Tetrahedron Vol. 41, No 6, pp 1101 to 1108, 1985
Printed in Great Britain

0040-4020/85 $3.00 + .00

CHEMICAL STUDIES OF BRITISH COLUMBIA NUDIBRANCHS

KIRK GUSTAFSON and RAYMOND J. ANDERSEN*
Departments of Chemistry and Oceanography, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

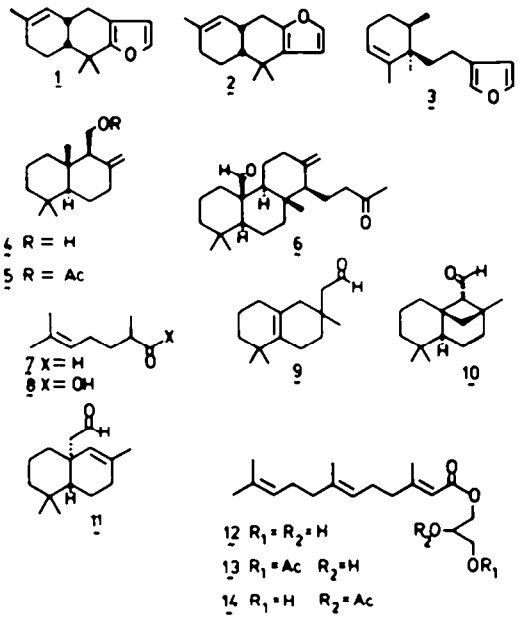
(*Received in USA 7 November* 1983)

Abstract The chemical constituents of the skin extracts of several nudibranchs have been examined. Extracts of *Archidoris montereyensis* contain the diterpenoic acid glyceride **20**, its two monoacetates **24** and **25**, the drimane sesquiterpenoic acid glyceride **26**, the monoacetate **28**, the monocyclofarnesic acid glyceride **30** and the glyceryl ether **31**. Glyceride **20** has also been isolated from extracts of *Archidoris odhneri*. The odoriferous principle of *Anisodoris nobilis* extracts was shown to be the degraded sesquiterpenoid **33**. *Polycera tricolor* extracts contain triophamine (**19**) as their major constituent. ^{14}C labelled mevalonic acid is incorporated into the diterpenoic acid glyceride **20** and the sesquiterpenoic acid glyceride **26** by *A. montereyensis*, and into the farnesic acid glyceride **12** by *A. odhneri*. The drimenoic acid glyceride **26** and the glyceryl ether **31** show antifeedant activity against the tide pool sculpin *Oligocottus maculosus*.

Nudibranchs have recently been the object of intense chemical scrutiny by several research groups.[1] The initial interest was prompted by biological reports that these colourful, shell-less molluscs, which appear highly vulnerable to predation, might utilize defensive allomones.[2] Burreson *et al.* provided the first confirmation of the biological predictions when they isolated the allomone 9-isocyanopupukeanane from the dorid *Phyllidia varicosa*.[3] Since this initial report, interest in nudibranch chemistry has been sustained by the discovery of numerous interesting metabolites. Many of these new compounds display antifeedant activity against fish.[1,4] During the course of the isolation and structural studies, several groups also examined the sponges, bryozoans, and coelenterates on which nudibranchs feed, thereby revealing that many of the metabolites present in their skin extracts were in fact sequestered from their diets.[1,3,4b] The recent demonstration by Cimino *et al.* that polygodial is elaborated directly by the dorid *Dendrodoris limbata* was the first proof that not all nudibranch metabolites have dietary origins.[5]

From the coastal waters of British Columbia approximately eighty species of indigenous nudibranchs have been described.[6] Many of these species are represented by sizable populations in the shallow intertidal zone. This easily accessible and abundant source of organisms, coupled with numerous literature reports of interesting nudibranch chemistry, encouraged us to examine the local species.

Our initial efforts were directed towards extracts of the dorid *Cadlina luteomarginata*. They were shown to contain furodysin (**1**), furodysinin (**2**), microcionin-2 (**3**), albicanol (**4**), albicanyl acetate (**5**) and luteone (**6**).[7] The three furanosesquiterpenoids **1**, **2** and **3**, which had been previously isolated from marine sponges,[8,9] were consistently found to be present in extracts of the gut contents of *C. luteomarginata* and were therefore assumed to be of dietary origin. Albicanol (**4**) was previously known from the liverwort *Diplophyllum albicans*,[10] whereas its acetate **5** and luteone (**6**) represented new natural products. None of these three compounds were detected in any collectable dietary organism of *C. luteomarginata*'s or in extracts of the gut contents of freshly collected animals. It therefore appeared possible that they may be elaborated by the nudibranch directly. Luteone was found to be responsible for the fragrant odour of B.C. specimens of *C. luteomarginata*, while albicanyl acetate and furodysin were found to exhibit fish antifeedant activity.



