Pergamon

TETRAHEDRON

Tetrahedron 56 (2000) 5247–5252

New Sesquiterpene and Brominated Metabolites from the Tropical Marine Sponge *Dysidea* sp.

George M. Cameron, Bronwin L. Stapleton, Shane M. Simonsen, Douglas J. Brecknell and Mary J. Garson*

Department of Chemistry, The University of Queensland, Brisbane QLD 4072, Australia

Received 22 March 2000; revised 3 May 2000; accepted 18 May 2000

Abstract—2D NMR spectroscopic data are reported for 6-hydroxyfurodysinin-*O*-methyl lactone (**3**), 2-(2′,4′-dibromophenoxy)-4,6-dibromoanisole (**4**), and dehydroherbadysidolide (**8**), all isolated for the first time from *Dysidea* sp. Revised NMR assignments are presented for the compounds dysetherin (**1**) and furodysinin-*O*-methyl lactone (**2**), previously reported from *Dysidea herbacea*. The full relative stereochemistry of spirodysin (**12**) is defined for the first time. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Natural products chemists have long been fascinated by marine sponges, given the diverse array of bioactive secondary metabolites isolated from these sessile marine invertebrates.[1] One of the most intensively studied species of sponge is the tropical marine sponge *Dysidea herbacea* (family Dysideidae, Order Dendroceratida), a ramulose or encrusting olive-green sponge that occurs in a number of distinct chemotypes as a result of an association with symbiotic microorganisms. The chemistry reported from this sponge, and related sponges in the genus, includes sesquiterpene spirolactol/lactones,[2] tricyclic furans,[3,4] some based on the furodysinin and furodysin skeletons[4] and their oxidised derivatives,[4b,4c,4g,5] modified steroids,[6] polychlorinated alkaloids,[7] brominated diphenyl ethers[8] and other metabolites.[9,10] The polychlorinated alkaloids and brominated diphenyl ethers produced by the sponge have been shown to be associated with the filamentous cyanobacterium *Oscillatoria spongeliae*,[11] and are therefore likely synthesised by the cyanobacterial symbiont;[12,13] in contrast, the terpene metabolites present in the sponge are localised in the sponge cells of *D. herbacea*.[12c]

In this paper we examine the structure and stereochemistry of some novel compounds isolated from collections of *Dysidea* spp. (Fig. 1) made at Lizard Island in Queensland. Two new sesquiterpenes and a new methoxy tetrabromodiphenyl ether are described for the first time. Our NMR investigation of these compounds also led to revision of the NMR assignments for some well-known *Dysidea* metabolites.

Keywords: alkaloids; bromophenols; NMR; sponges; terpenes.

* Corresponding author. Tel.: +61-7-3365-3605; fax: +61-7-3365-4299; e-mail: garson@chemistry.uq.edu.au

Results and Discussion

Sponge chemistry

Sponge specimens were collected at depths of 2–5 m at Horseshoe Reef and at depths of 10–15 m at the ‘Sponge’ Bommie, Lizard Island and carefully sorted, then extracted into DCM/MeOH 1:1. The combined organic extracts were concentrated in vacuo, then subjected to silica flash chromatography using a step gradient of hexane–EtOAc (from 5 to 100% EtOAc). Individual fractions were purified by normal phase (NP) HPLC using hexane–ethyl acetate 95:5 or by RPHPLC using MeOH/H_2O. Sponge sample 13-7-98-2-1 (*Dysidea* sp. 1519) was extracted to give dysetherin (**1**),[2c] furodysinin-*O*-methyl lactone (**2**)[5a,5b] and some acetylated sterols.[6d,6e] Sponge sample 14-7-98-1-2 (*Dysidea* sp. 1524) was extracted to give the new compounds 6-hydroxyfurodysinin-*O*-methyl lactone (**3**) and 2-(2′,4′-dibromophenoxy)-4,6-dibromoanisole (**4**), as well as the known 2-(3′,5′-dibromo-2′-methoxyphenoxy)-3,5-dibromoanisole (**5**),[8b–8d] and herbasterol (**6**).[6b] A second small sample of sp. 1524 was extracted to give 2-(2′,4′-dibromophenoxy)-4,6-dibromoanisole (**4**), 2-(3′,5′-dibromo-2′-methoxyphenoxy)-3,5-dibromoanisole (**5**),[8b–8d] 2-(3′,5′-dibromo-2′-methoxyphenoxy)-3,4,5-tribromoanisole (**7**),[8d,8f] dehydroherbadysidolide (**8**), 2-(3′5′-dibromo-2′-hydroxyphenoxy)-3,5,6-tribromophenol (**9**),[8c,8d] 2-(3′,5′-dibromo-2′-hydroxyphenoxy)-3,4,5,6-tetrabromophenol (**10**),[8c,8d] and herbamide A (**11**).[7a]

Sesquiterpenoids

Dysetherin (**1**) and furodysinin-*O*-methyl lactone (**2**) were identified by comparison of their ^{13}C and ^{1}H NMR data with the literature.[2c,5a,5b] Acquisition of 2D NMR data revealed

0040–4020/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0040-4020(00)00434-8

