New Halogenated Sesquiterpenes from South African Specimens of the Circumtropical Sea Hare *Aplysia dactylomela*

Kerry L. McPhail,[†] Michael T. Davies-Coleman,*[,†] Royston C. B. Copley,[‡] and Drake S. Eggleston[‡]

Department of Chemistry, Rhodes University, Grahamstown, South Africa, and SmithKline Beecham Pharmaceuticals, Department of Computational and Structural Sciences, New Frontiers Science Park (North), Third Avenue, Harlow, Essex CM19 5AW, United Kingdom

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An examination of the digestive gland extracts from two color variants of the circumtropical sea hare *Aplysia dactylomela*, collected off the Eastern Cape coast of South Africa, yielded four new halogenated sesquiterpenes: algoane (**1**), 1-deacetoxyalgoane (**2**), 1-deacetoxy-8-deoxyalgoane (**3**), and ibhayinol (**4**). The known sesquiterpenes, nidificene (**5**) and prepacifenol epoxide (**6**), were also isolated from the *A. dactylomela* extracts. Standard spectroscopic techniques were used to determine the structures of compounds **1**−**6**, with a single-crystal X-ray diffraction experiment establishing the structure of **1** and providing the absolute stereochemistry of this compound.

The sea hare, *Aplysia dactylomela*, is widely distributed throughout the tropical and subtropical oceans of the world and feeds predominantly on marine macro-algae.[1] The diet of this shell-less, molluskan herbivore often incorporates *Laurencia* species, which are well-known, prolific producers of structurally diverse, bioactive natural products (including halogenated sesquiterpenes).[2] *A. dactylomela*, in common with other *Aplysia* species, actively stores a variety of secondary metabolites from its algal diet in a large internal digestive gland. Although the first chemical investigation of an *Aplysia* species was reported over three and a half decades ago,[3] the ability of sea hares to concentrate natural products from several different algae species has continued to make these mollusks attractive study organisms for marine natural product chemists. Accordingly, *A. dactylomela* specimens collected from the Caribbean Sea,[4−6] eastern Pacific,[7] western Atlantic,[8,9] and northern Indian Oceans[10,11] have yielded a plethora of new and known compounds, including halogenated acetylenic ethers,[4] sesquiterpenes,[5,7,8,10,11] diterpenes,[6,9] brominated indoles and steroids.[11]

In southern Africa, *A. dactylomela* Rang, 1882 (Aplysiidae) occurs in the intertidal and shallow subtidal zones from Algoa Bay in the south to Mozambique in the north.[1] Despite its large size (400 mm) and reported common abundance,[1] we have found that the cryptic coloration of *A. dactylomela* and its elusive nocturnal behavioral patterns make collections of this mollusk difficult at the southern extremities of its range off South Africa. Fortunately, we have recently observed this species congregating on accessible shallow tidal platforms in Algoa Bay, South Africa, for a very brief period in late summer. Therefore, in continuation of our studies of the bioactive metabolites sequestered by South African opisthobranch mollusks,[12] we collected two color variants of *A. dactylomela* from the Cape Recife area of Algoa Bay and examined the acetone extracts of their freshly dissected digestive glands. This is the first investigation of the secondary metabolites from *A. dactylomela* specimens collected off the coast of Africa, and we report here the isolation and identification of four new halogenated sesquiterpenes: algoane (**1**), 1-deacetoxyalgoane (**2**), 1-deacetoxy-8-deoxyalgoane (**3**), and ibhayinol (**4**).[13] In addition, the known *Laurencia* metabolites nidificene (**5**)[14] and prepacifenol epoxide (**6**)[15] were identified among a group of several minor metabolites in the digestive gland extracts. The suspected red algal sources of these six compounds are unknown.

Results and Discussion

Of the four large specimens of *A. dactylomela* collected from the same site in Algoa Bay in March 1998, one was predominantly pale green, while the remaining three sea hares were a similar shade of red. As we were conscious that this color variation might reflect different dietary selectivity, and hence result in two different profiles of sequestered metabolites, the digestive gland of the green sea hare was dissected out of the animal, extracted, and worked up separately from those of the red specimens. The respective digestive glands were initially steeped in acetone for several days, the acetone extracts concentrated and partitioned between EtOAc and water. Si gel chromatography of each of the concentrated EtOAc partition layers, using a hexane-to-EtOAc solvent gradient, gave several crude fractions that were examined by TLC, ^{1}H NMR spectroscopy, and the brine shrimp general bioactivity assay.[16] Fortuitously, during both chromatographic separations, the major metabolite, **1**, crystallized slowly out of the combined, nonpolar chromatography fractions in similar high overall yield (ca. 6%, calculated from the mass of the respective concentrated EtOAc partition layers).

