavity is highly vascularised and provides the necessary respiratory surface for the uptake of oxygen from air. This clear evolutionary adaption to terrestrial life within the sub-class Pulmonata means that the few marine pulmonate genera are regarded as primitive members of this sub-class (Smith and Stansic 1998). Marine pulmonates are typically shelled inter-tidal molluscs and are very similar in appearance and habits to their distant relatives, the true limpets (Prosobranchia). In the southern hemisphere, studies of the secondary metabolites produced by pulmonate gastropods have been confined to the genera *Siphonaria* and *Trimusculus* occurring off the coasts of southern Africa, South America and Australia.

6.4.1
Southern African Marine Pulmonates

The natural products chemistry of four species of South African inter-tidal pulmonate molluscs has been reported.

Siphonarids (Order Basommatophora)

Molluscs of the genus *Siphonaria* (Family Siphonariidae) are air-breathing, shelled, inter-tidal herbivores often referred to as "false" limpets. Siphonariids have re-evolved a set of gill-like structures in the mantle cavity, enabling them to breathe effectively when they are submerged during high tides (Branch and Branch 1981). As the tide recedes, siphonariids revert to an air-breathing lifestyle by drawing air into the pulmonary cavity through a siphon situated on the right-hand side of the foot. During low tide, siphonariids graze on the algae and micro-organisms living on the rocks in the inter-tidal zone. In contrast to their distant "true" limpet relatives, siphonariids can be relatively easily displaced from the rock surface and are thus prone to predation by both terrestrial and aquatic predators at low and high tides, respectively (Beukes and Davies-Coleman 1999). In response to any perceived predatory pressure, siphonariids produce copious amounts of white mucus from lateral pedal glands. Although the mucus produced by siphonariids often contains a plethora of acyclic and cyclic propionate-derived metabolites, the role of these compounds or their acyclic

precursors in the chemical ecology of *Siphonaria* species is not clear (Davies-Coleman and Garson 1998).

S. capensis is the most common of the nine *Siphonaria* species known to occur off the coast of southern Africa. A C-2 epimeric mixture of *E* and *Z*-siphonarienfuranone (**82**, **83**), capensinone (**84**), capensifuranone (**85**) and a known polypropionate biogenetic precursor (2*E*, 4*S*, 6*S*, 8*S*)-2,4,6, 8-tetramethyl-2-undecenoic acid (**86**) were isolated from specimens of *S. capensis* collected off the eastern Cape coast of South Africa (Beukes and Davies-Coleman 1999). The biogenesis of polypropionates has been extensively reviewed (Davies-Coleman and Garson 1998) and is also discussed in this volume by Darias et al. (in the chapter titled "The Chemistry of Marine Pulmonate Gastropods"). The 1,3-*syn* arrangement of methyl substituents in the aliphatic chains of siphonariid metabolites has proved to be an interesting synthetic challenge; and the recent synthesis of **85** (Williams et al. 2004) first confirmed the (*S*)-configuration at each of the three chiral centres in the side-chain of **85**, originally proposed from biosynthetic arguments, and second provided the (4*S*)-configuration in this compound which was unassigned by Beukes and Davies-Coleman (1999). Acetone extracts of two other *Siphonaria* species, *S. concinna* and *S. costatus* collected from the same region of the South African coast, afforded the ubiquitous siphonariid metabolite pectinatone (**87**) and siserrone A (**88**) as the major metabolites, respectively, (Beukes and Davies-Coleman 1999; Brecknell et al. 2000). The non-contiguous polypropionate skeleton of **88** led Brecknell et al. (2000) to question the natural product status of this compound and to propose that **88** was possibly a product of the facile rearrangement of another *S. serrata* metabolite dihydrosiphonarin A (**89**), during chromatographic work-up of the *S. serrata* extract. Both dihydrosiphonarin A and its ethyl homologue, dihydrosiphonarin B (**90**), were present as minor metabolites in the *S. serrata* extract. The ecological role of compounds **82–90** in southern African *Siphonaria* species was not established. The chemical structures of compounds **82–90** are presented in Fig. 6.7.