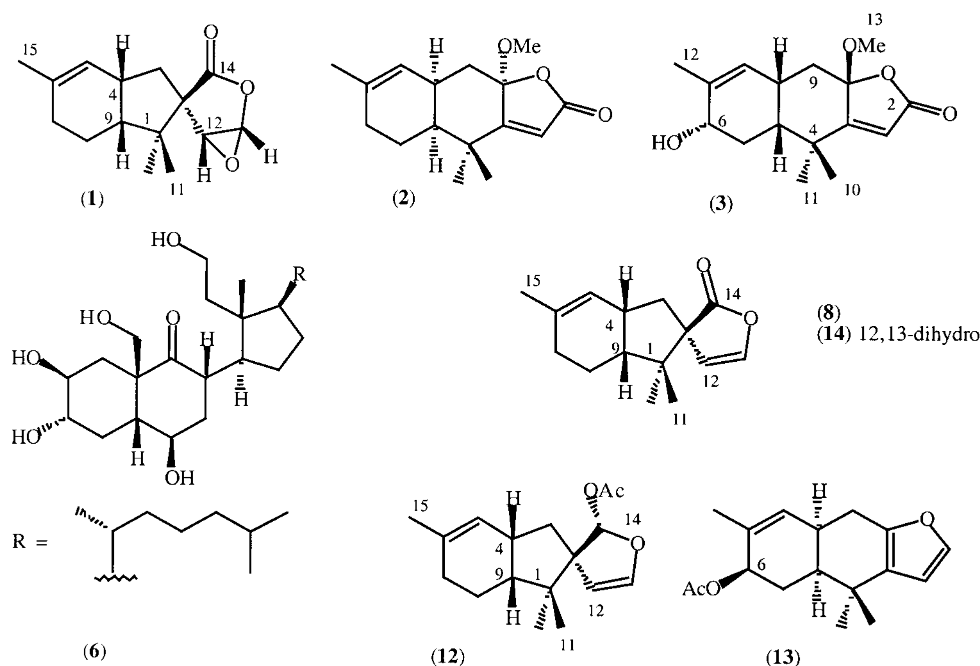

Figure 1. Terpenes from *Dysidea* spp.

some anomalies in the original ^{13}C assignments of dysetherin (**1**) and furodysinin-*O* methyl lactone (**2**); revised values are presented in Tables 1–3.

6-Hydroxyfurodysinin-*O*-methyl lactone (**3**) gave a molecular mass of 278.1527 corresponding to a molecular formula of $C_{16}H_{22}O_4$ and indicating a hydrogen deficiency of six. The ^{13}C and ^{1}H NMR (Table 3) indicated a 3,4-disubstituted 4-methoxybutenolide moiety (169.4 (s), 117.6 (d), 172.3 (s), 107.3 (s) and 50.4 (q) ppm; δ 5.82, 1H, s; δ 3.15, 3H, s) plus a trisubstituted double bond (136.9 (s), 126.6 (d) ppm; δ 5.44, 1H, ddd) therefore the remaining unsaturation was accounted for by a tricyclic system. Signals for a *gem* dimethyl group and a vinyl methyl were present on inspection of proton data (δ 1.24 and 1.34, both 3H, s; δ 1.71, 3H, d, J=1 Hz). Finally a proton signal at δ 4.10 (1H, ddd) linked to a methine carbon signal at 71.2 ppm, clearly suggested a hydroxyl-substituted carbon. These data strongly suggested a hydroxy analogue of furodysinin-*O*-methyl lactone (**2**). DQFCOSY, DEPT, HSQC and HMBC data confirmed the structure. In particular long range correlations between H8 and C6 and between H6 and both C7 and C8 placed the hydroxyl group adjacent to the trisubstituted double bond. The orientation of the substituted butenolide was confirmed by HMBC correlations between H3 and C4a, C10 and C11, while the position of the *O*-methyl group at

Table 1. Proton NMR assignments for dehydroherbadysidolide (**8**) compared with dysetherin (**1**), spirodysin (**12**) and herbadysidolide (**14**)

Atom#	δ ^{1}H^{a} (**8**)	NOESY	δ ^{1}H^{a} (**1**)	δ ^{1}H^{a} (**12**)	δ ^{1}H^{b} (**14**)
1	–	–	–	–	–
2	–	–	–	–	–
3α	1.68 (dd 13.5 9.5)	3β 4 10 12	1.60 (dd 13.3 10.0)	1.47 (dd 13.0 9.0)	[c]
3β	2.13 (dd 13.5 9.0)	3α 4	2.11 (dd 13.3 8.4)	2.31 (dd 13.0, 9.0)	[c]
4	3.05 (br q)	3α 3β 5 9 11	2.98 (br d, 10.0)	2.38 (br, m)	2.90 (br d 9.0)
5	5.35 (m 2.0)	3α 3β 4	5.33 (m, 1.6)	5.30 (m, 4.0)	5.34 (br s)
6	–	–	–	–	–
7	1.90 (br)	8α 8β 15	1.89 (br)	1.87 (m)	[c]
8α	1.77 (dddd 14.0 9.5)	7 8β 9	1.75 (dddd 13.5 6.1)	1.70 (dddd 13.2 6.4)	[c]
8β	1.53 (dddd 14.0 7.0)	7 8α 9	1.49 (dddd 13.5, 6.3)	1.49 (dddd 13.2 6.2)	[c]
9	2.41 (ddd 9.5 7.0 7.0)	4 7 8α 8β 10 11	2.32 (ddd 9.6 7.1 6.3)	1.86 (dd 6.7 3.2)	[c]
10	0.92 (s)	3 α 7 8α 8β	1.19 (s)	0.91 (s)	1.07 (s)[d]
11	0.97 (s)	4 8β 9	1.14 (s)	0.81 (s)	0.92 (s)[d]
12	5.41 (d 3.5)	3α 10 11 13	3.53 (d 2.4)	4.90 (d, 2.9)	[c]
13	6.76 (d 3.5)	12	5.46 (d 2.4)	6.30 (d, 2.9)	4.19 (m)
14	–	–	–	6.40 (s)	–
15	1.65 (br s)	7 8β	1.66 (br s)	1.64 (br s)	1.37 (s)
OAc			–	1.98 (s)	–