A molecular formula of $C_{17}H_{27}Br_2ClO_4$ was established for algoane (**1**) from HRFABMS data (*m*/*z* 489.0043 Δ +1 mmu). All 17 carbon signals were clearly resolved in the ^{13}C NMR spectrum of **1** (Table 1) which, in combination with a DEPT experiment, were assigned to five quaternary, four methine, three methylene, and five methyl carbon atoms. A ^{13}C NMR resonance at δ 168.8, a methyl singlet at δ 2.06 in the ^{1}H NMR spectrum, and a strong absorbance at 1721 cm^{-1} in the IR spectrum of **1** indicated the presence of a single acetate moiety. With one of the three degrees of unsaturation implied by the molecular formula thus accounted for, and no other sp^2 hybridized carbons evident in the ^{13}C NMR spectrum of **1**, a bicyclic structure was proposed for this compound.

Although the use of standard 2D NMR techniques quickly facilitated entry to the substitution pattern in

* To whom correspondence should be addressed. Tel.: 27-46-6038264. Fax: 27-46-6225109. E-mail: M.Davies-Coleman@ru.ac.za.
[†] Rhodes University.
[‡] SmithKline Beecham Pharmaceuticals.

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Table 1. ^{1}H (400 MHz, $CDCl_3$), ^{13}C (100 MHz, $CDCl_3$), and 2D NMR Data for Compound **1**

atom number	δ_H ppm (mult., *J*/Hz)	δ_C ppm (mult.)	COSY90 coupling to	HMBC correlation to
1	5.07 (d, 3)	74.3 d	H_2-2, H_2-5, H_3-12	C-2, C-3, C-5, C-6, C-16
2	2.55 (dd, 3, 15)	41.4 t	H-2, H-4	C-1, C-3, C-4, C-6, C-15
	2.83 (dd, 3, 15)		H-1, H-2, H_3-15	C-3, C-15
3		69.9 s		
4	4.98 (dd, 4, 12)	60.6 d	H-2, H_2-5, H_3-15	C-3, C-5, C-15
5	2.40 (dm, 14)	41.3 t	H-1, H-4, H-5	C-1, C-3, C-4, C-6
	2.65 (t, 13)		H-1, H-4, H-5	C-3, C-4, C-6
6		78.9 s		
7		52.6 s		
8	4.22 (p, 4)	81.7 d	H_2-9, OH-8	C-7, C-10, C-11, C-14
9	2.13 (td, 3, 10)	44.1 t	H-8, H-9, H-10	C-8, C-10
	2.94 (p, 8)		H-8, H-9, H-10	C-7, C-10, C-11
10	4.12 (t, 10)	60.9 d	H_2-9, H_3-13	C-9, C-11, C-12, C-17
11		47.1 s		
12	0.91 (s)	22.1 q	H-1, H_3-13	C-7, C-10, C-11, C-13
13	1.44 (s)	23.5 q	H-10, H_3-12	C-7, C-10, C-11, C-12
14	0.98 (s)	20.3 q	OH-6, H_3-13	C-6, C-7, C-8, C-11
15	1.75 (s)	27.5 q	H-2, H-4	C-2, C-3, C-4, C-5
16		168.8 s		
17	2.06 (s)	21.5 q		C-16
OH-6	5.79 (brs)		H-5, OH-8	C-1, C-5, C-6, C-7
OH-8	2.52 (d, 4)		H-4, H-8, OH-6	C-7, C-10

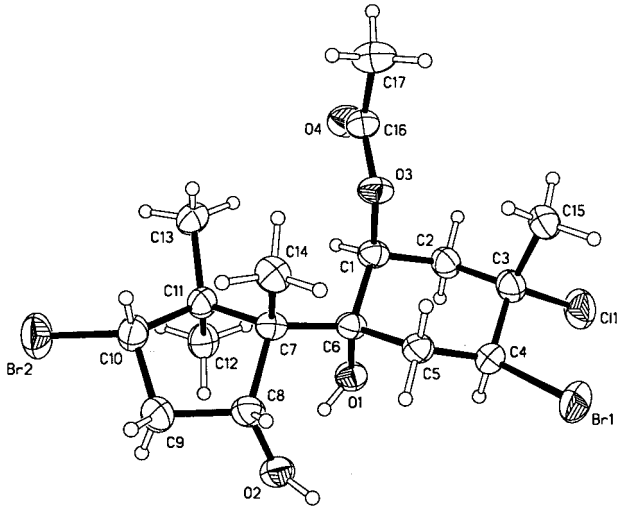


Figure 1. A view of a molecule of **1** from the crystal structure showing the numbering scheme employed. Anisotropic displacement ellipsoids for the nonhydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.

