Review

MARINE BIOTECHNOLOGY

Status and Perspective of Sponge Chemosystematics

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Abstract

In addition to their pharmaceutical applications, sponges are an important source of compounds that are used to elucidate classification patterns and phylogenetic relationships. Here we present a review and outlook on chemosystematics in sponges in seven sections: Secondary metabolites in sponges; Further applications of bioactive compound research in sponges; Sponge chemotaxonomy; Pitfalls of sponge chemotaxonomy; The chemotaxonomic suitability of sponge compounds; Potential synapomorphic markers in sponges; and The future of sponge chemotaxonomy.

Keywords: Bioactive compounds — chemosystematics — chemotaxonomy — Porifera — review — sponges

Secondary Metabolites in Sponges

Research on marine natural products has experienced explosive growth in the last decades, ranging from the pioneering work of Bergmann and coworkers on sponge arabinose nucleosides (Bergmann and Freeney, 1950) to the structure of maitotoxin, the most complex and poisonous marine compound known (Murata et al., 1994). Bergmann's work initiated an extensive search for potential marine drugs from sponges (and other marine invertebrates) after bioactive properties of those nucleosides were shown (Privat de Guarilhe and Rudder, 1964) and antiviral (Ara-A, viderabdine) or antitumor (Ara-C, cytarabine) analogs synthesized. This increased interest in sponge metabolites is reflected in the numerous recent reviews (e.g., Sarma et al., 1993).

To date, sponges are among the best sources of novel compounds with the greatest occurrence of potential pharmaceuticals (see Faulkner, 1998; Munro et al., 1999 and subsequent annual publications of this series). Sponges produce molecules with a variety of activities, e.g., antibacterial, anticoagulant, antifungal, antiinflammatory, antimalarial, antiplatelet, antituberculosis, and antiviral (e.g., Mayer and Hamann, 2004; Sipkema et al., 2005a). To date, several natural or prototypical compounds from sponges have passed the preclinical stage (Newman and Cragg, 2004). However, mostly synthesized analogs of natural compounds undergo clinical tests. Bergquist (1978, p. 203) remarked in late 1970s: "Effectively nature does the difficult part of the synthesis and the chemist does the rest." Indeed, no drug *directly* from marine origin has made it to the commercial sector yet.

Finding the bioactive compounds in sponges is relatively easy, and possibilities for prospecting still appear immense, but drug development requires such large amounts of metabolites (Munro et al., 1999) that it could result in extinction of the particular sponge species (Anderson, 1995). Solutions are sought in sponge farming (e.g., Duckworth et al., 1999 ; de Voogd 2005), primmorph systems (Müller et al., 2004), and especially the biosynthesis of compounds (e.g., Garson, 1994; see also Sipkema et al., 2005b). Considering the increasing efforts in combination with rapid methodological advances, it is evident that sponge biotechnology will remain a growing and important field in the future.

Further Applications of Bioactive Compound Research in Sponges

The search for bioactive sponge metabolites profits not only pharmaceutical sciences and industry; sponge taxonomists and systematists also benefit from new compound data for chemosystematic purposes (discussed in detail further below) and the discovery of new genera and species during the search for bioactive sponges. Currently more logistic

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and financial investments have been allocated to sampling from regions often inaccessible to the researcher such as deep seas. Special efforts combining sponge chemistry with taxonomy have been undertaken in New Caledonia (e.g., D'Ambrosio et al., 1996) and the Caribbean (e.g., Assmann et al., 2001), in particular the use of submersibles for deep-water sponge compounds. The Harbor-Branch Oceanographic Institute underlines in a cruise report the fruitful symbiosis of biochemistry and taxonomy: "Initial taxonomic analyses indicated that several sponges (...) may be species new to science, and many are certainly range extensions. Initial results of specimens tested for natural products indicate that many specimens show promising chemistry" (Pomponi et al., 2004).

Sponge Chemotaxonomy

The various natural products may also be employed as phylogenetic characters to aid in unraveling the numerous sponge classification problems. Difficulties in sponge morphological systematics are clearly due to their primitive features resulting in a shortage of clear morphological markers required for a robust phylogenetic reconstruction. Although use of DNA markers in sponge systematics began as early as 1991 (Kelly-Borges et al., 1991), sponge molecular systematics has become more established only recently. Secondary metabolites have been suggested as an alternative to morphological characters because they increase in number from year to year (van Soest and Braekman, 1999) and the structural complexity of the molecules promised a large source of new characters. Presence (or absence) of a particular compound or compound family among different sponge taxa may indicate a closer phylogenetic relationship.

It was especially the pioneering work of Bergquist and co-workers, who studied sponge compound compositions and their taxonomic distribution, that was extrapolated to numerous classification hypotheses. They studied (among others) free amino acids (Bergquist and Hartman, 1969), fatty acids (Bergquist et al., 1984; Lawson et al., 1984), sterols in Haplosclerida (Bergquist, 1980), calcareous and keratose sponges (Bergquist et al., 1986, 1991b), and compound composition in Dendroceratida and aaptamines in Hadromerida (Bergquist et al., 1990, 1991a). Subsequently, numerous biochemical markers were recruited and reviewed at all taxonomic levels to show coherence of sponge classification, e.g., protein banding in Microcionidae, Raspailiidae, and Axinellidae (Hooper et al., 1992); pyrrole-2-carboxylic acids and cyanoterpenes in Agelasida and Halichondrida (Braekman et al., 1992); sterols (Fromont et al., 1994); alkylpiperidines (Andersen et al., 1996) and straight-chain acetylenes (van Soest et al., 1998) in Haplosclerida; lipid biomarkers in Hexactinellida (Thiel et al., 2002); pyrroloquinolines in Latrunculiidae (Kelly in Urban et al., 2000); scalarane sesterterpenes in Spongiidae (Jaspars et al., 1997), suberitane in *Suberites* spp. (Diaz-Marrero et al., 2004); steroids (Barnathan et al., 2003 [2004]; Castellanos et al., 2003) and other compounds for Halichondrida and Suberitidae relationships (Erpenbeck and van Soest, 2005), as well as analyses on species and subspecies levels (e.g., Sennett et al., 1992; Kelly-Borges et al., 1994; Gauvin et al., 1998, 2004). Natural products have also been discussed with a view either to solve or likewise to raise lower-level taxonomic identification and assignment problems if distribution patterns were incongruent with morphological expectations (e.g., van Soest et al., 1996b; Jaspars et al. 1997; van Soest and Braekman, 1999; Kehraus et al. 2001; Miller et al., 2001).

The reviews of Bergquist (1978, 1979), Sarma et al. (1993), and van Soest and Braekman (1999) were the most comprehensive at the time and aimed to provide an overall picture of sponge chemotaxonomy.