[a] 500 MHz; solution in $CDCl_3$ referenced to ^{1}H=δ 7.25.
[b] See Ref. 2b.
[c] Not reported.
[d] Assignments may be reversed.

Table 2. ^{13}C NMR data and long-range $^{13}C-^{1}H$ correlations for dehydroherbadysidolide (**8**) compared with ^{13}C NMR data for dysetherin (**1**) and spirodysin (**12**)

Atom #	$\delta^{13}C^{a}$ (**8**)	HMBC[b]	$\delta^{13}C^{a}$ (**1**)	$\delta^{13}C^{a}$ (**12**)
1	48.3 (s)	–	47.5	46.9
2	60.6 (s)	–	59.7	63.1
3	40.3 (t)	2 4 5 12	39.1	36.0
4	35.3 (d)	[c]	31.1[d]	35.6
5	124.7 (d)	[c]	123.7	125.0
6	133.0 (s)	–	134.1	133.1
7	28.6 (t)	5 6	28.7[d]	28.6
8	21.3 (t)	1 4 6 7 9	21.2[d]	21.3
9	43.3 (d)	1 4 10 11	44.9[d]	44.5
10	22.0 (q)	1 2 9 11	22.3[d]	21.8
11	24.0 (q)	1 2 9 10	26.1[d]	23.0
12	112.6 (d)	1 13 14	57.8	107.1
13	141.0 (d)	1 12 14	76.1	142.5
14	181.0 (s)	–	177.6	99.3
15	23.8 (q)	5 6 7	23.7	23.8
–OAc	–	–	–	169.0, 21.0

[a] Inverse detection at 500 MHz (assigned by geHSQC); solution in $CDCl_3$; ^{13}C=77.0 ppm.
[b] Inverse detection at 500 MHz; correlations observed when $^{1}J_{13C-1H}$=135 Hz and long range $^{n}J_{13C-1H}$=8 Hz.
[c] Not observed.
[d] Revised assignments by HMBC.

C9 was implied by the chemical shift of the acetal carbon and confirmed by a correlation between H9 and C13.

The relative stereochemistry of (**3**) was determined from NOESY and coupling constant data (Table 3). The *cis* orientation of the two ring junction protons, H4a and H8a, was apparent from the strong NOESY correlation between them plus the small vicinal coupling constant (4.0 Hz). NOESY correlations between H8a and the methoxy group, and between H6 and H4a confirmed the relative stereochemistry at C6 and C9a. The W coupling of 1.5 Hz between H8a and H5β suggested a half chair conformation for the cyclohexene ring,[4g] which was confirmed by molecular modelling as was the chair conformation of the middle ring.[5d] The vicinal coupling constants calcuated from this analysis were in agreement with those measured from the proton NMR data. The absolute stereochemistry of (**3**) was suggested by comparison with literature data. Since (−)-furodysinin-*O*-methyl lactone (**1**) has been shown to have the same absolute stereochemistry as (+)-furodysinin by chemical correlation,[5b] we infer that our sample of 6-hydroxyfurodysinin-*O*-methyl lactone, with $[\alpha]_D$ of +146, belongs to the (−)-furodysinin series, secured as 4a*R*, 8a*R* by total synthesis from (+)-9-bromocamphor.[14] Searle et al. showed that (+)-6-acetoxyfurodysinin (**13**) possesses 4a*S*, 8a*S* configuration by conversion to (+)-furodysinin.[4g] Thus the presence or absence of a substituent at C6 does not appear to affect the sign of rotation within the furodysinin series. Therefore our sample of (**3**) from sp. 1524 was provisionally assigned as 4a*R*, 6*S*, 8a*R*, 9a*S*. The sample of furodysin-*O*-methyl lactone (**2**) isolated from sp. 1519 had $[\alpha]_D$ of −183, which is the same sign, but a different magnitude of rotation to that reported by Carte et al.[5b] and identified as 4a*S*, 8a*S*,9a*R* by Horton et al.[5d] The sample decomposed before the relative configuration could be checked by NOESY spectroscopy.