algoane (**1**), the sesquiterpene's structural class was not immediately apparent. The HMBC NMR data of **1** (Table 1) favored an unprecedented, nonaromatic, cuparane-type structure for this compound over a more common spiro-fused, chamigrane-type alternative skeleton, which was also broadly compatible with the NMR data. The cuparane-type structure of algoane (**1**), was unequivocally confirmed by a single-crystal X-ray diffraction study of this compound carried out at 150(2) K. The asymmetric unit contained one fully ordered molecule (Figure 1) in which the five- and six-membered rings adopted envelope and chair conformations, respectively. In addition, the absolute configuration at each of the seven chiral centers in **1** was established unambiguously as 1*S*, 3*S*, 4*S*, 6*R*, 7*S*, 8*R*, and 10*S* by refinement of the absolute structure parameter.[17] Two hydrogen bonding interactions, one intramolecular [O1 − H1 ··· O2; distance O1 ··· O2, 2.593 (4) Å] and the other intermolecular [O2 − H2 ··· O4; distance O2 ··· O4, 2.789 (4) Å], were evident from the crystal-structure determination.

The structure determination of **1** provided a valuable insight into the structures of two other crystalline compounds, **2** and **3**, isolated by semipreparative HPLC of other selected, bioactive Si gel chromatography fractions. The ^{1}H and ^{13}C NMR data of the latter two compounds (Table 2) were particularly useful and suggested that **2** and **3** contained the same basic skeleton as **1**, differing only in the degree of oxygenation around the five- and six-membered rings. Interestingly, **2** was isolated from the extracts of the red color variants of *A. dactylomela* and not from the pale green color-variant extract, which yielded only **3**.

Table 2. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR Data for Compounds **2** and **3**

	compound **2**		compound **3**	
atom number	δ_C ppm (mult.)	δ_H ppm (mult., *J*/Hz)	δ_C ppm (mult.)	δ_H ppm (mult., *J*/Hz)
1	30.3 t	1.67 (m, 3)	29.5 t	1.83 (m)
		1.92 (dm, 14)		1.89 (dd, 4, 14)
2	37.6 t	2.16 (dm, 4)	38.2 t	2.23 (dt, 3, 14)
		2.60 (td, 5, 14)		2.52 (td, 6, 14)
3	71.1 s		70.8 s	
4	61.5 d	4.86 (dd, 4, 12)	60.6 d	4.70 (dd, 4, 12)
5	43.9 t	2.29 (t, 13)	43.2 t	2.05 (m)
		2.45 (dm, 14)		2.14 (t, 13)
6	79.8 s		78.9 s	
7	52.3 s		50.2 s	
8	81.0 d	4.20 (dt, 3, 8)	30.7 t	1.30 (brm)
				2.36 (m)
9	44.4 t	2.13 (td, 3, 10)	30.8 t	1.99 (m)
		2.91 (p, 8)		2.29 (m)
10	61.3 d	4.14 (t, 10)	63.8 d	4.29 (dd, 8)
11	47.7 s		47.6 s	
12	23.7 q	1.47 (s)	22.2 q	1.19 (s)
13	23.7 q	1.06 (s)	22.6 q	1.04 (s)
14	20.4 q	0.90 (s)	23.3 q	1.02 (s)
15	23.3 q	1.67 (s)	23.2 q	1.66 (s)
OH-6		4.79 (d, 2)		1.44 (brs)
OH-8		2.39 (d, 4)		

The molecular formulas of **2** and **3** were established as $C_{15}H_{25}Br_2ClO_2$ (*m*/*z* 430.9987 Δ −1 mmu) and $C_{15}H_{25}Br_2ClO$ (*m*/*z* 415.0034 Δ −5 mmu), respectively, from HR-FABMS data. The difference of 58 atomic mass units ($C_2H_2O_2$) between the molecular masses of **1** and **2** implied the complete loss of the acetoxy functionality from C-1 in the latter compound. The absence of any contrary evidence from the IR, ^{13}C, and ^{1}H NMR spectra of **2**, indicative of the presence of an acetate moiety, corroborated this assumption. The emergence of a fourth methylene carbon signal (δ 30.3) and the disappearance of the C-1 oxymethine