Trimusculids (Order Eupulmonata)

Marine pulmonate gastropods of the family Trimusculidae congregate in large colonies on the under-surface of inter-tidal rocky overhangs on exposed shores (Gray et al. 1998). In response to the constraints of their sedentary way of life, trimusculids secrete a mucous net, which they use to filter out food particles present in the water column at high tide. In common with other shelled pulmonates, trimusculids are subject to predation by a variety of sub-tidal and inter-tidal predators. Diterpenes predominate in the secondary metabolites produced by trimusculid molluscs as a chemical defence against predation and the two bioactive diterpene acetates (**91**, **92**) isolated by Gray et al. (1998) from the only

trimusculid species known to occur off the southern African coast, *Trimusculus costatus*, were found to deter the feeding of *Pomadasys commersonni*, a common, omnivorous inter-tidal and sub-tidal southern African fish, at natural concentrations (ca. 2.5 mg per pellet).

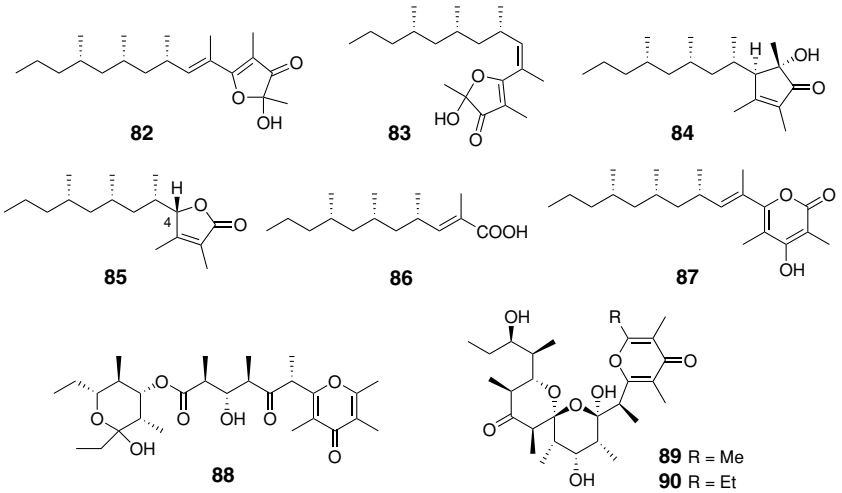


Fig. 6.7. Secondary metabolites isolated from the southern African pulmonates *S. capensis, S. costatus* and *S. concinna*

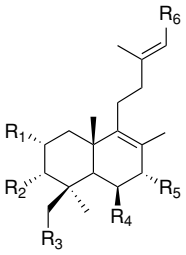
6.4.2 South American Marine Pulmonates

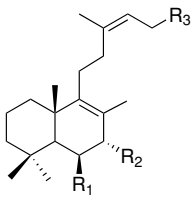
Studies of South American marine pulmonates have focussed exclusively on the trimusculid *T. peruvianus*.

Trimusculids (Order Eupulmonata)

A preliminary study of the diterpene secondary metabolites present in extracts of *T. peruvianus* specimens collected from the coast of Chile (Rovirosa et al. 1992) yielded the diterpene acetate (**93**). Additional investigations of the diterpene metabolites produced by this species afforded the four diterpene acetates (**94**–**97**) with a *Z* configuration assigned to the exocyclic olefin in these compounds (San Martin et al. 1996. Interestingly, the recent isolation of further four diterpene esters (**98**–**101**) containing a Δ^{13} *E*-olefin (Díaz-Marrero et al. 2003a) further confirmed that *T. peruvianus* biosynthesises both geometric isomers. All the diterpenes isolated thus far from *Trimusculus* species have been assumed to possess a labdane, as opposed to *ent*-labdane, stereochemistry and this assignment still requires corroboration. Finally, two polyhydroxylated steroids (**102**, **103**) with similar moderate in vitro cytotoxicity (IC_{50} 2.5 and 12.5 μg ml^{-1}, respectively) in two human colon

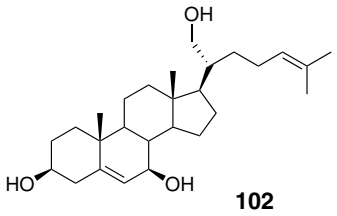
tumour cell lines (H-116, HT-29) were also isolated from *T. peruvianus* (Díaz-Marrero et al. 2003b). The chemical structures of the diterpene and sterol metabolites isolated from both *T. costatus* and *T. peruvianus* are presented in Fig. 6.8.