The second new sesquiterpenoid (**8**) was identified as the didehydro analogue of herbadysidolide (**14**). An observed molecular mass of 232.1469 corresponded to a molecular formula of $C_{15}H_{20}O_2$ and indicated a hydrogen deficiency of six. The ^{13}C NMR and ^{1}H NMR (Tables 1 and 2) indicated a lactone (181.0 ppm), a trisubstituted double bond (124.7 (d) 133.0 (s); δ 5.35 m), plus a polarised disubstituted double bond (112.6 (d), 141.0 (d); δ 5.41 d, 6.76 d) which together accounted for three degrees of unsaturation. Therefore the molecule was tricyclic. A geminal dimethyl group (δ 0.97 s, 0.92 s) and a vinylic methyl (δ 1.65 bs) were also apparent from the proton NMR. The structure (**8**) was apparent by comparison of these data with those of dysetherin (**1**), and of spirodysin (**12**) available from a previous study (Tables 1

Table 3. Selected NMR data for furodysinin-*O*-methyl lactone (**2**) and 6-hydroxyfurodysinin-*O*-methyl lactone (**3**)

Atom #	$\delta^{13}C^{a}$ (**2**)	$\delta^{13}C^{a}$ (**3**)	$\delta^{1}H^{b}$ (**3**)	COSY	HMBC[c,d]	NOESY
2	169.8 (s)[e]	169.4 (s)	–	–	–	
3	117.3 (d)	117.6 (d)	5.82 (s)	–	2 3a 4 9a	5α 11
3a	173.1 (s)	172.3 (s)	–	–	–	–
4	38.7 (s)	38.4 (s)	–	–	–	–
4a	47.7 (s)	46.7 (d)	1.80 (ddd 14.5 4.0 2.5)	5α 5β 8a	3a	5 α 5β 6 8a 10 11
5α	18.5 (t)	29.1 (t)	1.13 (ddd 10.0 12.5 14.5)	4a 5β 6	6 4a 8a	4a 5β 6 9α
5β	–	–	2.03 (dddd 12.5 7.5 2.5 1.5)	4a 5α 6	4a 6 7 8a	4a 5α 6 11
6	30.8 (t)	71.2 (d)	4.10 (ddd, 7.5 10.0 1.0)	5α 5β	7 8	4a 5α 5β 8 12
7	134.3 (s)	136.9 (s)	–	–	–	–
8	123.6 (d)	126.6 (d)	5.44 (ddd 5.5 1.0 1.0)	4a 8a 12	4a 6 8a 12	8a 9α 9β 12
8a	30.2 (d)	30.7 (d)	2.75 (m)	4a 8 9β 12	4a 5 7 8 9	4a 8 9α 10 13
9α	40.3 (t)	39.8 (t)	1.58 (dd 14.0 13.5)	6 8a 9β	4a 8 8a 9a	5′ 8 8a 9β
9β	–		2.36 (ddd 14.0 4.0)	8a 9α	3a 4a 8 8a 9a	8 8a 9α 13
9a	107.5 (s)	107.3 (s)	–	–	–	–
10	25.7 (q)[f]	25.4 (q)	1.34 (s)	–	3a 4 4a	4a 8a 11 13
11	25.3 (q)[f]	25.2 (q)	1.24 (s)	–	3a 4 4a	3 4a 5β 10
12	23.1 (q)	18.7 (q)	1.71 (d, 1.0)	8a	6 7 8	6 8
13	50.4 (q)	50.4 (q)	3.15 (s)	–	9a	–

[a] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; ^{13}C=77.0 ppm.
[b] 500 MHz; solution in $CDCl_3$ referenced to ^{1}H=δ 7.25.
[c] Inverse detection at 500 MHz; correlations observed when $^{1}J_{13C-1H}$=135 Hz and long range $^{n}J_{13C-1H}$=8 Hz.
[d] From ^{1}H to ^{13}C.
[e] Corrected from the value of 129.2 ppm cited in Ref. 5b
[f] Assignments may be interchanged within columns.

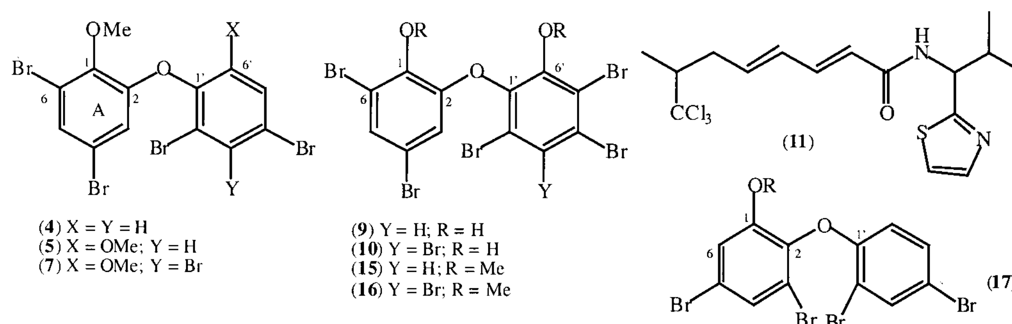

Figure 2. Selected halogenated metabolites from *Dysidea* spp.

and 2),[7b] and confirmed by analysis of DQFCOSY, HSQC and HMBC data for all three metabolites; the ^{13}C data for spirodysin are assigned here for the first time.