Table 3. ^{1}H (400 MHz, $CDCl_3$), ^{13}C (100 MHz, $CDCl_3$) and 2D NMR Data for Compound **4**

atom number	δ_H ppm (mult., *J*/Hz)	δ_C ppm (mult.)	COSY90 coupling to	HMBC correlation to
1	3.74 (brs)	75.4 d	H_2-2, H_2-5	C-2/5, C-3, C-6
2	2.41 (dd, 4, 15)	41.1 t	H-1, H-2, H_3-15	C-1, C-3, C-15
	2.64 (dd, 2, 15)		H-1, H-2	C-1, C-3, C-4, C-6, C-15
3		71.0 s		
4	4.71 (p, 8)	60.3 d	H_2-5, H_3-15	C-2/5, C-3, C-6, C-15
5	2.14 (d, 9)	41.3 t	H-1, H-4, OH	C-1, C-3, C-4, C-6, C-7, C-15
6		80.2 s		
7		55.5 s		
8	1.23 (p, 5)	34.7 t	H-8, H_2-9, H-10	C-7, C-9, C-10, C-11, C-14
	1.77 (m)		H-8	C-6, C-7, C-9, C-11, C-14
9	1.11 (q, 6)	31.9 t	H_2-8, H-9, H-10	C-8, C-10, C-13
	1.75 (m)		H_2-8, H-9, H-10	C-8
10	1.95 (sept, 6)	46.8 d	H-8, H_2-9, H_3-13	C-9, C-11, C-13
11		91.4 s		
12	1.05 (s)	19.5 q	H_3-13, H_3-14	C-7, C-10, C-11
13	0.94 (d, 7)	14.8 q	H-10, H_3-12	C-9, C-10, C-11
14	0.92 (s)	17.2 q	H_3-12	C-6, C-7, C-8, C-11
15	1.80 (s)	26.5 q		C-2/5, C-3, C-4
OH	1.39 (s)		H_2-5	C-1, C-2/5, C-6

resonance (δ 74.3) in the ^{13}C NMR spectrum of **2**, gave additional support to the 1-deacetoxyalgoane structure proposed for this compound.

Compound **3**, 1-deacetoxy-8-deoxyalgoane, differed in molecular formula by one oxygen atom from that of **2**, and lacked both the acetoxy and secondary hydroxyl substituent at C-1 and C-8, respectively, compared to **1**. The ^{13}C and ^{1}H NMR data for the six-membered ring of **3** were consistent with those of **2** (Table 2), tentatively attributing the molecular mass discrepancy between these two compounds to the loss of the hydroxyl functionality at C-8 in **3**. In accordance with the argument presented earlier for the structure of **2**, loss of the C-8 oxymethine carbon signal and its replacement with a methylene resonance (δ 30.7) in the ^{13}C NMR spectrum of **3** supported a deoxygenated five-membered ring in the latter compound. Furthermore, the HMBC correlations observed from the single hydroxyl proton in **3** to C-1, C-5, and C-6, and a long-range COSY coupling between this proton and one of the methylene protons resonating at δ 2.14 (H-5), confirmed that the tertiary hydroxyl group at C-6 was still intact. Interestingly, the large upfield shift of the tertiary hydroxyl proton in **3** (δ 1.44), relative to the deshielded chemical shifts observed for this proton in **1** and **2** (δ 5.79 and 4.79, respectively), could possibly be attributed to changes in hydrogen-bonding interactions brought about by the loss of the C-8 hydroxyl moiety in **3**. The potential for intramolecular hydrogen bonding between the tertiary hydroxyl proton and the secondary hydroxyl functionality at C-8, in the algoane group of compounds, was confirmed by the X-ray analysis of **1**.

The stereochemistry of compounds **2** and **3** was shown to be the same as that for algoane from a series of 1D NOE difference experiments. The configuration around the five-membered ring in **2** was established from reciprocal NOE enhancements of the proton resonances H-8 (δ 4.20), H-10 (δ 4.14), and H_3-14 (δ 0.90). The absence of an NOE enhancement of the tertiary hydroxyl proton signal on irradiation of the methyl singlet (H_3-14) confirmed the expected trans relationship between these two moieties. No NOE enhancement of the bromomethine signal (δ 4.86) was observed on irradiation of the vicinal methyl singlet (H_3-15, δ 1.67), as would be expected for the diequatorial halogen substitution pattern implied by the axial bromomethine proton coupling constants (J = 4, 12 Hz).[18] Similarly, NOE irradiation of the H-10 (δ 4.29) and H_3-14 (δ 1.02) resonances in **3** resulted in reciprocal enhancements, while no enhancement of the tertiary hydroxyl proton signal (δ 1.44) was observed. Diequatorial halogen substituents in the six-membered ring of **3** were also indicated by recourse to coupling constants and the absence of an NOE enhancement of the bromomethine proton signal on irradiation of the H_3-15 (δ 1.66) singlet.