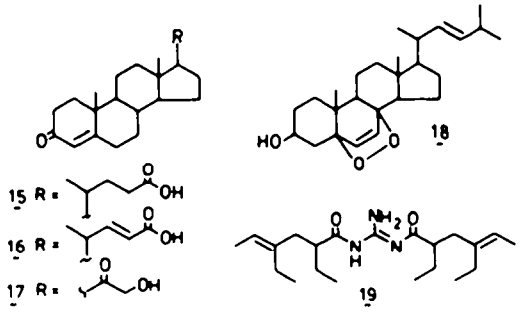
The dendronotid nudibranch, *Melibe leonina*, also has a very strong fruity odour that can be detected *in situ* by SCUBA divers operating in the vicinity of the densely packed ($\approx$50 animals/m^2) reproductive congregations of the animals. Nybakken and Ajeska's report that the primary defense of *M. leonina* was an odoriferous substance that was repugnant to predators prompted us to investigate its chemistry.[11] We were able to isolate the two degraded monoterpenes 2,6-dimethyl-5-heptenal (**7**) and 2,6-dimethyl-5-heptenoic acid (**8**) from chloroform extracts of freshly collected animals.[12] Neither the aldehyde **7**, which is the fragrant constituent, or the acid **8** had any demonstratable fish antifeedant activity.

Another pleasant smelling dorid which turned out to be a source of terpenoid chemistry is *Acanthodoris nanaimoensis*. Its extracts contained three isomeric sesquiterpenoid aldehydes. We proposed a structure for the major component, nanaimoal (**9**), that was consistent with its spectral data.[13] An unambiguous synthesis of its *p*-bromophenylurethane derivative verified that our proposed structure was correct. We have recently solved the structures of the two minor *A. nanaimoensis* metabolites, acanthodoral (**10**) and isoacanthodoral (**11**), by X-ray diffraction analysis.[14] All three *A. nanaimoensis* sesquiterpenoids have new carbon skeletons.

One of the most abundant intertidal nudibranchs in B.C. waters is the large snow white nudibranch *Archidoris odhneri*. We discovered that its extracts contained a series of terpenoic acid glycerides. The major component was the farnesic acid glyceride (**12**), which was accompanied by its two monoacetylated derivatives **13** and **14**.[15]

Two of the B.C. nudibranchs that we have examined contained interesting steroids. Specimens of the bright orange dorid *Aldisa sanguinea cooperi* are usually found deeply embedded in the sponge *Anthoarcuata graceae* from which they apparently obtain cryptic pigmentation and nutrition. Extracts of these animals yielded two steroids: 3-oxo-chol-4-ene-24-oic acid (**15**) and its unsaturated analog **16**.[16] The sponge *A. graceae* is devoid of either of the acids **15** or **16**, but it does contain significant amounts of cholestenone. We were able to show that **15** has fish antifeedant activity, while cholestenone is totally inactive. It is conceivable that *A. sanguinea cooperi* is obtaining an inactive metabolite from its diet and chemically modifying it to produce an active antifeedant. There is a striking chemical similarity between the acid **15** and the steroid **17** which is employed by the great diving beetle *Dytiscus marginalis* as a fish repellent.[17]

Specimens of the bryozoan eating dorid *Adalaria* sp. were collected from the surface of kelp leaves which were heavily encrusted with their primary dietary organism *Membranipora membranacea*. The major components of *Adalaria* extracts were 5,8α-epidioxy-(22*E*)-24-nor-5α-cholesta-6,22-dien-3β-ol (**18**) and a series of previously reported steroidal peroxides.[18]



A second bryozoan predator that we have investigated is the clown nudibranch *Triopha catalinae* (Cooper). Triophamine (**19**), which contains the rare diacylguanidine functionality, is the only novel metabolite found in skin extracts of *T. catalinae*.[19] The gross structure of triophamine was established from consideration of its spectral data and degradation products. A total synthesis established the alkene geometry and verified the overall structure.[20]

Our ongoing chemical investigations of British Columbia nudibranchs have three major objectives. The primary goal of our work is to determine the chemical structures of the major skin metabolites of new species. In addition to this, we have also begun to utilize radio-isotope incorporation studies to investigate the biosynthetic origin (i.e. nudibranch or dietary) of the metabolites, and we are continuing to evaluate new compounds for fish antifeedant activity. In this paper we report the results of our recent studies on several Northeast Pacific nudibranchs.

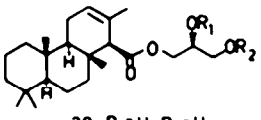
Isolation and structure elucidation

Archidoris montereyensis. Specimens of the dorid *Archidoris montereyensis* were collected from a variety of very exposed intertidal and subtidal locations in the Deer Group of Islands, Barkley Sound, B.C. Freshly collected specimens were immediately immersed in methanol. The chloroform soluble portion of the methanol extract was fractionated by sequential application of flash chromatography, radial thin layer chromatography, and HPLC to give a number of pure metabolites.