The relative stereochemistry of herbadysidolide (**14**) has been deduced by single crystal X-ray analysis[2b] and matches that of dysetherin (**1**), isolated from *Dysidea etheria*, determined by difference nOe experiments.[2c] A NOESY spectrum run on the sample of dysetherin isolated in this study confirmed these results, giving the anticipated nOe correlations, notably between H4 and H9, between H9 and H11, and between H10 and H12, confirming the relative stereochemistry reported in the literature. When dehydroherbadysidolide was examined by NOESY spectroscopy, the various nOe's between H4, H9 and H11 confirmed these were on the same face of the molecule. An nOe from H3α to H12 led to the conclusion that the C14 carbonyl group was *syn* to the H4/H9 ring junction, as in (**1**) and (**14**). Conformational analyses of (**8**) and its C2 epimer were carried out using MM3-92[15] and revealed that both epimers exist as a mixture of conformers (populations of 50, 27 and 23% respectively for (**8**) and 64, 29, 4 and 3% for the C2 epimer). The agreement between the conformationally-averaged calculated[16] vicinal coupling constants for (**8**) and the experimental values for dehydroherbadysidolide shown in Table 1 was quite satisfactory, while that for the epimer was poor. Finally we compared these data with that of spirodysin (**12**) whose relative configuration at C4 and C9 was originally established by conversion to furodysinin and furodysin,[4a] and at C14 by an NMR study.[2a] The relative configuration at the spiro centre C2, which was unknown, has now been reinvestigated using a sample previously isolated from *Dysidea herbacea*. NOe's between H4/H9, between H9/H11, between H11/H14, and between H9/H14 implied these protons all lie on the same face of the molecule. There was also a strong nOe between H10 and H12, consistent with spirodysin possessing the same C2 stereochemistry relative to C4/C9 as herbadysidolide, dehydroherbadysidolide and dysetherin.

The absolute stereochemistry of the metabolites (**1**), (**8**), (**12**) and (**14**) is unknown. Since the nmr data for dysetherin (**1**) and the relative stereochemistry determined by NOESY match that in the literature, but the $[\alpha]_D$ is opposite in sign, we may have isolated *ent*-dysetherin. Dehydroherbadysidolide, with $[\alpha]_D$ of −65, and herbadysidolide, with $[\alpha]_D$ of −47,[2b] likely share the same absolute configuration, while the sample of spirodysin used in this study had $[\alpha]_D$ of −15°, opposite to the literature value of+24,[2a] and may therefore be the *ent* isomer (Fig. 2).

Bromo metabolites

Bromoanisoles (**5**) and (**7**) and bromophenols (**9**) and (**10**)

Table 4. ^1H, ^{13}C NMR Data and long-range ^{13}C–^1H correlations for bromoanisole (**4**) compared with selected ^1H, ^{13}C NMR data for bromoanisoles (**5**), (**7**), and (**17**)

Atom #[a]	$\delta^{13}C^b$ (**4**)	δ^1H^c (**4**)	$\delta^{13}C^{d,e}$ (**5**)	$\delta^1H^{d,e}$ (**5**)	$\delta^{13}C^{d,e}$ (**7**)	$\delta^1H^{d,e}$ (**7**)	δ^1H^f (**17**)
1	147.6 (s)	–	144.8	–	144.9	–	–
2	150.0 (s)	–	150.9	–	150.3	–	–
3	121.9 (d)	6.90 (d 2.5)	116.4	6.45 (d)	116.3	6.43	–
4	116.7 (s)	–	116.0	–	116.3	–	–
5	131.0 (d)	7.49 (d 2.5)	128.1	7.35 (d)	128.5	7.31	–
6	119.3 (s)	–	118.5	–	118.7	–	–
1′	152.1 (s)	–	–		–	–	–
2′	115.4 (s)	–	–		–	–	–
3′	136.2 (d)	7.78 (d 2.5)	–		–	–	7.72 (d)
4′	117.2 (s)	–	–		–	–	–
5′	131.9 (d)	7.38 (dd 8.5 2.5)	–		–	–	7.21 (dd)
6′	120.3 (d)	6.74 (d 8.5)	–		–	–	6.30 (d)
–OMe (ring A)	61.3 (q)	3.89 (s)	60.4	3.99 (s)	60.7	3.99	–

[a] Numbering scheme of Refs. 8b–8d.
[b] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; ^{13}C=77.0 ppm.
[c] 500 MHz; solution in $CDCl_3$ referenced to $^1H=\delta$ 7.25.
[d] ^{13}C data at 15.04 MHz; solution in DMSO-d_6.
[e] See Refs. 8b–8d.
[f] 200 MHz. See Ref. 8g.