The digestive gland extract from the red variant yielded the new minor halogenated sesquiterpene **4**, ibhayinol. The ^{13}C and ^{1}H NMR data of ibhayinol (**4**, Table 3) were similar to those of the unsaturated sesquiterpenes **2** and **3**, and we initially assumed that this compound was a fourth member of the algoane series. However, the molecular formula of **4** ($C_{15}H_{24}BrClO_2$), established from HRFABMS data (*m*/*z* 351.0726 Δ −1 mmu), implied three degrees of unsaturation and, with no olefinic or carbonyl signals evident in the ^{13}C NMR spectrum of **4**, required this compound to be tricylic. From a comparison of the 1D and 2D NMR data of **4** (Table 3) with those of **1**−**3**, both a six- and a five-membered saturated ring could be delineated for the former compound.

Although the ^{13}C NMR spectrum of ibhayinol (**4**) revealed five deshielded resonances (δ 60.3, 71.0, 75.4, 80.2, 91.4), attributable to carbons attached to heteroatoms, only one exchangeable hydroxyl proton was evident in the ^{1}H NMR spectrum of this compound. The former two carbon signals were assigned to brominated methine and chlorinated quaternary carbon atoms, respectively, in accordance with the related C-4 and C-3 ^{13}C chemical shifts established for compounds **1**−**3**. A two-bond HMBC correlation from the hydroxyl proton to the quaternary carbon C-6 (δ 80.2) established the tertiary position of the single hydroxyl moiety. With three of the five deshielded carbon resonances therefore accounted for, the remaining oxygen atom was placed in a cyclic ether position between C-1 (δ 75.4) and C-11 (δ 91.4) to form the third ring required by the molecular formula. The unusually deshielded, latter chemical shift is consistent with the equivalent quaternary ether carbon chemical shift (δ 97.1) reported for the tricyclic laurane sesquiterpene, isoaplysin (**7**)[2] and suggested a similar nonaromatic, cyclic laurane ether structure for **4**. Two- and three-bond HMBC correlations from the doublet methyl (δ 0.94) to C-9−C-11 placed this methyl group in a vicinal position relative to the ether moiety. Similarly, HMBC correlations and COSY couplings (Table 3) were used to position the remaining substituents and thus establish the structure of ibhayinol (**4**).

The relative stereochemistry of ibhayinol was established from a combination of a NOESY experiment (supported by 1D NOE difference experiments) and molecular modeling

studies.[19] NOESY correlations between H_3-12 (δ 1.05), H_3-13 (δ 0.94), and H_3-14 (δ 0.92) placed all three methyl groups on the same side of the molecule and suggested cis fusion of rings A and B. No NOESY correlations were observed between these three methyl groups and H-1 (δ 3.74). The latter proton, however, showed strong NOESY correlations to H-8β (δ 1.23) and H-10 (δ 1.95) and a weaker correlation to H_3-15 (δ 1.80). A NOESY correlation between the bromomethine proton H-4 (δ 4.71) and H-2α (δ 2.41) suggested a trans dihalogen substitution pattern in ring C. The NOE correlations observed between the protons on rings A and C limited the number of conformational models possible for ibhayinol. Interestingly, a single weak NOESY correlation was observed from H_3-14 to the hydroxyl proton (δ 1.39). In the most probable cis fused ring-B and -C model, calculated from molecular modeling studies and corroborated by the extensive NOE data, the hydroxyl proton is separated from the H_3-14 methyl protons by an interatomic distance of 2.7 Å. In the trans fused alternative model the interatomic distances calculated from the hydroxyl proton to the two alpha methyl protons H_3-12 and H_3-14 are both less than 2 Å. No NOE correlations were observed from the hydroxyl proton to H_3-12, implying that the former alternative with a β-alcohol functionality is more acceptable. Exhaustive calculations of interatomic distances in both the cis and trans fused ring-B and -C models of ibhayinol (**4**) and comparison of these distances with the NOE data further supported the former model.