Pitfalls of Sponge Chemotaxonomy

Interest in the use of chemotaxonomy in sponges has declined in recent years. As every other phylogenetic reconstruction method, sponge chemotaxonomy revealed peculiar pitfalls (Bergquist, 1978, 1979; van Soest et al., 1996a), especially with characters recruited from the literature (van Soest and Braekman, 1999; Erpenbeck and van Soest, 2005).

Absence/Presence. The classical chemotaxonomic approach is classification according to shared compounds or compound groups (synapomorphies). Such presence/absence coding appears relatively straightforward, but requires thorough investigation. The ideal chemotaxonomic marker should be present in all members of the taxon and shown to be absent in others. The importance of the latter is frequently underestimated. The following are two examples: A demosponge order, Nepheliospongida, was constructed based on the presence of cyclopropene sterols in some taxa and subsequently extrapolated as a combining character of further taxa (Bergquist, 1980), but later abandoned after examination of other putative nepheliospongids. Isolation of bromoisoxazoline alkaloids in *Aplysina* showed overlapping traits among species of the genus, which subsequently diminished their suitability

for species level chemotaxonomy (Fendert et al., 1999).

The isolation of a particular compound is useless for applications in classification as long as its exclusiveness remains ambiguous. This fact reflects a conflict of interest between compound research for pharmaceutical and taxonomic purposes. There is a bias toward the report of "new" compounds in the chemical literature, leading to the accumulation of chemotaxonomically uninformative (autapomorphic) characters, as the *absence* of other compounds is unreported. To date, analyses explicitly scoring both absence *and* presence of compounds in sponges are few and originate mostly from chemosystematic work (e.g., Bergquist et al., 1986, 1991b; Blumenberg et al., 2002; Castellanos et al., 2003).

Further, the report of a pharmaceutically valuable compound focuses the research on congenerics of a "bioactive" species because chances of finding other valuable compounds appear higher.

Prevalent analytical methods likewise contribute to a bias in species size. Smaller species cannot provide sufficient tissue material for thorough analyses, if specimens are not abundant. Those species will remain a blank spot in chemotaxonomy under the current analytical methods.

Homology of Compounds. The uncertainty of whether a particular compound is homologous or not is probably the greatest problem in chemosystematics (we use the term homology in the biological sense, referring here to similar precursors and pathways). Even if compounds or compound groups appear exclusive for a particular taxon, they are not necessarily homologous and derived from a common ancestor and therefore do not necessarily reflect a genealogical relationship. They might originate from different precursors and biochemical pathways. The same or different pathways might have evolved in different phyla in cases in which a bioactive compound can be produced in an energetically favorable manner from relatively simple precursors (Salomon et al., 2001). Several hypotheses on biogenetic pathways have been proposed to reconstruct sponge phylogenies (e.g., van Soest et al. 1998), but their correctness frequently remained speculative.

Nevertheless, a peculiar compound distribution in sponges might be explained by convergent biosynthesis. While the amount of newly discovered compounds increases exponentially, background knowledge does not. Much effort has been directed to synthesizing natural compound analogs to lessen dependence on sponge material, but comparatively small effort to investigation of the biogenetic pathways. The latter simply appears less interesting for further applications. How to treat compound information phylogenetically remains a decision for the systematist.

Variability. Suitable chemotaxonomic markers require stability in their occurrence. The production of terpenes (Thompson et al., 1987) and some fatty acids (Garson et al., 1994) underlies variability. Such variability might be based on (1) biotic factors (key signals) initiating the production defensive compounds, (2) the abundance of symbionts to produce the precursors, and (3) abiotic factors such as season and temperature. Compound variability can have a severe influence on phylogenetic coding, especially if compound compositions are scored as different characters.

Origin of the Compounds—Sponge or Symbiont?. Another problem in chemotaxonomy is the correct assignment of a compound as sponge- and not associate-derived. When the high abundance of microorganismal symbionts in sponges was discovered (e.g., Reiswig, 1975 ; Vacelet, 1975) it was demonstrated that sponge microsymbionts may also be the source of some of the bioactive compounds (e.g., Faulkner et al., 1994). Numerous substances bear remarkable structural similarities to bacterial compounds, implying a bacterial origin despite their high complexity (see Piel, 2004). Researchers are therefore intensively studying microbial communities in sponges (e.g., Friedrich et al., 1999 ; Hentschel et al., 2002).

The microbial origin of the compounds could provide great opportunities for marine pharmacology because obtaining sponge material from the sea is difficult (see above) and mariculture is not sufficiently established yet (Proksch et al., 2002). Isolation and culture of symbiotic microorganisms as producers of the secondary metabolites as well as transfer of symbiont biosynthetic genes into cultivable bacteria are subjects of ongoing research (e.g., Piel et al., 2004; see also Proksch et al., 2003). Many "sponge compounds" are isolated from associated fungi, which also appear to be a valuable and effective source of bioactive metabolites (Kobayashi et al., 1993; see Kelecom, 2002 and Höller et al. 2000 for a review).

However, compound production by sponge associates bears severe difficulties for chemotaxonomy. Principally, symbiont compounds cannot be used as taxon markers as long as the symbionts may switch between hosts—the genealogic information would be lost. Successful applications of symbiont compounds in chemotaxonomy are dependent on the stability of the host-symbiont relationship (Bewley and Faulkner, 1998). Some symbionts might display

such high host specificity that their compounds could be considered as suitable markers. Mechanisms for coevolution of associates with their sponge hosts, such as vertical transmission of bacteria with sponge larvae, have been discovered (e.g., Lévi and Lévi, 1976). Their phylogenetic patterns are currently studied via molecular tools (e.g., Erpenbeck et al., 2002).

Nevertheless, the assignment of a compound as sponge or symbiont compound is difficult. A very small percentage of bacteria are cultivable (Amann et al., 1995), which makes culture-based surveys problematic. Several analyses are performed to locate the compounds in the sponge (e.g., Lawson et al., 1986, 1988; Garson et al., 1992, 1994; Faulkner et al., 1994; Unson et al., 1994; Bewley et al., 1996; Uriz et al., 1996a,b; Marin et al., 1998; Gillor et al., 2000; Turon et al., 2000; Salomon et al., 2001; Richelle-Maurer et al., 2001, 2003). Uncertainty remains whether the localization of the compounds also reveals the location of biosynthesis (Piel, 2004), because compounds found in sponge cells might just be stored there. Nevertheless, the storage of large quantities of metabolites from other cells is questioned (Salomon et al., 2001) and biosynthetic studies of cell cultures yielded evidence that metabolites are stored at their location of biosynthesis (Andrade et al., 1999).