91 $R_1 = R_2 = R_3 = H$, $R_4 = R_5 = OAc$, $R_6 = CH_2OH$
92 $R_1 = R_4 = R_5 = OAc$, $R_2 = R_3 = H$, $R_6 = CH_2OH$
93 $R_1 = R_2 = R_3 = H$, $R_4 = OAc$, $R_5 = OH$, $R_6 = CH_2OH$
98 $R_1 = R_5 = H$, $R_2 = R_3 = R_4 = OH$, $R_6 = COOH$
99 $R_1 = R_5 = H$, $R_2 = OCOC_4H_9$, $R_3 = R_4 = OH$, $R_6 = COOH$
100 $R_1 = R_5 = H$, $R_2 = R_4 = OCOC_4H_9$, $R_3 = OH$, $R_6 = COOH$
101 $R_1 = R_5 = H$, $R_2 = R_3 = OCOC_4H_9$, $R_4 = OH$, $R_6 = COOH$

94 $R_1 = OH$, $R_2 = R_3 = OAc$
95 $R_1 = R_2 = R_3 = OAc$
96 $R_1 = R_2 = OAc$, $R_3 = OH$
97 $R_1 = OAc$, $R_2 = H$, $R_3 = OH$



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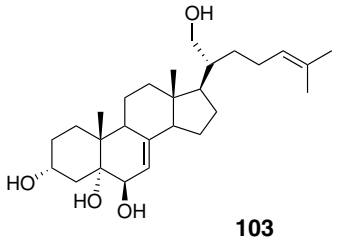

103

Fig. 6.8. Secondary metabolites isolated from the southern African and South American pulmonates *Trimusculus costatus* and *T. peruvianus*

6.5 Conclusions

Ironically, despite the relative inaccessibility of Antarctica and the obvious logistic difficulties in collecting gastropod molluscs from under the sea ice or from great depths in harsh and unpredictable climatic conditions that prevail in the southern Oceans, more appears to be known about the chemical ecology of Antarctic gastropod molluscs than the chemical ecology of the relatively more accessible marine molluscs of southern Africa and South America. Studies of the natural product chemistry of the marine gastropod fauna of South America and, to a lesser extent, southern Africa are still in a state of relative infancy. From

the handful of natural products investigations of South American gastropod molluscs already carried out, it would appear that the extensive South American coastline (exceeding 140,000 km in length) holds enormous promise for future marine mollusc natural product studies. Supporting evidence for a putative evolutionary link between cool temperate, shallow-water South American marine invertebrate fauna and those of Antarctica, first alluded to by Dayton et al. (1994), could possibly be achieved from a study of the diterpene metabolites produced by specimens of *A. kerguelenensis* known to inhabit the cold waters off southern South America.

References

Avila C, Iken K, Fontana A, Cimino G (2000) Chemical ecology of the Antarctic nudibranch *Bathydoris hodgsoni* Eliot, 1907: defensive role and origin of its natural products. J Exp Mar Biol Ecol 252:27–44

Beesly PL, Ross GJB, Wells A (1998) Mollusca: the southern synthesis. Part B (Fauna of Australia, vol 5). CSIRO, Canberra, pp. 565–1234

Berlinck RGS, Hajdu E, Rocha RM da, Oliveira JHHL de, Hernandez ILC, Seleghim MHR, Granato AC, Almeida EVR de, Nunez CV, Muricy G, Peixinho S, Pessoa C, Moraes MO, Cavalcanti BC, Nascimento GGF, Thiemann O, Silva, M, Souza AO, Silva CL, Minarini PRR (2004) Challenges and rewards of research in marine natural products chemistry in Brazil. J Nat Prod 67:510–522

Beukes DR, Davies-Coleman MT (1999) Novel polypropionates from the South African marine mollusk *Siphonaria capensis*. Tetrahedron 55:4051–4056

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2005) Marine natural products. Nat Prod Rep 22:15–61

Branch G, Branch M (1981) The living shores of southern Africa. Struik, Cape Town

Branch GM, Griffiths CL, Branch ML, Beckley LE (1994) Two Oceans. David Philip, Cape Town