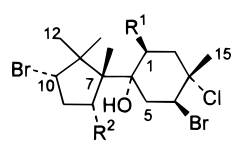
HRFABMS data secured the molecular formula of **5** as $C_{15}H_{23}Br_2Cl$ (*m/z* 395.9858 Δ +2 mmu). The optical rotation, IR, NMR, and MS data for this compound were consistent with those reported for nidificene.[14b] The other known metabolite isolated from the green *A. dactylomela* extract, compound **6**, yielded a molecular formula of $C_{15}H_{21}Br_2ClO_3$ (*m/z* 441.9552 Δ +11 mmu) from HRFABMS data. The melting point and NMR data of **6** are in accordance with the limited data reported for the diepoxychamigrane sesquiterpene, prepacifenol epoxide (mp 98 °C), isolated from the digestive gland of the sea hare *A. californica*.[15] The ^{1}H and ^{13}C NMR data of compounds **5** and **6** were previously unassigned, and the assigned NMR data for these two known compounds, established from exhaustive 2D NMR experiments, are presented in the Experimental Section.

The metabolites of South African specimens of the sea hare *A. dactylomela* described here have provided an insight into the potentially interesting chemistry of the relatively unexplored Southern African *Laurencia* species. To date only one chamigrane sesquiterpene (**8**) has been isolated from a South African *Laurencia* species.[20] Unfortunately, the insolubility of the pure compounds **1**−**6** in ethanol/seawater or DMSO/seawater mixtures precluded a comparative study of their bioactivity in the brine shrimp bioassay.

Experimental Section

General Experimental Procedures. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AMX400 spectrometer. Optical rotations were measured on a Perkin−Elmer 141 polarimeter; IR data, on a Perkin−Elmer spectrum 2000 FT-IR spectrometer, and LRMS, on a Finnigan GCQ spectrometer. HREIMS and HRFABMS were obtained by Dr. P. Boshoff and Prof. L. Fourie of the Mass Spectrometry Units at the Cape Technikon, Cape Town, and the University of Potchefstroom, Potchefstroom, respectively. HPLC separations were performed on a Whatman Magnum 9 Partisil column.

Invertebrate Material. *A. dactylomela* is the largest sea hare occurring off the Southern African coast, growing to a length of 400 mm, with distinctive general markings and dorsal appendages.[1] Although *A. dactylomela* co-occurs with *A. oculifera* at the limit of its range, the latter species is much smaller and has different dorsal appendages and cryptic coloration. Four large specimens of *A. dactylomela* were collected by hand from the intertidal zone at the same site in the Cape Recife Nature Reserve, Algoa Bay, on the Eastern Cape coast of South Africa in March 1998, and identified by Ms. S. Kuiters of the Zoology Department, University of Port Elizabeth. Of this collection, one was a green color variant in which the predominant background color was pale green. The other three specimens had a background color that was predominantly red-brown. A voucher specimen of *A. dactylomela* is retained in the Rhodes University marine invertebrate collection (Aply98-001).



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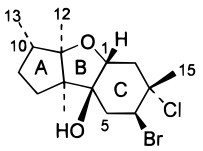

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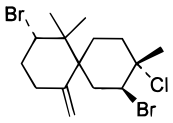

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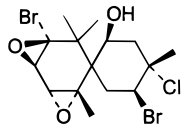

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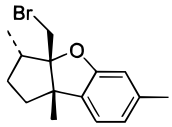

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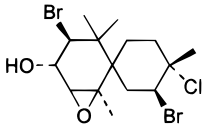

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Extraction and Isolation. The single specimen of the green color variant was dissected and the digestive gland removed and placed in Me_2CO for one week, while the digestive glands of the three specimens of the red color variant were separately dissected out and placed together in Me_2CO, also for one week. The two Me_2CO extracts were worked up separately and initially partitioned between EtOAc and H_2O. The EtOAc partition fraction of the green color variant was concentrated (0.9 g) and subjected to Si gel column chromatography (hexanes−EtOAc). The crude chromatograhy fractions were monitored by TLC, ^{1}H NMR, and the standard brine shrimp bioactivity assay.[16] Algoane (59 mg, 6.6% calcd from mass of concentrated EtOAc partition layer) crystallized slowly from one of the combined fractions (8:2 hexanes−EtOAc). Exhaustive normal-phase semipreparative HPLC (19:1 and 9:1 hexanes−EtOAc) of the more polar and bioactive column chromatography fractions yielded compounds **3** (19 mg, 2.1% of the crude extract), **5** (6 mg, 0.7% of the crude extract), and **6** (11 mg, 1.2% of the crude extract).