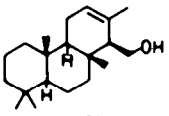
The major constituent of the extracts (≈2 mg/animal), glyceride **20**, was an optically active ($[\alpha]_D = -12.5°$, $CHCl_3$) crystalline compound (m.p. 125–126°), which had a molecular formula of $C_{23}H_{38}O_4$. Its IR spectrum showed hydroxyl (3700–3000 cm^{-1}) and ester (1730 cm^{-1}) absorption bands. The 400 MHz ^1H-NMR spectrum of **20** contained a series of resonances at δ 2.48 (bs, 2H, exchangeable), 3.63 (dd, 1H, J = 12, 6 Hz), 3.70 (dd, 1H, J = 12, 4 Hz), 3.95 (m, 1H), 4.15 (dd, 1H, J = 12, 7 Hz), and 4.22 (dd, 1H, J = 12, 5 Hz) that were virtually identical to those exhibited by the 1-acyloxy-2,3-dihydroxypropane fragment in glyceride **12**, which we had previously isolated from *A. odhneri*.[15] A series of singlet methyl resonances at δ 0.82, 0.87, 0.92, 0.96 and 1.61 suggested that the remaining twenty carbon atoms were part of a diterpenoid residue. Two of the five total sites of unsaturation required by the molecular formula of **20** could be accounted for by the glyceryl ester carbonyl and the one olefinic functionality that was indicated by the vinyl Me resonance at δ 1.60, the single vinyl proton at 5.54, and ^{13}C-NMR resonances at 124.3 (d) and 128.6 (s) ppm. The remaining sites of unsaturation were presumed to be rings, and hence it was clear that **20** was a tricyclic diterpenoic acid glyceride. Further support for this hypothesis could be found in the mass spectral fragments at *m/z* 286 (loss of $-O-CH_2-CHOH-CH_2OH+H$), 258 (loss of $-CO_2-CH_2-CHOH-CH_2OH+H$) and 192 (base peak).

The regular isoprenoid structure **20** was an obvious proposal that would explain all observed spectral data. A limited quantity of material, coupled with some spectral uncertainty caused by a persistent minor contamination by **26**, prevented us from verifying the structure *via* chemical and spectroscopic means. The correct structure was therefore shown to be **20** by a single crystal X-ray diffraction analysis.[21,22]

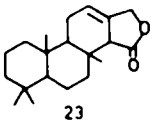
The absolute configuration of glyceride **20** was determined by reducing it with DIBAL to the known alcohol **21** ($[\alpha]_D = -9°$, lit.[23] $[\alpha]_D = -9°$). Its ring system therefore has the standard steroidal configuration. The carbon skeleton of the diterpenoid residue of **20** is rather rare in nature, the only previous examples



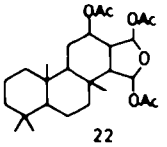
20 R_1=H R_2=H
24 R_1=H R_2=Ac
25 R_1=Ac R_2=H



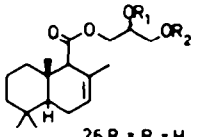
21



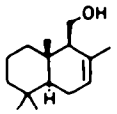
23



22



26 R_1=R_2=H
28 R_1=H R_2=Ac
29 R_1=Ac R_2=H



27

being the family of sponge metabolites related to aplysillin (**22**)[24] and isoagathalactone (**23**).[23a]

Two minor metabolites of *A. montereyensis* extracts were shown to be monoacetylated derivatives of glyceride **20**. Acetate **24** had a molecular formula of $C_{25}H_{40}O_5$, indicating the addition of a $CH_2C{=}O$ residue to glyceride **20**. The ^{1}H-NMR spectrum of **24** showed a Me resonance at δ 2.11, only a single exchangeable proton at 2.41, resonances for the diterpenoid acyl residue which were virtually identical to those found in **20**, and a complex five proton multiplet between 4.08 and 4.25 corresponding to the 1-acyloxy-2-hydroxy-3-acetoxypropane residue. The chemical shifts and coupling patterns observed for the glyceryl ester residue protons were identical to those previously observed in the monoacetyl glyceride **13**.[15] Reduction of acetate **24** with DIBAL gave alcohol **21**. The second monoacetate **25** also had a molecular formula of $C_{25}H_{40}O_5$ and its ^{1}H-NMR spectrum showed a Me resonance at δ 2.09, all the required resonances for the diterpenoid acyl residue of **20**, and a series of resonances at 3.76 (d, 2H, J = 6 Hz), 4.26 (dd, 1H, J = 7, 12 Hz), 4.32 (dd, 1H, J = 6, 13 Hz) and 5.06 (p, 1H, J = 6.5 Hz) appropriate for a 1-acyloxy-2-acetoxy-3-hydroxy propane residue. The resonances for the 2-acetylated glyceryl ester residue were identical to those previously observed for the monoacetate **14**.[15] Reduction of **25** with DIBAL also gave alcohol **21**.