Brecknell DJ, Collett LA, Davies-Coleman MT, Garson MJ, Jones DD (2000) New non-contiguous polypropionates from marine molluscs: a comment on their natural product status. Tetrahedron 56:2497–2502

Carte B, Faulkner DJ (1983) Defensive metabolites from three nembrothid nudibranchs. J Org Chem 48:2314–2318

Cimino G, Ghiselin MT (1998) Chemical defense and evolution in the Sacoglossa (Mollusca: Gastropoda: Opisthobranchia). Chemoecology 8:51–60

Cimino G, Ghiselin MT (1999) Chemical defense and evolution in biosynthetic capacity among dorid nudibranchs (Mollusca: Gastropoda: Opisthobranchia). Chemoecology 9:187–207

Cimino G, Sodano G (1993) Biosynthesis of secondary metabolites in marine mollusks. Top Curr Chem 167:77–115

Cimino G, De Rosa S, De Stefanon S, Sodano G, Villani G (1983) Dorid nudibranch elaborates its own chemical defense. Science 219:1237–1238

Cimino G, Fontana A, Gavagnin M (1999) Marine opisthobranch molluscs: chemistry and ecology in sacoglossans and dorids. Curr Org Chem 3:327–372

Cimino G, Ciavatta ML, Fontana A, Gavagnin M (2001) Metabolites of marine opisthobranchs: chemistry and biological activity. In: Tingali C (ed) Bioactive compounds from natural sources. Taylor and Francis, London, pp. 577–637

Copley RCB, Davies-Coleman MT, Edmonds DR, Faulkner DJ, McPhail KL (2002) Absolute stereochemistry of ibhayinol from a South African sea hare. J Nat Prod 65:580–582

Davies-Coleman MT, Faulkner DJ (1991) New diterpenoic acid glycerides from the Antarctic nudibranch *Austrodoris kerguelensis*. Tetrahedron 47:9743–9750

Davies-Coleman MT, Garson MJ (1998) Marine polypropionates. Nat Prod Rep 15:477–493

Dayton PK, Robilliard GA, Paine RT, Dayton LB (1974) Biological accommodation in the benthic community at McMurdo Sound, Antarctica. Ecol Monogr 44:105–128

Dayton PK, Mordida BJ, Bacon F (1994) Polar marine communities. Am Zool 34:90–99

Díaz-Marrero AR, Dorta E, Cueto M, Rovirosa J, San-Martin A, Loyola A, Darias J (2003a) Labdane diterpenes with a new oxidation pattern from the marine pulmonate *Trimusculus peruvianus*. Tetrahedron 59:4805–4809

Díaz-Marrero AR, Dorta E, Cueto M, Rovirosa J, San-Martin A, Loyola A, Darias J (2003b) New polyhydroxylated steroids from the marine pulmonate *Trimusculus peruvianus*. ARKIVOC 10:107–117

Faulkner DJ (2002) Marine natural products. Nat Prod Rep 19:1–48

Faulkner DJ, Ghiselin MT (1983) Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. Mar Ecol Prog Ser 13:295–301

Fontana A, Muniain C, Cimino G (1998) First chemical study of patagonian nudibranchs: a new seco-11,12-spongiane, tyrinnal, from the defensive organs of *Tyrinna nobilis*. J Nat Prod 61:1027–1029

Fretter V, Graham A, Ponder WF, Lindberg DR (1998) Prosobranchs. In: Beesly PL, Ross GJB, Wells A (eds) Mollusca: the southern synthesis. Part B (Fauna of Australia, vol 5). CSIRO, Canberra, pp. 605–638

Gavagnin M, Fontana A (2000) Diterpenes from marine opisthobranch molluscs. Curr Org Chem 4:1201–1248

Gavagnin M, Trivellone E, Castelluccio F, Cimino G, Cattaneo-Vietti R (1995) Glyceryl ester of a new halimane diterpenoic acid from the skin of the Antarctic nudibranch *Austrodoris kerguelensis*. Tetrahedron Lett 36:7319–7322

Gavagnin M, De Napoli A, Cimino G, Iken K, Avila C, Garcia FJ (1999a) Absolute configuration of diterpenoid diacylglycerols from the Antarctic nudibranch *Austrodoris kerguelenensis*. Tetrahedron Asymm 10:2647–2650