Similar chromatographic workup of the concentrated EtOAc partition layer (3.05 g) from the red color variants yielded **1** (158 mg), again through spontaneous crystallization from the nonpolar chromatography fractions. Further algoane (24 mg) was obtained by normal-phase semipreparative HPLC (7:3 hexanes−EtOAc) of the crystallization mother liquors. Semipreparative HPLC (9:1 and 7:3 hexanes−EtOAc) of other column chromatography fractions yielded compounds **2** (17 mg, 0.6% of the crude extract) and **4** (5 mg, 0.2% of the crude extract).

Algoane (1): colorless prisms (hexane−EtOAc), mp 188−192 °C; $[\alpha]^{22}_D$ +51.1° (*c* 0.60, $CHCl_3$); IR (film) ν_{max} 3604−3115, 2985, 1721, 1373, 1232, 1074, 1034, 756 cm^{-1}; ^{1}H and ^{13}C NMR

data, see Table 1; EIMS (70 eV) *m/z* (int %) 411 (5), 315 (10), 253 (20), 188 (35), 173 (34), 161 (26), 125 (19), 119 (14), 109 (92), 108 (69), 107 (17), 93 (21), 69 (45), 43 (100); HRFABMS *m/z* 489.0043 (calcd for $C_{17}H_{27}Br_2ClO_4$, 489.0042)

1-Deacetoxyalgoane (2): colorless prisms (hexane−EtOAc), mp 152−155 °C; $[\alpha]^{22}_D$ +18.0° (*c* 0.40, $CHCl_3$); IR (film) ν_{max} 3496−2481, 2973, 1460, 1387, 1076, 994, 785 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* 430.9987 (calcd for $C_{15}H_{25}Br_2ClO_2$, 430.9988).

1-Deacetoxy-8-deoxyalgoane (3): colorless prisms (hexanes−EtOAc), mp 138−142 °C; $[\alpha]^{22}_D$ +22.5° (*c* 1.15, $CHCl_3$); IR ν_{max} (film) 3618−3136, 2976, 1463, 1386, 1089, 1008, 781 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* 415.0034 (calcd for $C_{15}H_{25}Br_2ClO$, 415.0039).

Ibhayinol (4): white amorphous powder; $[\alpha]^{22}_D$ +8.8° (*c* 0.57, $CHCl_3$); IR ν_{max} 3679−3099, 2956, 1460, 1387, 1093, 1060, 630 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRFABMS *m/z* 351.0725 (calcd for $C_{15}H_{24}BrClO_2$, 351.0726).

Nidificene (5): white amorphous powder; $[\alpha]^{22}_D$ +27° (*c* 0.22, $CHCl_3$); IR ν_{max} 2971, 1638, 1451, 1390, 1380, 1092, 905, 866 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.95 (3H, s, H_3-12), 1.14 (3H, s, H_3-13), 1.69 (3H, s, H_3-15), 1.72 (1H, m, *J* = 14 Hz, H-1), 1.94 (1H, m, *J* = 14 Hz, H-5), 1.97 (1H, m, H-1), 2.02 (1H, m, *J* = 5, 13 Hz, H-9), 2.14 (3H, m, H-2, H-5, H-8), 2.25 (1H, m, *J* = 6, 19 Hz, H-9), 2.31 (1H, m, *J* = 5, 14 Hz, H-8), 2.44 (1H, m, *J* = 4, 14 Hz, H-2), 4.43 (1H, dd, *J* = 5, 13 Hz, H-10), 4.71 (1H, dd, *J* = 5, 13 Hz, H-4), 4.86 (1H, s, H-14), 5.25 (1H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.5 (q, C-12), 23.6 (q, C-13), 24.1 (q, C-15), 25.4 (t, C-1), 33.5 (t, C-8), 35.8 (t, C-9), 38.6 (t, C-5), 38.7 (t, C-2), 43.8 (s, C-11), 51.1 (s, C-6), 61.4 (d, C-4), 63.6 (d, C-10), 71.8 (s, C-3), 114.7 (t, C-14), 145.6 (s, C-7); EIMS *m/z* (rel int) 396 (12), 319 (32), 283 (43); 281 (44), 275 (23), 237 (35), 201 (56), 159 (22), 145 (20), 119 (20), 109 (100), 105 (39), 95 (70), 93 (41), 69 (83); HRFABMS *m/z* 395.9858 (calcd for $C_{15}H_{23}Br_2Cl$, 395.9856).