A third minor component ($\simeq$0.2 mg/animal) of the *A. montereyensis* extracts was an optically active ($[\alpha]_D$ = +23.10°) solid which had a molecular formula of $C_{18}H_{30}O_4$. Its IR spectrum showed OH (3475 cm^{-1}) and ester CO (1730 cm^{-1}) absorptions. It was immediately obvious from its 400 MHz ^{1}H-NMR spectrum, which showed characteristic resonances at δ 2.48 (b, 2H, exchanges), 3.63 (dd, 1H, J = 12, 6 Hz), 3.70 (dd, 1H, J = 12, 4 Hz), 3.95 (bm, 1H), 4.15 (dd, J = 12, 7 Hz) and 4.22 (dd, 1H, J = 12, 5 Hz), that the molecule contained a 1-acyloxy-2,3-dihydroxypropane fragment. This was supported by its mass spectrum which showed peaks at *m/z* 218

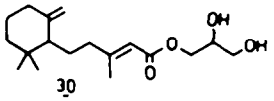
(loss of $-O-CH_2-CHOH-CH_2OH$)

and 190

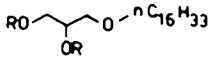
(loss of $-CO_2CH_2-CHOH-CH_2OH$ + H).

Four methyl resonances at δ 0.89, 0.92, 0.98 and 1.62 suggested that the remaining fifteen carbon atoms comprised a sesquiterpenoid acyl residue. A single deshielded proton at δ 5.57 implied the presence of one olefinic functionality in the terpenoid segment which in conjunction with the ester carbonyl accounts for two of the four required sites of unsaturation. Structure **26**, which contains a drimane type acyl residue, accounts for all the spectral features of the compound. We proved that this structure was correct by reducing glyceride **26** to drimenol (**27**). Our reduction product was identical by GC, MS and ^{1}H-NMR comparison to an authentic sample.[24] The absolute configuration of **26** was proven to be the one shown by comparing the optical rotation of the reduction product **27** ($[\alpha]_D$ = −20°, $CHCl_3$) to the literature values[26] ($[\alpha]_D$ = −20°, $CHCl_3$). We have also been able to isolate the monoacetylated glyceride **28** in very low yields from these extracts. Its structure could be routinely assigned from its spectral data. The corresponding secondary acetate **29** was not detected.

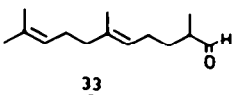
A fourth minor metabolite could only be isolated in very low yields after extensive HPLC purification. High resolution mass spectrometry indicated that it had a molecular formula of $C_{18}H_{30}O_4$ and a ^{1}H-NMR (400 MHz) spectrum revealed that it too was a sesquiterpenoic acid glyceride. The ^{1}H-NMR signals for the glyceryl residue clearly showed that it was acylated on a primary OH. Resonances at δ 4.55 (1H, bs) and 4.79 (1H, bs) suggested that the terpenoic acid fragment contained an exocyclic methylene, and a second trisubstituted olefinic functionality was indicated by resonances for a single olefinic proton at 5.70 and a vinyl Me at 2.16 ppm. The deshielded position of the vinyl Me resonance was consistent with its being attached to the β position of an $\alpha\beta$ unsaturated ester. Two aliphatic Me singlets at δ 0.85 and 0.93 ppm were also clearly discernible in the ^{1}H-NMR spectrum. Based on the above spectral data we have assigned structure **30** to this minor metabolite. Its mass spectrum, which shows prominent fragment ions at *m/z* 176 ($C_{13}H_{20}$), 109 (C_8H_{13}) and 82 (C_5H_6O), is consistent with this proposal.



30



31 R = H
32 R = Ac



33

The final minor metabolite that we were able to isolate from *A. montereyensis* extracts was the known compound 1-O-hexadecylglycerol (**31**).[27] Its spectral data were in good agreement with the reported literature values. We have also found this compound as a minor component of the extracts from the dorid *Aldisa sanguinea cooperi* and the sponge *Halichondria panicea* on which *A. montereyensis* feeds. Compound **31** shows potent *in vitro* antibiotic activity against *Staphylococcus aureus* and *Bacillus subtilis*, and it alone appears to be responsible for the antibacterial activity observed for crude *A. sanguinea cooperi* and *A. montereyensis* extracts.

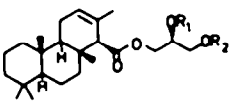
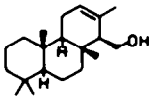
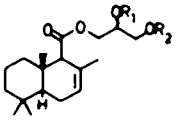
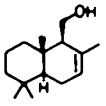
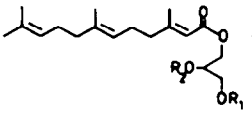
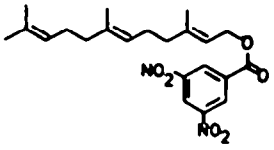
Archidoris odhneri. We have re-examined the extracts of *A. odhneri* paying particular attention to a number of very minor components. As previously reported, the major constituents of *A. odhneri* extracts were the farnesic acid glycerides **12**, **13**, and **14**.[15] We have now been able to isolate the diterpenoic acid glyceride **20** from these extracts in low yield, and careful examination *via* HPLC indicates that the sesquiterpenoic acid glyceride **26** and the monocyclic glyceride **30** are also present in extremely low yields.