Misidentifications. An additional problem for the implementation of compound results in systematics is the potential misidentification of the samples. Identifying sponges is a task for specialists, and misidentifications lead to severe errors in subsequent chemotaxonomical conclusions. Long preservation times can also affect the correct redetermination negatively, especially in sponges without a mineral skeleton. The initially perceived morphological "similarities" of sponges can quickly result in samples being placed in different orders. An example is an exceptional report of diterpene isonitriles from an *Amphimedon* demosponge (Haplosclerida), which was a new, morphologically very similar *Cymbastela* species (Halichondrida, van Soest et al., 1996a). van Soest and Braekman (1999) and later Erpenbeck and van Soest (2005) pointed out several other potential misidentifications masking or faking (chemo)systematic relationships. van Soest et al. (1996a) and van Soest and Braekman (1999) suggested solutions such as morphological descriptions included in the chemical publications. Still, sponge species can be redetermined only out of a few biochemical publications and only the request of original voucher material ensures secure identification.

Sponge-Sponge Contamination. Inadequate sampling limits the use of biochemical data. Overgrowth by other species is a common phenomenon in sponges. One specimen might produce a metabolite whose origin will be described for the wrong species. Although such occasions are rare, they can have an impact on chemotaxonomical conclusions. This problem can be solved only with adequate sampling, a sufficiently large sample size, and familiarities in voucher identification.

Insufficient Amount of Characters. Finally, it remains unlikely that the number of potential chemical synapomorphies will be sufficient to resolve an entire phylogeny. A popular way to increase character numbers is to include compound concentrations in the matrix. While this approach might be suitable to indicate differences between taxa (e.g., Thiel et al., 2002), reconstructing phylogenies with compound concentrations is ambiguous as no objective criteria can be applied for their definition. Castellanos et al. (2003) screened the sterol composition of several halichondrid demosponges and expanded the character set to more than 300 characters by assigning categories of relative amount. This led to a large number of uninformative autapomorphic characters, from which no supported phylogeny could be reconstructed. Chemotaxonomic characters are therefore used mostly in combination with morphology (e.g., van Soest, 1991).

The Chemotaxonomic Suitability of Sponge Compounds

Several decades after the first chemotaxomomic exercises, knowledge regarding the suitability of chemotaxonomic markers increased, especially as a result of research on their potential biosynthetic origin (structures in Figure 1).

Fatty acids have been used several times in sponge chemotaxonomy (e.g., Bergquist et al., 1984). Long-chain fatty acids (LCFAs, Litchfield et al., 1976) were chemosystematically used to for order- and class-level sytematics (Thiel et al. 1999, 2002). Thiel et al. (2002) showed that possession of $\Delta^{5,9}$ LCFAs discriminates Hexactinellida and Demospongiae from Calcarea. However, chemotaxonomic application of fatty acids might remain problematic. A large fraction of the total sponge fatty acids might be of bacterial origin (Gillan et al., 1988). Although LCFAs appear to be produced and stored in sponge cells (Garson et al., 1994) and their occurrence appears independent from bacteria (Lawson et al., 1988), their precursors are considered to be bacterial (Hahn et al., 1988). Linear short-chain fatty acids are

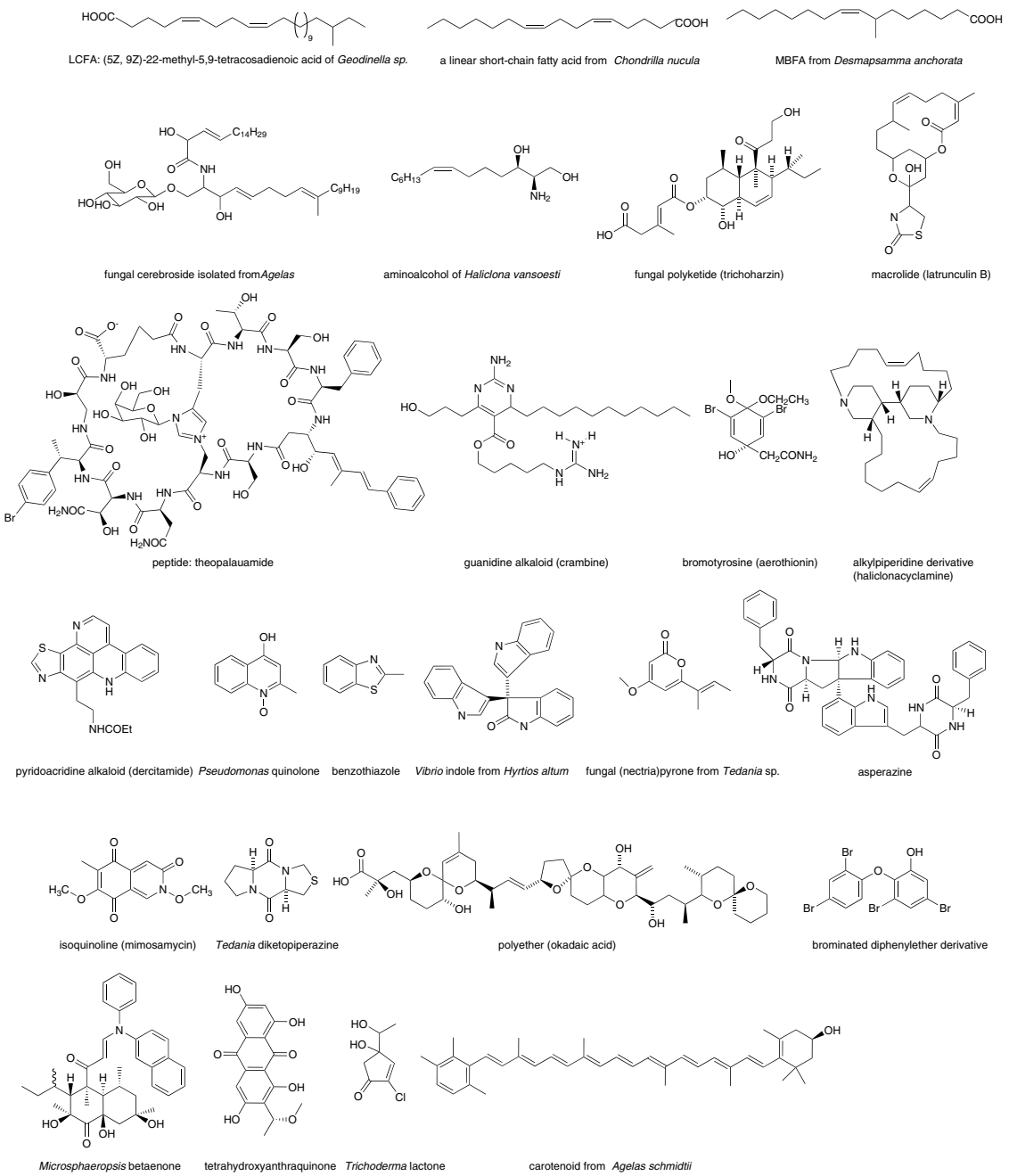

Figure 1. Selected compounds isolated from sponges. Their chemotaxonomic value is discussed in the text.