were identified by comparison of their spectroscopic data with the literature;[8b–8d,8f] methylation of (**9**) and (**10**) with diazomethane yielded bromoanisoles (**15**) and (**16**) identical in all respects to the literature.[8b–8d] The new bromoanisole (**4**) gave a five line molecular ion pattern consistent with the presence of four bromine substituents and a molecular mass of 515.7211 corresponding to a molecular formula of $C_{13}H_8Br_4O_2$. A DQFCOSY experiment revealed that the proton signal at δ 6.90 (J=2.5 Hz) was coupled to the signal at δ 7.49 (J=2.5 Hz), consistent with a 1,2,4,6- or 1,2,3,5-tetrasubstituted aromatic ring A; comparative ^{1}H and ^{13}C data for ring A of (**4**) were available from compounds (**5**) and (**7**) (Table 4).[8b–8d] In the B ring, the proton data clearly suggested a 1,2,4-trisubstituted ring (δ 6.74, J=8.5 Hz; δ 7.38, J=8.5, 2.5 Hz; δ 7.78, J=2.5 Hz). Anjaneyulu et al.[8g] have previously reported compound (**17**), the ring A isomer of (**4**); the ^{1}H and ^{13}C NMR data reported for ring B by these workers matches those of (**4**) (Table 4) except at the sterically-hindered positions *ortho* to the ether linkage. The structure of (**4**) was confirmed by geHMBC. Calculated chemical shift values were in agreement with the assigned structure except at the positions *ortho* to the ether linkage.

Taxonomic considerations

Although the chemistry reported in this paper is strikingly similar to that described for the sponge commonly known as *Dysidea herbacea*, the sponge samples investigated cannot at this time be formally described to species level. The chemical variability, notably the co-occurrence of brominated and chlorinated metabolites together with terpenes in sp. 1524, was unexpected, and clearly suggests the sample was not uniform despite careful sorting. Two collections of sp. 1524 were made, from sites near to each other, yet different metabolites were present in the two collections. These data, which highlight the chemical variation associated with *D. herbacea* at Lizard Island, are in striking contrast to our recent work at Heron Island where we have identified four distinct chemical populations of the sponge usually identified as *Dysidea* spp., each of which occupies a distinct geographic niche.[7b,17,18]

Experimental

Isolation of metabolites

Sponge samples were collected using SCUBA at about 3–5 m depth at Horseshoe Reef and the Sponge Bommie at Lizard Island on the Great Barrier Reef. For a brief description of the sponge and the cyanobacterial symbiont, see Refs. 11,12c. Voucher samples of *Dysidea* sp. (G314230; G314231) are held at the Queensland Museum, Brisbane.

Frozen sponge (36 g wet wt. G314230), collected at Horseshoe Reef, was cut into pieces and left in DCM/MeOH 1:1 (3×80 mL) at room temperature. The organic solution was filtered through a plug of cotton wool and the solvent removed by rotary evaporation to give a green semi-solid (441 mg) which was further purified by flash chromatography on silica using a step gradient of hexane/DCM 1:1 to neat DCM, then DCM/EtOAc through neat EtOAc, then EtOAc/MeOH through neat MeOH. Early eluting fractions containing metabolites were purified by silica HPLC using 5% EtOAc in hexane to give 2.6 mg of dysetherin (**1**), $[\alpha]_D$=+54 (c=0.005),[2c] and 3.5 mg of furodysinin-*O*-methyl lactone (**2**), $[\alpha]_D$=−183 (c=0.0035).[5a,5b]

Frozen sponge (105 g wet wt. G314231), collected at Horseshoe Reef, was cut into pieces and left in DCM/MeOH 1:1 (3×300 mL) at room temperature. The organic solution was filtered through a plug of cotton wool and the solvent removed by rotary evaporation to give a green semi-solid (3.1 g) which was further purified by flash chromatography on silica using a step gradient of hexane/DCM 1:1 to neat DCM, then DCM/EtOAc through neat EtOAc, then EtOAc/MeOH through neat MeOH. Early eluting fractions containing halogenated metabolites were further purified by silica HPLC using 5% EtOAc in hexane to give 1.0 mg of 2-(2′,4′-dibromophenoxy)-4,6-dibromoanisole (**4**) and 0.9 mg of 2-(3′,5′-dibromo-2′methoxyphenoxy)-3,5-dibromoanisole (**5**)[8b–8d] while later eluting fractions were purified by C18 HPLC using MeOH/H_2O 1:1 to give 5.4 mg of 6-hydroxyfurodysinin-*O*-methyl lactone (**3**) and 59.0 mg of herbasterol (**6**).[6b]

Frozen sponge (40 g wet wt. G314231), collected at the Sponge Bommie, was cut into pieces and left in DCM/MeOH 1:1(3×200 mL) at room temperature. The organic solution was filtered through a plug of cotton wool and the solvent removed by rotary evaporation to give a green semi-solid (532 mg) which was further purified by flash chromatography on silica using a step gradient of hexane/DCM 1:1 to neat DCM, then DCM/EtOAc through neat EtOAc, then EtOAc/MeOH through neat MeOH. Early eluting fractions containing bromophenols were purified by silica HPLC using 2% or 5% EtOAc in hexane to give 0.8 mg of dehydroherbadysidolide (**8**), 4.6 mg of 2-(2′,4′-dibromophenoxy)-4,6-dibromoanisole (**4**), 11.2 mg of 2-(3′,5′-dibromo-2′-methoxyphenoxy)-3,5-dibromoanisole (**5**)[8b,8d] and 2.4 mg of 2-(3′,5′-dibromo-2′-methoxyphenoxy)-3,4,5-tribromoanisole (**7**).[8d,8f] Later-eluting fractions contained 18.7 mg of 2-(3′,5′-dibromo-2′-hydroxyphenoxy)-3,5,6-tribromophenol (**9**),[8c,8d] 2.5 mg of 2-(3′,5′-dibromo-2′-hydroxyphenoxy)-3,4,5,6-tetrabromophenol (**10**)[8c,8d] and 3.3 mg of herbamide (**11**), $[\alpha]_D$=−52 (c=2.5).[7a]