Prepacifenol epoxide (6): colorless prisms, mp 95−97 °C; $[\alpha]^{22}_D$ +109.9° (*c* 0.73, $CHCl_3$); IR ν_{max} 3619−3122, 2978, 1447, 1387, 1089, 1027, 805 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.98 (3H, s, H_3-12), 1.48 (3H, s, H_3-14), 1.53 (3H, s, H_3-13), 1.91 (3H, s, H_3-15), 2.14 (1H, m, *J* = 2, 4 Hz, H-5), 2.52 (2H, m, *J* = 3, 5 Hz, H-2), 2.54 (1H, t, *J* = 14 Hz, H-5), 3.06 (1H, s, H-8), 3.65 (1H, s, H-9), 4.03 (1H, sept, *J* = 3 Hz, H-1), 4.73 (1H, dd, *J* = 4, 13 Hz, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q, C-14), 24.3 (q, C-12), 27.2 (q, C-13), 28.0 (q, C-15), 33.9 (t, C-5), 46.8 (s, C-11), 47.2 (t, C-2), 50.2 (s, C-6), 55.6 (d, C-9), 56.7 (d, C-8), 61.0 (s, C-7), 62.1 (d, C-4), 69.8 (d, C-1), 71.5 (s, C-3), 75.5 (s, C-10); EIMS (70 eV) *m/z* (rel int) 365 (6), 319 (26), 317 (20), 277 (26), 201 (18), 194 (35), 178 (23), 159 (19), 135 (18), 121 (22), 109 (47), 107 (20), 105 (22), 97 (19), 79 (19), 71 (33); 69 (28), 53 (43), 43 (100); HRFABMS *m/z* 441.9552 (calcd for $C_{15}H_{21}Br_2ClO_3$, 441.9541).

X-ray Diffraction of 1. Large crystals of **1** were grown via the slow evaporation of an EtOAc−hexane solution. A single crystal was cleaved several times to obtain appropriate dimensions and flash cooled in a stream of N_2 gas to 150(2) K. Lattice parameters were determined from the setting angles of 25 reflections well distributed in reciprocal space and measured using a Nonius MACH3 diffractometer. Intensity data were collected using graphite monochromated copper radiation and an $\omega-2\theta$ variable scan speed technique. Three check reflections were monitored to assess any crystal movement during the experiment. The intensities of these three reflections were measured every hour of exposure time and showed a variation of 10.5%. Data were corrected for this variation and for Lorentz and polarization effects. An absorption correction was applied using psi-scan data. The structure was solved and refined using the SHELXTL package.[21] Atomic coordinates and anisotropic displacement parameters were refined for the nonhydrogen atoms. Coordinates were refined for the hydroxyl hydrogen atoms, with O−H distances being restrained to 0.84(2) Å. The remaining hydrogen atoms were included in idealized positions, either riding on the carbon atoms to which they were attached or, for the methyl hydrogen atoms, being treated as rigid, rotating groups. For all hydrogen atoms, isotropic displacement parameters were assigned as an appropriate multiple (either 1.2 or 1.5) of *U*(eq) for the atom to which they were bonded. The full-matrix least-squares refinement (on F^2) of 229 variables converged ($\Delta/\sigma_{max} < 0.001$) to values of the conventional crystallographic residuals $R1 = 0.0365$ ($wR2 = 0.0965$) for 3733 observed data with $I \geq 2\sigma(I)$ and $R1 = 0.0372$ ($wR2 = 0.1008$) for all data. The goodness-of-fit was 1.078. The function minimized was $\sum w(F_o^2 - F_c^2)^2$. Weights, *w*, were assigned to the data as $w = 1/[\sigma^2(F_o^2) + (0.0579P)^2 + 2.2595P]$ where $P = [\max(F_o^2,0) + 2F_c^2]/3$. A final difference Fourier map showed residual density between 0.603 e $Å^{-3}$ and −0.609 e $Å^{-3}$. The absolute configuration was assigned on the basis of the absolute structure parameter,[17] which refined to a value of 0.02(2). Values of the neutral atom scattering factors and real and imaginary dispersion corrections were taken from the *International Tables for X-ray Crystallography*.[22]

Crystal data:[23] colorless block; 0.50 × 0.43 × 0.37 mm; orthorhombic; space group $P2_12_12_1$ (no. 19); unit cell dimensions $a = 8.2536(5)$ Å, $b = 11.4458(7)$ Å, $c = 21.063(2)$ Å, $V = 1989.8(2)$ $Å^3$; $Z = 4$; $d_{calc} = 1.638$ Mg m^{-3}; μ (Cu Kα, $\lambda = 1.54178$ Å) = 6.554 mm^{-1}.

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(23) Crystallographic data for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB21EZ, UK (Fax: +44-(0)1223-336033 or E-mail: deposit@ccdc.cam.ac.uk).

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