present in both symbiont and sponge cells but their origin cannot be assigned unambiguously (Thiel et al., 1999). Mid-chain branched fatty acids (MBFAs, C_{15}–C_{25}) are known from various bacteria (e.g., Campbell and Naworal, 1969) and sponge-associated fungi (e.g., Yu et al., 1996). The complex mixtures of MBFAs as potential LCFA precursors are unique and a result of a special symbiosis between bacteria and sponges (Thiel et al., 1999). Further, environmental conditions influence fatty acid composition (Garson et al. 1994). Several other lipid groups have been isolated from sponge-associates and their use in chemotaxonomy requires great care. Among these are cerebrosides (Keusgen et al., 1996), additional glycerolipids (Bultel-Ponce et al., 1999), and glycoglycerolipids (Wicke et al., 2000). Aminoalcohols from *Haliclona vansoesti* were found associated with the sponge cells and not with prokaryotic endobionts (Richelle-Maurer et al., 2001). Many polyketides isolated from sponges are suspected to derive from associates such as fungi (e.g., Kobayashi et al., 1993). Piel et al. (2004) report

on the isolation of prokaryotic genes in *Theonella swinhoei* for a *Pseudomonas*-polyketid resembling *Theonella* compounds. Macrolides are widespread in sponges, but difficult to use for chemotaxonomy. Both sponge and symbiont might be responsible for their biosynthesis. Latrunculin B was localized in the sponge cells of *Negombata magnifica* but not in its prokaryotic symbionts (Gillor et al., 2000). However, Piel (2004) lists a number of sponge-derived macrolides with prokaryotic counterparts, e.g., swinholide A and tolytoxin. Many bioactive sponge peptides are shown to be synthesized by bacteria, e.g., theopalauamides (Schmidt et al., 1998), andrimids (Oclarit et al., 1994), or cyclic depsipeptides (Kalinovskaya et al., 1995) or resemble bacteria compounds and are likewise of minor suitability for chemosystematics (see Piel, 2004). Several alkaloids were localized as sponge compounds and appear as suitable chemotaxonomic markers, e.g., the guanidine alkaloids crambine and crambinescidine from *Crambe crambe* (Uriz et al., 1996a) and bromotyrosine metabolites (e.g., aerothionin) from *Aplysina* spp. (Faulkner et al., 1999; Turon et al., 2000). The alkylpiperidine derivative haliclonacyclamine was localized within the sponge rather than the associated dinoflagellate cells (Garson et al., 1998). However, *Micromonospora* isolated from a marine sponge produces manzamine A (Kasanah et al. and Youssaf et al. in Newman and Cragg, 2004). Pyridoacridine alkaloids are widespread among Metazoa, suggesting a microbial origin. Dercitamide, however, is located exclusively in bacteria-free sponge cells of *Oceanapia sagittaria* (Faulkner et al., 1999; Salomon et al., 2001). Quinolones have been extracted from sponges and their associated bacteria simultaneously (Debitus et al., 1998). Other *Pseudomonas* quinolones have been isolated out of *Homophymia* (Bultel-Ponce et al., 1997). Benzothiazoles, indoles, and certain lactames are frequently synthesized from microorganisms (Stierle et al., 1991; Kobayashi et al., 1994; Kelecom, 2002). Sponge fungi frequently produce pyrones (Abrell et al., 1994) and asperazine (e.g., Varoglu et al., 1997). The isoquinoline mimosamycin from *Reniera* and *Xestospongia* spp. was previously isolated from *Streptomyces* (Fukumi et al., 1977). Other isoquinolines such as renieramycin from *Reniera* spp. resemble bacterial counterparts (see Piel, 2004). Diketopiperazines are common protein degradation products, implying a low chemotaxonomic value (Faulkner et al., 1993) and characteristic metabolites of microbial origin (Sammes, 1975). They have been located and isolated from sponge bacteria (e.g., Stierle et al., 1988). The pyrrole-2-carboxylic acid derivatives oroidin and sceptrin were recently localized in the sponge cells and not in the bacterial associates (Richelle-Maurer et al., 2003). The polyether okadaic acid from, e.g., *Halichondria okadai*, is produced by the dinoflagellate *Prorocentrum* (e.g., Murakami et al., 1982). *Vibrio* spp. associated with *Lamellodysidea* spp. produce brominated diphenyl ethers (e.g., Elyakov et al., 1991). Trichlorohydroxyphenylethers were obtained from *Micrococcus luteus* isolated from *Xestospongia* spp. (Bultel-Ponce et al., 1998). *Microsphaeropsis* fungi produce betaenone and tetrahydroxyanthraquinone derivatives in *Aplysina aerophoba* (Brauers et al., 2000). Lactones, chlorolactones, and lactone amides have frequently been isolated from sponge-associated bacteria and fungi (e.g., Amagata et al., 1998). Bioactive glyceroles are produced by *Micrococcus luteus* out of *Xestospongia* spp. (Bultel-Ponce et al., 1997, 1998).

Carotenoids in sponges are probably of bacterial origin (Liaaen-Jensen, 1967) and were frequently isolated from sponge-associated bacteria (e.g., Miki et al., 1994). The composition of sesqui- di- sester- and triterpenes (see examples in Figure 2) may change as a result of environmental influences (Thompson et al., 1987). Many are sponge-derived (Kelecom, 2002) such as various *Dysidea* sesquiterpenes (e.g., Unson and Faulkner, 1993) and various diterpenoids (e.g., Garson et al., 1992). However, other terpenes are clearly associate derived (e.g., Cheng et al., 1994). Several terpenes such as isocyanoterpenoids or carbonimidic dichlorides are rare in nature (Karuso and Scheuer, 1989; Simpson et al., 1997) and potentially suitable as chemotaxonomic markers (e.g., Braekman et al., 1992; Jaspars et al., 1997; van Soest and Braekman, 1999; Diaz-Marrero et al., 2004).

Most sterols (see examples in Figure 2) isolated from sponges are sponge derived (see Kelecom, 2002; Piel, 2004) although several cytotoxic sterols were isolated from sponge associates (e.g., Amagata et al., 1999). Sterol biosynthesis can follow several pathways in sponges, making their homologization difficult (Blumenberg et al., 2002). Sterols were frequently used for chemotaxonomic purposes (Bergquist, 1978, 1980; Bergquist et al., 1986, 1991b; Fromont et al., 1994; Blumenberg et al., 2002; Castellanos et al., 2003; Barnathan et al., 2003 [2004]). Rare features of their side chain such as cyclosterols, additional isopropyl groups (see Umeyama et al., 2000a,b), or two degrees of unsaturation (Kerr et al., 1997) found application in chemotaxonomy (e.g., Bergquist, 1980). Other modifications might be abundant among sponges (such as sulfation).