6-Hydroxyfurodysinin-*O*-methyl lactone (3). $[\alpha]_D$= +146 (CH_2Cl_2, c=0.0035); ^{1}H and ^{13}C NMR, see Table 3; HREIMS, found 278.1527, $C_{16}H_{22}O_4$ requires 278.1518 (+3.3 ppm); GCMS m/z (intensity %) 278, (M+, 23), 246 (16), 228 (14), 202 (14), 175 (23), 149 (16), 109 (48), 95 (51), 67 (72), 41 (100).

2-(2′,4′-Dibromophenoxy)-4,6-dibromoanisole (4). ^{1}H and ^{13}C NMR, see Table 4; DQFCOSY H3 with H5, H5′ with H3′ and H6′; NOESY H5′ with H6′; geHMBC, C1 with H3 and H5, C2 with H3, C3 with H5, C4 with H3 and H5, C5 with H3, C6 with H5, C1′ with H3′, H5′, and H6′, C2′ with H3′ and H5′, C3′ with H5′, C4′ with H3′ and H6′, C5′ with H3′; HREIMS, found 515.7211, $C_{13}H_8{}^{79}Br_2{}^{81}Br_2O_2$ requires 515.7220 (−1.8 ppm); LREIMS m/z (intensity %) 516, (M+, 100), 422 (51), 356 (19), 313 (19), 210 (9), 178 (13), 75 (17), 62 (9), 67 (72), 50 (10).

Dehydroherbadysidolide (8). $[\alpha]_D=-66$ ($CHCl_3$, $c=0.0004$); ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS, found 232.1469, $C_{15}H_{20}O_2$ requires 232.1463 (+2.5 ppm); GCMS *m/z* (intensity %) 232, (M+, 6), 217 (3), 189 (3), 136 (60), 108 (100), 93 (39), 69 (41).

Acknowledgements

We thank the Department of Chemistry, The University of Queensland and the Australia Research Council for financial support, including scholarships to GMC and BLS. The director and staff of Lizard Island Research Station provided assistance with field work, and Jamie Simpson and Richard Clark helped with collection of sponge samples. Mr John Kennedy (Queensland Museum) provided taxonomic advice. This research was performed under permit G98/227 issued jointly by GBRMPA and QNPWS.