Gavagnin M, De Napoli A, Castelluccio F, Cimino, G (1999b) Austrodorin-A and -B: first tricyclic diterpenoid 2′-monoglyceryl esters from an Antarctic nudibranch. Tetrahedron Lett 40:8471–8475

Gavagnin M, Ungur N, Castelluccio F, Muniain C, Cimino G (1999c) New minor diterpenoid diacylglycerols from the skin of the nudibranch *Anisodoris fontaini*. J Nat Prod 62:269–274

Gavagnin M, Fontana A, Ciavatta ML, Cimino G (2000) Chemical studies on Antarctic nudibranch mollusks. Ital J Zool 67[Suppl 1]:101–109

Gavagnin M, Carbone M, Mollo E, Cimino G (2003a) Further chemical studies on the Antarctic nudibranch *Austrodoris kerguelenensis*: new terpenoid acylglycerols and revision of the previous stereochemistry. Tetrahedron 59:5579–5583

Gavagnin M, Carbone M, Mollo E, Cimino G (2003b) Austrodoral and austrodoric acid: *nor*-sesquiterpenes with a new carbon skeleton from the Antarctic nudibranch *Austrodoris kerguelenensis*. Tetrahedron Lett 44:1495–1498

Gosliner T (1987) Nudibranchs of southern Africa: a guide to the opisthobranch molluscs of southern Africa. Sea Challengers, Monterey

Granato AC, Berlinck RGS, Magalhaes A, Schefer AB, Ferreira AG, De Sanctis B, De Freitas JC, Hajdu E, Migotto AE (2000) Natural products from the marine sponges *Aaptos* sp. and *Hymeniacidon* aff. *heliophila*, and from the nudibranch *Doris* aff. *verrucosa*. Quim Nov 23:594–599

Gray CA, Davies-Coleman MT, McQuaid C (1998) Labdane diterpenes from the South African marine pulmonate *Trimusculus costatus*. Nat Prod Lett 12:47–53

Graziani EI, Andersen RJ, Krug PJ, Faulkner DJ (1996) Stable isotope incorporation evidence for the de novo biosynthesis of terpenoic acid glycerides by dorid nudibranchs. Tetrahedron 52:6869–6878

Gustafson K, Andersen RJ (1985) Chemical studies of British Columbia nudibranchs. Tetrahedron 41:1101–1108

Iken K, Avila C, Ciavatta ML, Fontana A, Cimino G (1998) Hodgsonal, a new drimane sesquiterpene from the mantle of the Antarctic nudibranch *Bathydoris hodgsoni*. Tetrahedron Lett 39:5635–5638

Iken K, Avila C, Fontana A, Gavagnin M (2002) Chemical ecology and the origin of defensive compounds in the Antarctic nudibranch *Austrodoris kerguelenensis* (Opisthobranchia: Gastropoda). Mar Biol 141:101–109

Kaiser CR, Pitombo LF, Pinto AC (1998) C-13 and H-1 NMR assignments of the chamigrenes prepacifenol and dehydroxyprepacifenol epoxides. Spec Lett 31:573–585

Kaiser CR, Pitombo LF, Pinto AC (2000) NMR analysis of a complex spin system from a spiro-chamigrene. Spec Lett 33:457–467

Kaiser CR, Pitombo LF, Pinto AC (2001) Complete ^{1}H and ^{13}C NMR assignments of chamigrenes from *Aplysia dactilomela*. Magn Reson Chem 39:147–149

Kay EA, Wells FE, Ponder WF (1998) Class Gastropoda. In: Beesly PL, Ross GJB, Wells A (eds) Mollusca: the southern synthesis. Part B (Fauna of Australia, vol 5). CSIRO, Canberra, pp. 565–604

McClintock JB (1994) The trophic biology of Antarctic echinoderms. Mar Ecol Progr Ser 111:191–202

McClintock JB, Baker BJ (1997) A review of the chemical ecology of Antarctic marine invertebrates. Am Zool 37:329–342

McClintock JB, Janssen J (1990) Pteropod abduction as a chemical defence in a pelagic Antarctic amphipod. Nature 346:462–464

McClintock JB, Heine J, Slattery M, Weston J (1990) Chemical bioactivity in common shallow-water Antarctic marine invertebrates. Antarct J US 25:204–206