Potential Synapomorphic Markers in Sponges

We used the MarinLit software (Blunt and Munro, 2003) to review the current status of sponge chemo-

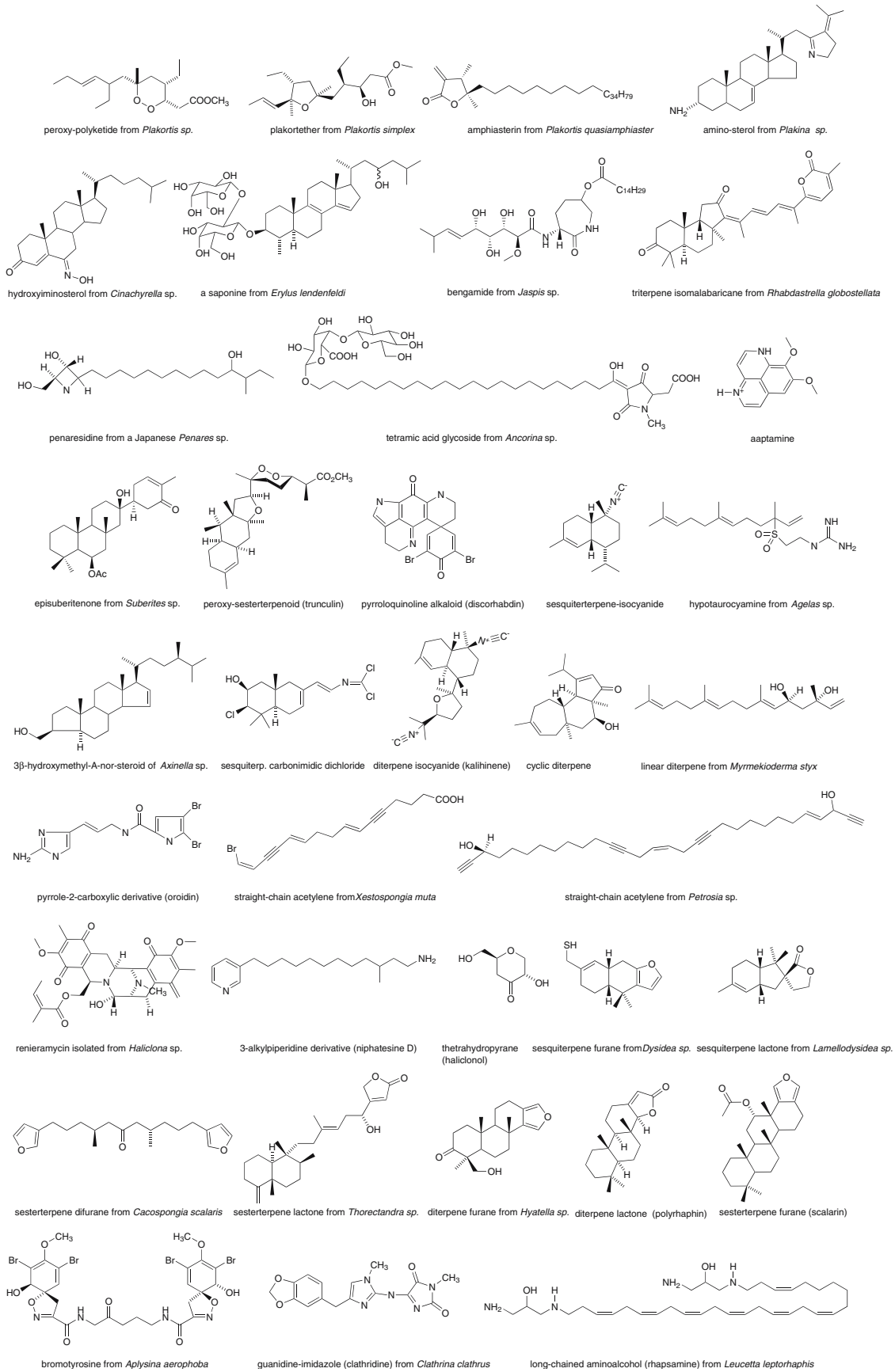

Figure 2. Selected compounds with potential chemotaxonomic value.

taxonomy. MarinLit is a compilation of marine biochemical literature ranging from 1940 until the present. As of the summer of 2004 MarinLit comprised 15,489 records—some 4500 of them include sponges, with more than 5600 structures. Entries for the years before 1997 were taken precompiled from the survey of van Soest and Braekman (1999). We tried to surmount the above-described problems on sponge chemotaxonomy as well as possible: Classification and taxon names have considerably changed since the first records and we tried to cope with this as much as possible. The classification was adopted from the Systema Porifera (Hooper and van Soest, 2002) and used throughout our considerations. As major problem remained the erroneous identification of the specimens. In several cases vouchers were reexamined. Other taxonomic corrections were made using the geographic sample location as a clue for the likely correct taxonomical assignment of the sponge. Further, we aimed to identify symbiont compounds, as far as they were known from the literature. In most cases we regard symbiotic compounds as phylogenetically uninformative if no obligate symbiosis and patterns of coevolution are demonstrated. Every potential synapomorphy was checked against MarinLit for its distribution among the Porifera and compared with the previous chemotaxonomic survey of van Soest and Braekman (1999, Table 1). If a compound is reported from wide a range of taxa or from taxa with only a distant relationship we did not consider it a suitable marker. However, we cannot rule out that compounds might be synapomorphic characters on a narrower taxonomic scale, assuming convergent evolution in major taxa.

Potential Synapomorphic Markers for Demospongiae

Peroxy-polyketides and derivatives were noted as ambiguous markers for Homosclerophorida (found in *Plakinastrella* and *Plakortis* spp.) and Chondrosida (*Chondrilla* and *Chondrosia* spp.; see van Soest and Braekman, 1999). However, there were reports from Haplosclerida (*Callyspongia* and *Cladocroce* spp. and recently *Xestospongia* spp., e.g., Murakami et al., 2002), and from Poecilosclerida (*Mycale* and *Acarnus* spp., e.g., Capon et al., 1997). We can clearly disregard peroxy-polyketides as markers for any demosponge group.

Plakortethers have been isolated from *Plakortis simplex* and presented as a new class of metabolites (Campagnuolo et al., 2002). They are related to plakortin and can be considered as potential *Plakortis* or Homosclerophorida markers only if this cycloperoxide is shown to be an unambiguous chemotaxonomic marker for its group (see above).

Amphiasterins are a new group of lactones extracted from the Homosclerophorida *Plakortis quasiamphiaster* (Zampella et al., 2001) and to date unknown from other taxa. Their chemotaxonomic suitability remains to be shown.

Aminosterols are a potential Homosclerophorida marker (van Soest and Braekman, 1999) and are still reported only from several species of *Corticium* and *Plakina* (plakinamides).