Anisodoris nobilis. The large dorid *Anisodoris nobilis* is commonly called the speckled sea lemon due to its bright yellow–orange colour and its persistent fruity odour. We have examined *A. nobilis* in an attempt to determine the nature of the chemical responsible for its smell. Specimens of this animal were collected in numerous rocky subtidal locations in Barkley Sound, B.C. The freshly collected whole animals were immediately immersed in chloroform. Concentration of the chloroform *in vacuo* produced an odoriferous crude oil which was fractionated by radial TLC and preparative gas chromatography to give one major odoriferous compound, aldehyde **33**, in low yield. The mass spectrum of **33** showed a parent ion at *m/z* 208 appropriate for a molecular formula of $C_{14}H_{24}O$. It also showed a fragment ion at *m/z* 150 ($M^+ - CH_3—CH—CHO + H$) resulting from a McLafferty rearrangement, and a base peak at 69 resulting from a doubly allylic cleavage which expels the terminal isoprene residue. In its ^{1}H-NMR spectrum (400 MHz) **33** showed methyl resonances at δ 1.10 (d, J = 7 Hz), 1.60 (bs), 1.61 (bs) and 1.69 (bs), a pair of geminal methylene protons at 1.42 and 1.78, six allylic protons at 1.96–2.13, one methine proton at 2.36, two olefinic protons at 5.12, and an aldehyde proton at 9.58 ppm (d, J = 1 Hz). The ^{1}H-NMR resonances of **33** were reminiscent of those previously observed for the degraded monoterpene **7**.[12] By analogy we concluded that **33** was the degraded sesquiterpenoid 2,6,10-trimethyl-5,9-undecadienal. Aldehyde **33** has not been previously reported as a natural product, but it has apparently found use in the perfume industry.

Polycera tricolor. One of the most exquisite nudibranchs in B.C. waters is the extremely delicate and brightly coloured dorid, *Polycera tricolor*. Specimens of this organism were collected subtidally on Mutine Reef, Barkley Sound, B.C. Freshly collected specimens were extracted in the usual manner with methanol. The extracts of *P. tricolor* contained one major metabolite which we were able to show was identical to triophamine (**19**), a metabolite originally isolated from the dorid *Triopha catalinae*.[19] *P. tricolor*, like *T. catalinae*, feeds on bryozoans, which leads to the speculation that triophamine may have a dietary origin.

Incorporation studies. An important question that must be asked about all nudibranch skin metabolites is

Table 1. Results of ^{14}C mevalonic acid incorporation experiments

Nudibranch	Metabolite	Derivative used in counting	Activity DPM/mg[a]
Archidoris montereyensis			550
	20 R_1 = H, R_2 = H	**21**	
			3260
	26 R_1 = R_2 = H	**27**	
Archidoris odhneri			1810
	12 R_1 = R_2 = H	**35**	

[a] Disintegrations per minute/milligram.

Table 2. Fish antifeedant bioassay results.

Compound	Concentration μg/mg	Activity	Concentration μg/mg	Activity
Diterpenoic acid glyceride **20**	5	−*	160	−
1°-acetate **24**	5	−	110	−
Sesquiterpenoic acid glyceride **26**	18	+	NT	NT
Farnesic acid glyceride **11**	26	−	111	−
Glyceryl ether **31**	18	+	NT	NT

* +, pellet not eaten; −, pellet eaten.

whether they are derived from the animal's diet or from direct biosynthesis. We have sought to answer this question for a number of the substances isolated from *A. montereyensis* and *A. odhneri* by feeding ^{14}C labelled mevalonic acid and checking for incorporation. The radio labelled substrate was in all instances administered to freshly collected live nudibranchs by direct injection into their digestive glands. After injection, the nudibranchs were placed in a running seawater aquarium for 24 hours, and then extracted in the normal fashion. The metabolites of interest were all taken to HPLC purity, and then converted to a derivative which was also purified via HPLC before being crystallized and counted.

The results of the incorporation studies are shown in Table 1. Significant levels of ^{14}C mevalonic acid incorporation were observed for the farnesic acid glyceride **12**, the sesquiterpenoic acid glyceride **26**, and the diterpenoic acid glyceride **20**.