References

1. Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155 and earlier articles in this review series.
2. Spirolactols/lactones: (a) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1978**, 4949. (b) Charles, C.; Braekman, J. C.; Daloze, D.; Tursch, B.; Declerq, J. P.; Germain, G.; van Meerscche, M. *Bull. Soc. Chim. Belg.* **1978**, *87*, 481. (c) Schram, T. J.; Cardellina, J. H. *J. Org. Chem.* **1985**, *50*, 4155.
3. Other sesquiterpenes: (a) Sarma, N. S.; Rambabu, M.; Anjaneyulu, A. S. R.; Rao, C. B. S.; Saito, I. *Ind. J. Chem.* **1986**, *25B*, 1001. (b) Venkateswarlu, V.; Farooq Biabani, M. A.; Venkat Rami Reddy, M.; Chavakula, R.; Venkateswara Rao, J. *J. Nat. Prod.* **1994**, *57*, 827. (c) Sera, Y.; Adachi, K.; Nishida, F.; Shizuri, Y. *J. Nat. Prod.* **1999**, *62*, 395.
4. Furodysin/furodysinin skeleton: (a) Kaslauskas, R.; Murphy, P. T.; Wells, R. J.; Daly, J. J.; Schönholzer, P. *Tetrahedron Lett.* **1978**, 4951. (b) Dunlop, R. W.; Kazlauskas, R.; March, G.; Murphy, P. T.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 95. (c) Guella, G.; Mancini, I.; Guerriero, A.; Pietra, F. *Helv. Chim. Acta* **1985**, *68*, 1276. (d) Capon, R. J.; MacLeod, J. K. *J. Nat. Prod.* **1987**, *50*, 1136. (e) van Altena, I. A.; Millar, D. A. *Aust. J. Chem.* **1989**, *42*, 2181. (f) Ksebati, M.; Schmitz, F. J. *J. Nat. Prod.* **1988**, *51*, 857. (g) Searle, P. A.; Jamal, N. M.; Lee, G. M.; Molinski, T. F. *Tetrahedron* **1994**, *50*, 3879.
5. Lactones and autoxidised derivatives of furodysinin/furodysin: (a) Grode, S. H.; Cardellina, J. H. *J. Nat. Prod.* **1984**, *47*, 76. (b) Carte, B.; Kernan, M. R.; Barrabee, E. B.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 3528. (c) Carte, B.; Mong, S.; Poehland, B.; Sarau, H.; Westley, J. W.; Faulkner, D. J. *Tetrahedron Lett.* **1989**, *30*, 2725. (d) Horton, P.; Inman, W. D.; Crews, P. *J. Nat. Prod.* **1990**, *53*, 143.
6. Modified sterols: (a) Delseth, C.; Tolela, L.; Scheuer, P. J.; Wells, R. J.; Djerassi C. *Helv. Chim. Acta* **1979**, *62*, 101. (b) Capon, R. J.; Faulkner, D. J. *J. Org. Chem.* **1985**, *50*, 4771. (c) Rambabu, M.; Saram, N. S. *Ind. J. Chem.* **1987**, *26B*, 1156. (d) West, R. R.; Cardellina, J. H. *J. Org. Chem.* **1988**, *53*, 2782. (e) West, R. R.; Cardellina, J. H. *J. Org. Chem.* **1989**, *54*, 3234. (f) Kobayashi, M.; Krishna, M. M.; Ishida, K.; Ayaneyulu, V. *Chem. Pharm. Bull.* **1992**, *40*, 72.
7. Recent references to chlorinated alkaloids include: (a) Clark, W. D.; Crews P. *Tetrahedron Lett.* **1995**, *36*, 1185. (b) Dumdei, E. J.; Simpson, J. S.; Garson, M. J.; Byriel, K. S.; Kennard, C. H. L. *Aust. J. Chem.* **1997**, *50*, 139. (c) Fu, X.; Zeng, L.-M.; Su, J.-Y.; Pais, M. *J. Nat. Prod.* **1997**, *60*, 695. (d) Fu, X.; Ferreira, M. L. G.; Schmitz, F. J.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 1226. (e) J. B. MacMillan, T. F. Molinski, *J. Nat. Prod.* **2000**, *63*, 155.
8. Bromophenols: (a) Sharma, G. M.; Vig, B. *Tetrahedron Lett.* **1972**, 1715. (b) Norton, R. S.; Wells, R. J. *Tetarhedron Lett.* **1980**, *21*, 3801. (c) Carte, B.; Faulkner, D. J. *Tetrahedron* **1981**, *37*, 2335. (d) Norton, R. S.; Croft, K. D.; Wells, R. J. *Tetrahedron* **1981**, *37*, 2341. (e) Salva. J.; Faulkner, D. J. *J. Nat. Prod.* **1990**, *53*, 757. (f) Fu, X.; Schmitz, F. J.; Govindan, M.; Abbas, S. A.; Hanson, K. M.; Horton, P. A.; Crews, P.; Laney, M.; Schatzman, R. C. *J. Nat. Prod.* **1995**, *58*, 1384. (g) Anjaneyulu, V.; Nageswara Rao, K.; Radhika, P.; Muralikrishna, M.; Connelly, J. D. *Ind. J. Chem.* **1996**, *35B*, 89. (h) Fu, X.; Schmitz, F. D. *J. Nat. Prod.* **1996**, *59*, 1102. (i) Handayani, D.; Edrada, R. A.; Proksch, P.; Wray. V.; Witte, L.; Van Soest, R. W. M.; Kunzmann, A.; Soedarsono *J. Nat. Prod.* **1997**, *60*, 1313.
9. Isolated reports of terpenes from sponge specimens originally classified as Dysidea herbacea: (a) Kashman, Y.; Zviely, M. *Tetrahedron Lett.* **1979**, 3879. (b) Kashman, Y.; Zviely, M. *Experientia* **1980**, *36*, 1279.
10. Miscellaneous reports of amino acid-derived metabolites include: (a) Bandaranayake, W. M.; Berris, J. E.; Bourne, D. J. *Comp. Biochem. Physiol.* **1996**, *115*, 281. (b) Sakai, R.; Kamiya, H.; Murata, M.; Shimamoto, K. *J. Am. Chem. Soc.* **1997**, *119*, 4112.
11. (a) Berthold, R. J.; Borowitzka, M. A.; Mackay, M. A. *Phycologia* **1982**, *21*, 327. (b) Larkum, A. W. D.; Cox, G. C.; Hiller, R. G.; Parry, D. L.; Dibbayawan, T. P. *Mar. Biol.* **1987**, *95*, 1–13.
12. (a) Faulkner, D. J.; He, H.; Unson, M. D.; Bewley, C. A.; Garson, M. J. *Gazz. Chim. Ital.* **1993**, *123*, 301. (b) Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349. (c) Flowers, A. E.; Garson, M. J.; Webb, R. I.; Dumdei, E. J.; Charan, R. D. *Cell. Tiss. Res.* **1998**, *292*, 597.
13. Unson, M. D.; Holland, N. D.; Faulkner, D. J. *Mar. Biol.* **1994**, *119*, 1.
14. (a) Richou, O.; Vaillancourt, V.; Faulkner, D. J.; Albizati, K. F. *J. Org. Chem.* **1989**, *54*, 4729. (b) Vaillancourt, V.; Agharahimi, M. R.; Sundram, U. N.; Richou, O.; Faulkner, D. J.; Albizati, K. F. *J. Org. Chem.* **1991**, *56*, 378.
15. Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551.
16. Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.
17. Trousdale, E. K.; Taylor, S. W.; Parkin, S.; Hope, H.; Molinski, T. F. *Nat. Prod. Lett.* **1998**, *12*, 61.
18. Flowers, A. E.; Garson, M. J.; Byriel, K. S.; Kennard, C. H. L.; Aust *J. Chem.* **1998**, *51*, 195.