Hydroxyimminosteroids were isolated from several species of the Spirophorida genus *Cinachyrella* (Tethyidae, Rodriguez et al., 1997). These unusual steroids are a potential marker for this genus. Further potential Spirophorida compound markers have not been discovered yet.

Saponines are discussed as potential but ambiguous marker for Astrophorida because of their patchy taxonomic distribution. There are reports from species of Geodiidae (*Erylus*), Pachastrelliade (*Pachastrella*), and Ancorinidae (*Melophlus* and *Penares*) (see van Soest and Braekman, 1999). However, saponines isolated from Poecilosclerida (*Mycale laxissima* and *Ectyplasia ferox*, e.g., Campagnuolo et al., 2001) and Haplosclerida (*Niphates olemda* Yeung et al., 1994 as *Cribochalina*) nullify their suitability as Astrophorida markers.

Bengamide and bengazole derivatives are known from the Astrophorida families Calthropellidae (*Pachastrissa* sp., Fernandez et al., 1999) and Ancorinidae (e.g., *Jaspis carteri*, D'Auria et al., 1997) but similarities with bacterial fatty acids imply a dubious chemotaxonomic quality (Adamczeski et al., 1989).

Isomalabaricane triterpenes were assigned as potential markers for Astrophorida of the family Ancorinidae (*Stelletta s.l., Rhabdastrella*, van Soest and Braekman, 1999). Redetermination of voucher material showed isomalabricane as a chemotaxonomical maker for the genus *Rhabdastrella* only (Tasdemir et al., 2002). Reports from other, morphologically similar taxa are probably misidentified *Rhabdastrella*.

Penaresidins are straight-chained azetidine alkaloids and still exclusive markers for *Penares* (Ancorinidae, van Soest and Braekman, 1999).

Tetramic acid glycosides are reported from several species of the Astrophorida familiy Ancorinidae (*Ancorina* and *Penares*; e.g., Ohta et al., 1997) and several "Lithistida" of the families Theonellidae (e.g., *Theonella swinhoei*, Matsunaga et al., 1991) and Neopeltidae (*Homophymia conferta*, Wolf et al., 1999). Tetramic acid derivatives are frequently fungal associate compounds (Wright et al., 2003).

Although there is as yet no such report for tetramic acid glycosides, the chemotaxonomic value remains ambiguous.

Aaptamine-type alkaloids are a suggested marker for Hadromerida (Bergquist et al., 1991a), respectively its family Suberitidae (van Soest and Braekman, 1999). As aaptamine alkaloids have only recently been isolated from species of *Xestospongia* (Haplosclerida, Calcul et al., 2003), *Hymeniacidon* (Halichondrida, Pettit et al., 2004), and *Luffariella* (Dictyoceratida, Park et al., 1995), aaptamines cannot be considered as valid Hadromerida markers.

Suberitane-derived sesterterpenes were further considered as potential markers for the Hadromerida genus *Suberites* (Diaz-Marrero et al., 2004) in addition to high amounts of 5-α-stanols in the overall sterol composition (Barnathan et al., 2003 [2004]).

Peroxy-sesterterpenoids and pyrroloquinoline alkaloids might be suitable markers for Poecilosclerida based on the current sponge classification. Peroxy-sesterterpenoid derivatives, isolated from various species of the genus *Mycale*, were discussed as potential but ambiguous Poecilosclerida markers because of their abundance in *Latrunculia* spp. (van Soest and Braekman, 1999) previously assigned to the order Hadromerida. However, latrunculid sponges are currently classified with Poecilosclerida (Hooper and van Soest, 2002). Peroxy-sesterterpenoids have also been isolated from the Podospongiidae *Diacarnus* spp. (e.g., D'Ambrosio et al., 1998) and *Sigmoscreptella laevis* (Albericci et al., 1979), both previously assigned to Latrunculiidae and currently to Poecilosclerida (Hooper and van Soest, 2002). Similar pyrroloquinoline alkaloids have been isolated from *Latrunculia* and several Poecilosclerida genera (see van Soest and Braekman, 1999; Kelly in Urban et al., 2000). Thus peroxy-sesterterpenoids and pyrroloquinoline alkaloids are potential Poecilosclerida markers.

Polycyclic guanidine alkaloids were proposed as markers for the Poecilosclerida family Crambeidae (van Soest and Braekman, 1999) after taxonomic revision of similar reports from other species (van Soest et al., 1996a). Taking those taxonomic reconsiderations into account, this marker still can be assumed as valid. Polycyclic guanidine alkaloids are found in starfish (Palagiano et al., 1995) and probably derive from a sponge diet or other sponge contamination.

Sesquiterpene isocyanides are known from several Halichondrida (van Soest and Braekman, 1999). They are regarded as potential but ambiguous markers for this order as there are reports from *Theonella swinhoei* ("Lithistida," Nakamura et al., 1984) and an unspecified Verongida (Hamann and Scheuer, 1991), which strongly diminish their chemotaxonomic value. Convergent pathways have to be assumed to consider sesquiterpenes isocyanides as markers for Halichondrida.

Hypotaurocyamines are sesquiterpene-derived compounds, and appear to be unique for the Agelasida genus *Agelas* (Braekman et al., 1992). They are unknown from other sponges and therefore a marker for this genus.

Hydroxy-nor-sterols are reported from Halichondrida of the family Axinellidae (e.g. Bergquist, 1978, 1979; Barnathan et al., 2003 [2004]). Erpenbeck and van Soest (2005) provided evidence for the 3β-hydroxymethyl-A-nor-steroids as potential chemotaxonomic markers for the family Axinellidae by reanalyzing their taxonomic distribution.

Sesquiterpenoid carbonimidic dichlorides represent rare examples of terpene compounds carrying functional groups containing both nitrogen and carbon (Simpson et al., 1997). They were previously found in Halichondrida (*Stylissa* spp. and *Axinyssa* spp., e.g., Wratten and Faulkner, 1977) and therefore regarded as potential halichondrid markers (Erpenbeck and van Soest, 2005). *Ulosa spongia*, in which terpenic carbonimidic dichlorides were also found (Kehraus et al., 2001), was consequently assigned to Halichondrida, but the correct genus and species assignment remains ambiguous.

Diterpene isocyanides are regarded as halichondrid markers (Braekman et al., 1992), and have been found in Haplosclerida *Cribrochalina* spp. (Niphatidae, Ciavatta et al., 1999). However, the skeletal similarity of niphatid taxa to certain Halichondrida (Axinellidae) genera has caused much (chemo-) taxonomic confusion (van Soest et al., 1996b), and an additional misidentification is likely. Until reexamination of the voucher occurs, we still can assume the diterpene isocyanides as potential markers for Halichondrida (Erpenbeck and van Soest, 2005).