Fish antifeedant bioassay. One of the prime motivations for studying nudibranch skin chemistry is the possibility that the isolated metabolites may play a defensive role for the organisms. Fish represent one group of potential predators on nudibranchs and it is therefore of interest to determine whether nudibranch skin chemicals have any antifeedant activity towards fish. We have previously used a goldfish feeding bioassay to successfully test compounds from *C. luteomarginata* and *A. sanguinea cooperi*.[7a]

In an attempt to make the fish bioassay ecologically more realistic, we have modified our testing protocol to employ the tide pool sculpin *Oligocottus maculosus*. These fish are commonly encountered in the same field habitats as many of the nudibranch species we have studied; they are hearty laboratory animals; and they are active and voracious feeders. Fish that have been starved for 24 hours are alternatively presented with shrimp pellets which are either untreated or coated with low levels of the compounds of interest. A positive antifeedant result involves mouthing and eventual rejection of the coated food pellets by a group of fish in a fixed period of time during which an untreated pellet would have been completely consumed.

Our bioassay results for several *A. montereyensis* and *A. odhneri* metabolites are shown in Table 2. The terpenoic acid glyceride **26**, which has a drimane terpenoid residue, is active, while both the diterpenoic acid glyceride **20** and the farnesic acid glyceride **12** are inactive. Glyceryl ether **31** has previously been demonstrated to be toxic to fish, and we have now shown that it also inhibits feeding behaviour.

DISCUSSION

As a consequence of our research and that of others, a number of features of nudibranch chemistry are emerging. It is now clear that the majority of nudibranch skin metabolites are terpenoid. Sesquiterpenoids are the most abundant, although a number of diterpenoids[28] are starting to appear. There have also been reports of a number of irregular terpenoids containing 9,[12] 14, 23,[7] and 26[31] carbon atoms. They apparently have arisen by degradation or alkylation of regular isoprenoid precursors. A series of polyacetylenes from *Peltodoris atromaculata*[29] and *Diaulula sandiegensis*,[30] triophamine from *T. catalinae*,[19] and the bipyrroles, tambjamines A-D,[4b] represent notable non-terpenoid nudibranch metabolites.

It is also now evident that the chemicals extracted from nudibranchs have a variety of origins. Many of the compounds are sequestered unchanged from the sponges, bryozoans and coelenterates on which the nudibranchs feed.[1,3,29,31] Other compounds, such as the bile acid derivatives **15** and **16**, may be dietary metabolites that have been chemically modified by the nudibranch.[16] The work by Cimino *et al.* on polygodial synthesis by *D. limbata*,[5] and our studies on the terpenoic acid glycerides from *A. montereyensis* and *A. odhneri*, have shown that nudibranchs are capable of the direct biosynthesis of both sesquiterpenoids and diterpenoids. Preliminary results in our laboratory also indicate that albicanyl acetate (**5**), nanaimoal (**9**), acanthodoral (**10**), and isoacanthodoral (**11**) are being biosynthesized by *C. luteomarginata* and *A. nanaimoensis*, respectively.[32] It is clear that nudibranchs have well developed biosynthetic capabilities for generating terpenoids and therefore it should not be immediately assumed that interesting metabolites found in their skin extracts are of dietary origin.

The defensive potential of nudibranch metabolites is of great interest, but it is also very difficult to evaluate. We have attempted to examine the effectiveness of *A. montereyensis* and *A. odhneri* metabolites at thwarting the feeding behaviour of small intertidal fish that are a potential predator. One has to exercise extreme caution in interpreting the results of any behavioral bioassay. However, when a metabolite shows antifeedant activity in our current bioassay, the results are so dramatically clear-cut that we have confidence in our results.

It is interesting to note that of the three terpenoic acid glycerides tested for antifeedant activity (Table 2), only compound **26**, which is a drimane sesquiterpenoid

derivative, showed any activity. Previous studies have demonstrated that the drimane sesquiterpenoids polygodial,[33] albicanyl acetate[7] and olepupuane[34] also have fish antifeedant activity. It appears that the carbon skeleton of these molecules may be just as important as any particular functional group in determining their biological activities. It is also interesting to note that a second antifeedant metabolite in *A. montereyensis* extracts, the glyceryl ether **31**, is also found in the sponge *H. panicea* and is therefore probably of dietary origin. Conceivably *A. montereyensis* utilizes two compounds as part of its defensive arsenal, one which is being made by the nudibranch, and one which it concentrates from its diet.

EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded on Bruker WH 400 and WP80 spectrometers. TMS was used as an internal standard. Low resolution mass spectra were recorded on an AEI MS902 spectrometer and high resolution mass spectra were recorded on an AEI MS50 spectrometer. IR spectra were recorded on a Perkin-Elmer model 710 B spectrometer and optical rotations were measured on a Perkin Elmer 141 polarimeter using a 1 dm cell. M.ps were obtained on a Fisher-Johns apparatus and values are uncorrected. Merck Silica Gel 230–400 mesh was used for flash chromatography, Merck Silica Gel PF 254 with $CaSO_4 \cdot 1/2\ H_2O$ Type 60 was used for radial TLC and a Whatman Magnum 9-Partisil 10 column was used for preparative HPLC. All chromatography solvents were HPLC grade.