McClintock JB, Baker BJ, Hamann MT, Yoshida W, Slattery M, Heine JN, Bryan PJ, Jayatilake GS, Moon BH (1994a) Homarine as a feeding deterrent in common shallow-water Antarctic lamellarian gastropod *Marseniopsis mollis*: a rare example of chemical defense in a marine prosobranch. J Chem Ecol 20:2539–2549

McClintock JB, Baker BJ, Slattery M, Heine JN, Bryan PJ, Yoshida W, Davies-Coleman MT, Faulkner DJ (1994b) Chemical defense of common Antarctic shallow-water nudibranch *Tritoniella belli* Eliot (Mollusca: Tritonidae) and its prey *Clavularia frankliniana* Rouel (Cnidaria: Octocorallia). J Chem Ecol 20:3361–3372

McPhail K, Davies-Coleman MT (1997) New spongian diterpenes from the east African nudibranch *Chromodoris hamiltoni*. Tetrahedron 53:4655–4660

McPhail KL, Davies-Coleman MT (2005) (3*Z*)-Bromofucin from a South African sea hare. Nat Prod Res 19:449–452

McPhail K, Davies-Coleman MT, Coetzee P (1998) A new furanosesterterpene from the South African nudibranch *Hypselodoris capensis* and a Dictyoceratida sponge. J Nat Prod 61:961–964

McPhail KL, Davies-Coleman MT, Copley RCB, Eggleston DS (1999) New halogenated sesquiterpenes from South African specimens of the circumtropical sea hare *Aplysia dactylomela*. J Nat Prod 62:1618–1623

McPhail KL, Rivett DEA, Lack DE, Davies-Coleman MT (2000) The structure and synthesis of tsitsikammafuran: a new furanosesquiterpene from a South African *Dysidea* sponge. Tetrahedron 56:9391–9396

McPhail KL, Davies-Coleman MT, Starmer J (2001) Sequestered chemistry of the arminacean nudibranch *Leminda millecra* in Algoa Bay, South Africa. J Nat Prod 64:1183–1190

Pettit GR (1997) The dolastatins. Fortschr Chem Organ Naturst 70:1–79

Pika J, Faulkner DJ (1994) Four sesquiterpenes from the South African nudibranch *Leminda millecra*. Tetrahedron 50:3065–3070

Pika J, Faulkner DJ (1995) Unusual chlorinated homo-diterpenes from the South African nudibranch *Chromodoris hamiltoni*. Tetrahedron 51:8189–8198

Pitombo LF, Kaiser CR, Pinto AC (1996) Occurrence of chamigrenes in *Aplysia dactylomela* from Brazilian waters. Bol Soc Chil Quim 41:433–436

Rapson TD (2004) Bioactive 4-methoxypyrrolic natural products from two South African marine invertebrates. MSc thesis, Rhodes University, Rhodes

Rovirosa J, Quezada E, San-Martin A (1992) New diterpene from the mollusc *Trimusculus peruvianus*. Bol Soc Chil Quim 37:143–145

Rudman WB, Willan RC (1998) Opisthobranchia. In: Beesly PL, Ross GJB, Wells A (eds) Mollusca: the southern synthesis. Part B (Fauna of Australia, vol 5). CSIRO, Canberra, pp. 915–1035

San-Martin A, Quezada E, Soto P, Palacios Y, Rovirosa J (1996) Labdane diterpenes from the marine pulmonate gastropod *Trimusculus peruvianus*. Can J Chem 74:2471–2475

Smith BJ, Stansic J (1998) Pulmonata. In: Beesly PL, Ross GJB, Wells A (eds) Mollusca: the southern synthesis. Part B (Fauna of Australia, vol 5). CSIRO, Canberra, pp. 1037–1125

Ungur N, Gavagnin M, Mollo E, Cimino G (1999) Absolute stereochemistry of anisodorin 5. Tetrahedron Asymm 10:1635–1636

Williams DR, Nold AL, Mullins RJ (2004) Asymmetric conjugate addition for the preparation of *syn*-1,3-dimethyl arrays: synthesis and structure elucidation of capensifuranone. J Org Chem 69:5374–5382

Yoshida WY, Bryan PJ, Baker BJ, McClintock JB (1995) Pteroenone: a defensive metabolite of the abducted Antarctic pteropod *Clione antarctica*. J Org Chem 60:780–782