Cyclic diterpenes (more precisely with 5,6,7,tricarboyclic "cyanthiwigin" structure; see Erpenbeck and van Soest, 2005) are potential markers for the Halichondrida family Desmoxyidae (van Soest and Braekman, 1999) and still exclusively reported from its genera *Higginsia* and *Myrmekioderma*.

Linear diterpenes are autapomorphic characters for *Myrmekiodema* (van Soest and Braekman, 1999) and until present not reported from other taxa.

Pyrrole-2-carboxylic acid derivatives are discussed as biochemical markers for the Agelasida and Axinellidae (Braekman et al., 1992). Regarding the occurrence of pyrrole-2-carboxylic acid derivatives in other taxa (Rudi et al., 1994), the marker should be restricted to pyrrole-2-carboxylic acid derivatives with an aminopropylimidazole moiety

that still combines the Halichondrida families Axinellidae and Dictyonellidae with the Agelasida (both Agelasidae and Astroscleridae, Erpenbeck and van Soest, 2005).

Straight-chain acetylenes were used for phylogenetic approaches with the order Haplosclerida (van Soest et al., 1998). They are still regarded as sponge rather than symbiont compounds (Kelecom, 2002) despite being reported from fungi (e.g., Parish et al., 2004) and the occurrence of huge numbers of bacteria in Haplosclerida (Brantley et al., 1995). However, the report of related (see van Soest and Braekman, 1999), similar, or identical compounds (e.g., the Astrophorida *Theonella swinhoei*, Fu et al., 1999) underlines the initial suspicion of microbial origin (but note the discussion on acetylenic compounds). van Soest and Braekman (1999) pointed out a taxonomically relevant distribution of brominated straight-chain acetylenes for *Xestospongia* (Haplosclerida), which is corroborated by our screening, and hydroxyl side chains for *Petrosia* (Haplosclerida), which is diminished by the *Theonella* (Astrophorida) compounds.

Renieramycins are isoquinolones and display a distribution throughout Haplosclerida. They have been isolated from species of *Haliclona*, *Xestospongia*, and *Neopetrosia* (He and Faulkner, 1989). However, their chemotaxonomic suitability is ambiguous because renieramycins resemble bacterial compounds such as safracin (*Pseudomonas*) and saframycin (*Streptomyces*, see Piel, 2004).

3-Alkylpiperidine derivatives are frequently reported from all five marine Haplosclerida families and regarded as good markers for the order (Andersen

Table 1. Comparison of the chemotaxonomical value of the compounds from the previous estimation of 1998 (van Soest and Braekman, 1999) with those discussed in the text.

Taxon	*Compound class*	*1998*	*2004*
Plakina and *Corticium*	Aminosterols	+	+
Homosclerophorida and Chondrosida	Peroxy-polyketides	O	–
Cinachyrella	Hydroxyimminosteroids	n.a.	+
Astrophorida	Saponins	O	–
Stelletta s.l. (Astrophorida)	Isomalabaricane triterpenes	+	Astrophorida
Penares	Penaresidins	+	+
Pachastrellidae	Sulfated sterols	O	–
Suberitidae	Aaptamines	+	–
Suberites	Suberitane-derived sesterterpenes	n.a.	+
Spirastrellidae and Clionaidae	4,8,12-Trimethyltridecanoic acid	O	–
Mycale	Peroxy-sesterterpenoids	O	Poecilosclerida
Latrunculiidae (Poecilosclerida)	Peroxy-sesterterpenoids	O	Poecilosclerida
Latrunculiidae (Poecilosclerida)	Pyrroloquinoline alkaloids	O	Poecilosclerida
Crambeidae	Polycyclic guanidine alkaloids	+	+
Halichondrida	Sesqui- and diterpene isocyanides	+	O
Halichondriidae	Sulfated sterols	O	–
Axinellidae	Hydroxy-nor-sterols	n.a.	+
Halichondrida	Sesquit. carbonimidic dichlorides	n.a.	O
Halichondrida	Diterpene isocyanide	+	+
Desmoxyidae	Cyclic diterpenes	+	+
Myrmekioderma	Linear diterpenes	O	+
Axinellidae and Agelasida	Pyrrole-2-carboxylic derivatives	+	+
Agelasidae	Hypotaurocyamines	+	+
Haplosclerida s.l.	Straight-chain acetylenes	+	–
Xestospongia s.s.	Brominated acetylenes	+	+
Petrosia	Polyhydroxylated acetylenes	+	–
Haplosclerida s.l.	3-Alkylpiperidine derivatives	+	–
Haliclona	Tetrahydropyrans	O	–
Petrosiidae and Phloeodictyidae	Cyclopropene sterols	O	–
Dictyoceratida and Dendroceratida	Furano/lactone terpenes	+	–
Spongiidae, Thorectidae, and Irciniidae	Furano/lactone sesterterpenes	O	–
Dysideidae	Furano/lactone sesquiterpenes	O	–
Dendroceratida and Dictyoceratida	Furano/lactone diterpenes	O	+
Spongiidae, Thorectidae, and Irciniidae	Scalarane sesterterpenes	n.a.	+
Verongida	Bromotyrosine derivatives	+	–
Clathrinida	Guanidine imidazoles	+	–
Clathrinida	Long-chain aminoalcohols	+	O

A plus indicates compounds assigned as biochemical markers in 1998 and exclusive markers in the present review. A circle indicates a nonexclusive status of a marker. Compounds with a dash are regarded unsuitable in the present review.

et al., 1996), considering reports from Dictyoceratida and "Lithistida" a result of overgrowth (van Soest and Braekman, 1999). However, recent reports from Poecilosclerida (*Echinochalina*, Jimenez et al., 2000) and Halichondrida species (*Halichondria*, Chill et al., 2002) diminish their suitability as Haplosclerida markers.

Tetrahydropyranes are probably not suitable markers for the Haplosclerida genus *Haliclona* as suggested by van Soest and Braekman (1999). They are abundant in various compounds such as the phorboxazoles (Paterson and Luckhurst, 2003).

Furan- and lactone- sesquiterpenes, diterpenes, and sesterterpenes are biogenetically related and suggested markers for both Dictyoceratida and Dendroceratida (van Soest and Braekman, 1999). There is a record on bisfuranoterpenes from the Hadromerida *Spheciospongia papillosa* (Capon et al., 1981 as *Spirastrella*) identical to those isolated from a Western Australian *Spongia*. If misidentification or overgrowth can be ruled out, the reliability of this marker is seriously diminished.

Sesquiterpene furanes were suggested Dictyoceratida and Dendroceratida compounds but are also reported from species of the Haplosclerida genus *Aka* (Sullivan and Faulkner, 1983 as *Siphonodictyon*) and the Halichondrida *Axinella* (Anjaneyulu et al., 1994).