Collection data. All specimens were collected by hand using SCUBA. Collections were made at a depth of 1–15 m in Barkley Sound, British Columbia. Samples were immediately immersed in MeOH or $CHCl_3$ and stored at low temp.

Extraction and chromatography. A total of 80 specimens of *Archidoris montereyensis* were extracted with MeOH (3 × 1 l) for 3 days. The combined MeOH extracts were evaporated to an aqueous suspension and extracted with $CHCl_3$ (3 × 500 ml). The combined extracts were dried over Na_2SO_4 and evaporated to yield a brown gum (2.7 g, 8.3% dry weight, 33.7 mg/animal).

The gum (1.5 g) was subjected to flash chromatography on a column of silica gel (130 g) with a step gradient of hexane–EtOAc mixtures. Elution with 20% EtOAc in hexane removed non-polar fats and pigments. The acetylated glycerides were eluted with a 1:1 soln of hexane–EtOAc while the non-acetylated glycerides were eluted with 100% EtOAc.

Material eluted with (1:1) hexane–EtOAc was fractionated by radial TLC (silica gel), eluting solvent $CHCl_3$–MeOH (99:1). Final separation was achieved by LC on Partisil-10 with hexane–EtOAc–MeOH (85:15:1) eluant to yield in the order of their elution, compounds **24** (24 mg), **28** (2 mg) and **25** (3 mg).

Compound **24** m.p. 117–119° (hexane–Et_2O); $[\alpha]_D$ = −53.7° (*c* = 0.13, $CHCl_3$); HRMS, obs. 420.2884, $C_{25}H_{40}O_4$ cal. 420.2876; IR ($CHCl_3$) 3600–3350, 2930, 1730, 1230 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.87 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.61 (s, 3H), 2.11 (s, 3H), 2.41 (bs, 1H, exchangeable), 2.97 (bs, 1H), 4.08–4.25 (m, 5H), 5.53 (bs, 1H); MS *m/z* 420 (2), 402 (5), 347 (5), 286 (100), 192 (65), 177 (70), 117 (60).

Compound **28** oil; $[\alpha]_D$ = +15.3° (*c* = 1.2, $CHCl_3$); HRMS, obs. 352.2221, $C_{20}H_{32}O_5$ cal. 352.2250; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.92 (s, 3H), 0.98 (s, 3H), 1.61 (s, 3H), 2.11 (s, 3H), 2.48 (bs, 1H exchangeable), 2.95 (bs, 1H), 4.05–4.24 (m, 5H), 5.55 (bs, 1H); MS, *m/z* 352 (3), 334 (4), 279 (5), 218 (55), 190 (45), 124 (80), 117 (80), 109 (100), 95 (80).

Compound **25** oil; $[\alpha]_D$ = −33.0° (*c* = 0.83, $CHCl_3$); HRMS, obs. 420.2876, $C_{25}H_{40}O_5$ cal. 420.2876; IR ($CHCl_3$) 3600–3210, 2925, 1725, 1230, 1055 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.87 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 1.60 (s, 3H), 2.09 (s, 3H), 2.94 (bs, 1H), 3.76 (d, J = 6 Hz, 2H), 4.26 (dd, J = 7 and 12 Hz, 1H), 4.32 (dd, J = 6 and 13 Hz, 1H), 5.06 (m, 1H), 5.52 (bs, 1H); MS, *m/z* 420 (5), 402 (3), 286 (90), 258 (20), 192 (55), 191 (50), 177 (75), 117 (100), 95 (80), 81 (80).

The 100% EtOAc fraction from the flash column was subjected to radial TLC eluting with $CHCl_3$–MeOH (95:5). Final separation was achieved by LC on Partisil-10 with hexane–EtOAc–MeOH (50:40:2) eluant to yield in the order of their elution, compounds **20** (38 mg), **31** (2 mg), **26** (3 mg) and **30** (2 mg). *Compound* **20**, m.p. = 125–126° (hexane–Et_2O), $[\alpha]_D$ = −12.5° (*c* = 0.4, $CHCl_3$); HRMS, obs. 378.2772, $C_{23}H_{38}O_4$ cal. 378.2770; IR ($CHCl_3$) 3600–3300, 2900, 2850, 1730, 1460, 1170 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.87 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.61 (bs, 3H), 1.92–1.99 (m, 2H), 2.44 (bs, 2H, exchangeable), 2.96 (bs, 1H), 3.63 (dd, J = 6 and 12 Hz, 1H), 3.70 (dd, J = 4 and 12 Hz, 1H), 3.95 (m, 1H), 4.15 (dd, J = 7 and 12 Hz, 1H), 4.22 (dd, J = 5 and 12 Hz, 1H), 5.54 (bs, 1H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) 15.63 (q), 15.73 (q), 18.52 (q), 18.73 (q), 21.15 (t), 21.68 (t), 22.74 (t), 33.20 (s), 33.43 (q), 36.70 (s), 37.53 (s), 40.01 (t), 41.95 (t), 54.45 (d), 56.58 (d), 62.76 (d), 63.64 (t), 65.17 (t), 70.47 (d), 124.32 (d), 128.60 (s), 173.35 (s) ppm; MS, *m/z* 378 (30), 363 (5), 347 (10), 286 (65), 258 (35), 192 (100), 177 (80), 95 (75).