Sesquiterpene lactones are known from several species of the Dictyoceratida *Dysidea* and *Lamellodysidea* (both Dysideidae) and the Dendroceratida *Dictyodendrilla* spp. (Dictyodendrillidae; Tran et al., 1995). However, the sesquiterpene lactone picrotoxin has been reported from the Hadromerida *Cliona inconstans* (Sarma et al., 1987 as *Spirastrella*) and is also known from terrestrial sources. Therefore the chemotaxonomic suitability of sesquiterpene lactones is dubious.

Sesterterpene furanes were isolated from the Dendroceratida *Igernella* and *Spongionella* spp. (Dictyodendrillidae), from several species of the Dictyoceratida families Irciniidae, Spongiidae, and Thorectidae, but not from Dysideidae. An exception of ambiguous relevance are reports from sesterterpene furane sulfates in an unspecified Halichondrida of the family Halichondriidae (Kernan and Faulkner, 1988), almost identical to compounds reported from the Dendroceratida *Darwinella australensis* (Makarieva et al., 2003).

Sesterterpene lactones are known from Dictyoceratida species of the families Irciniidae, Spongiidae, and Thorectidae but not from Dysideidae. There are reports from sesterterpene lactone sulfates from the Dendroceratida *Darwinella australensis* (Makarieva et al., 2003), but with an uncertain relationship to the Dictyoceratida compounds.

Diterpene furanes are reported from Dendroceratida Darwinellidae (*Aplysilla* and *Dendrilla* spp.), but not from Dictyodendrillidae. For the Dictyoceratida there are records from Spongiidae, Thorectidae, and Dysideidae but not from Irciniidae.

Diterpene lactones have been frequently isolated from the Dendroceratida Darwinellidae and Dictyodendrillidae and from the Dictyoceratida Spongiidae and Thorectidae (only *Dactylospongia* and *Luffariella*), but not from Dysideidae and Irciniidae.

Scalarane sesterterpenes (e.g., scalarin) are markers for Dictyoceratida and reported from Spongiidae, Thorectidae, and Dysideidae but not Irciniidae. Jaspars et al. (1997) suggested 20,22-bishomosesterterpenes as chemotaxonomic markers for foliose Spongiidae (now Thorectidae).

Bromotyrosine derivatives are suggested as combining markers for the order Verongida, despite records from Poecilosclerida *Iotrochota* and the Agelasida *Agelas* spp. (see van Soest and Braekman, 1999). Recent reports from a Haplosclerida *Oceanapia* sp. (Nicholas et al., 2001), the Astrophorida *Poecillastra* and *Jaspis* spp. (Kim et al., 1999), and the Dicyoceratida *Dysidea rhax* (Shin et al., 2000 as *Aplysinella rhax*) compromise this compound as Verongida marker.

Potential Synapomorphic Markers for Calcarea and Hexactinellida. Guanidine imidazoles previously combined Calcarea families (van Soest and Braekman, 1999) but findings of related metabolites in the astrophorid demosponge *Stelletta* spp. (Tsukamoto et al., 1999) raise doubts on their suitability.

Aminoalcohols with chain length of greater than C29 have been reported for species of the Clathrinidae and Leucettidae (Calcarea, order Clathrinida, van Soest and Braekman, 1999). Aminoalcohols with chain lengths only up to C20 are known from demosponges (*Haliclona vansoesti*, Devijver et al., 2000).

Sterols of Hexactinellida were examined by Blumenberg et al. (2002), who stated that unconventional modifications of the ring or side-chain carbon skeletons, as found in many demosponges, appeared absent from the hexactinellids, and that overlap in steroid compositions among its members would prevent further chemosystematic classifications at the intraclass level.

The Future of Sponge Chemotaxonomy

Reviewing the literature and comparing the results of our approach with previous reviews (e.g., van Soest and Braekman, 1999) clearly projects the

picture of a steady come-and-go of biochemical compounds as phylogenetic markers (Table 1). Numerous compounds were presented as markers for sponge classification and later abandoned after further study on compound nature, biogenetic (microorganismal) origin, pathway, or taxonomic distribution. Only a few compounds appear as rather solid markers for higher taxa.

Ironically, this situation reflects the status of sponge morphological systematics. In recent decades many phylogenetic hypotheses have been proposed on various morphological characters or reproductive features. They were extrapolated to the entire taxon and later abandoned when subsequent analyses revealed contradictions. Questions of homology or homoplasy are a great challenge for both sponge morphologists and chemotaxonomists (van Soest, e.g., 1991; Chombard et al., 1998). We demonstrate that sponge chemosystematics at its present state is not suitable to outperform morphology but it might be a source for additional characters when used in an equivalent matter.

Nevertheless, the question remains whether increased efforts in chemotaxonomy will ever fulfill the huge expectations in this field. These expectations were raised in the last decades, but did not yet result in major breakthroughs in sponge systematics. Sponge molecular systematics is becoming a more and more established and mature field and has the power to outperform both morphological systematics and chemotaxonomy. DNA sequence comparison offers the systematist an overwhelming amount of characters clearly exceeding the resolution power of biochemical compounds. The nucleotide substitution models provide acknowledged hypotheses on character evolution. Assessment of character homology appears easier in a DNA alignment (especially with protein-coding sequences) than in morphology or biogenetic pathway studies. Contamination with associates might be more easily recognized by comparison against DNA data banks (Erpenbeck et al., 2002) than the identification of sponge associates as true producers of a compound. Certainly, molecular systematics has specific pitfalls especially in sponges, leading to "unexplainable" results, but clearly offers larger perspectives. Full-genome research is currently about to be established for other organismal groups and promises an even higher phylogenetic resolution in the future.

Does that mean chemosystematics is a "breadless art" and useless for further applications? We clearly do not think so. Although systematic questions might be better solved by DNA data, chemosystematics provides many important insights into compound distribution, when plotted against a robust and comprehensive phylogenetic (DNA) tree. Chemotaxonomy, especially on a narrower taxonomic level, remains important to elucidate biosynthetic pathways of valuable components. If a certain compound is present at the base of a phylogenetic clade, it might be a potential precursor for modified or polymeric molecules. It will enable us to draw conclusions about their distribution among sponge groups. If a specific bioactive molecule is produced in a group of sponges but *not* by their close relatives, other techniques can be employed to screen for the responsible gene combinations. Conclusions on homologous or convergent pathways of compound biosyntheses can be drawn, which will be of particular interest when screening for further suitable target taxa. In addition, the successful application of stereochemistry in phylogeny has been shown repeatedly and may provide a powerful character source for lower taxonomic levels (Pietra, 2003).

For this purpose, several things have to be considered for chemotaxonomy. Comprehensive sets of specimens must be sampled, which comprise preferably all sponge species of the taxon regarded. After careful determination and taxonomic identification, the whole range of the particular compounds group must be monitored for all specimens such as done for sterols (Bergquist et al., 1986, 1991b; Blumenberg et al., 2002; Castellanos et al., 2003) or fatty acids (Thiel et al., 2002).

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