Compound **31** HRMS, obs. 316.2973, $C_{19}H_{40}O_3$ cal. 316.2977; IR ($CHCl_3$) 3600–3250, 2900, 1110 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J = 7 Hz, 3H), 1.26 (bs, 28H), 1.59 (m, 2H), 3.44 (t, J = 6 Hz, 2H), 3.50 (m, 2H), 3.64 (dd, J = 6, 12 Hz, 1H), 3.67 (dd, J = 4, 12 Hz, 1H), 3.82 (m, 1H), ppm; MS, *m/z* 316 (2), 285 (5), 255 (15), 225 (15), 71 (80), 57 (100). *Compound* **26** $[\alpha]_D$ = +23.1 (*c* = 0.93, $CHCl_3$); HRMS, obs. 310.2142, $C_{19}H_{30}O_4$ cal. 310.2144; IR ($CHCl_3$) 3600–3300, 2900, 1730, 1165 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.89 (s, 3H), 0.92 (s, 3H), 0.98 (s, 3H), 1.62 (bs, 3H), 2.48 (bs, 2H, exchangeable), 2.96 (bs, 1H), 3.63 (dd, J = 6 and 12 Hz, 1H), 3.70 (dd, J = 4 and 12 Hz, 1H), 3.95 (m, 1H), 4.15 (dd, J = 7 and 12 Hz, 1H), 4.22 (dd, J = 5 and 12 Hz, 1H), 5.57 (bs, 1H) ppm; MS, *m/z* 310 (15), 295 (5), 279 (10), 218 (75), 190 (45), 187 (40), 124 (70), 109 (100), 95 (75).

Compound **30** oil; $[\alpha]_D$ = +9.7° (*c* = 0.3, $CHCl_3$) HRMS, obs. 310.2146, $C_{19}H_{30}O_4$ cal. 310.2144; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 3H), 0.93 (s, 3H), 2.16 (s, 3H), 3.62 (dd, J = 12 and 7 Hz, 1H), 3.70 (dd, J = 12 and 5 Hz, 1H), 3.95 (m, 1H), 4.18 (dd, J = 12 and 7 Hz, 1H), 4.24 (dd, J = 12 and 5 Hz, 1H), 4.55 (s, 1H), 4.79 (s, 1H), 5.70 (s, 1H); MS, *m/z* 310 (2), 295 (5), 279 (5), 237 (10), 219 (35), 176 (85), 124 (30), 109 (85), 95 (90), 82 (100), 69 (90).

Ten specimens of *Archidoris odhneri* were extracted with MeOH and subjected to a separation and purification scheme identical to that used with *A. montereyensis*. Two major metabolites were isolated, **20** (5 mg) and **12** (43 mg).

Careful examination of the HPLC trace showed minor peaks with retention times appropriate for glycerides **26** and **30**. Isolation and purification of these compounds was precluded by their very low yields in *A. odhneri* extracts.

A total of 22 specimens of *Anisodoris nobilis* were extracted with $CHCl_3$ (2 × 800 ml). The chloroform was dried over Na_2SO_4 and evaporated to obtain a pleasant smelling light brown oil (820 mg). The oil was separated by flash chromatography on silica gel (hexane–$CHCl_3$ step gradient). Material eluting with 15% $CHCl_3$ in hexane was further purified by radial TLC using 100% hexane as the eluant. Final purification was achieved by preparative GC (3% OV-17 on Chromosorb, initial temp 100°, rate 4°/min, RT 14 min) providing 2.8 mg of **33** as a sweet smelling, colourless oil.

Compound **33** oil; HRMS, obs. 208.1825, $C_{14}H_{24}O$ cal. 208.1823; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.10 (d, J = 7 Hz, 3H), 1.42 (m, 1H), 1.60 (s, 3H), 1.61 (s, 3H), 1.69 (s, 3H), 1.78 (m, 1H), 1.96–2.13 (m, 6H), 2.36 (m, 1H), 5.12 (m, 2H), 9.58 (d, J = 1 Hz, 1H) ppm; MS, *m/z* 208 (5), 190 (5), 175 (3), 165 (50), 150 (20), 95 (25), 81 (70), 69 (100).

A total of 30 specimens of *Polycera tricolor* were extracted with MeOH (3 × 300 ml). The extracts were evaporated to an aqueous suspension and extracted with $CHCl_3$ (3 × 100 ml).

The $CHCl_3$ soln was dried over Na_2SO_4 and concentrated to a brown oil (385 mg) which was subjected to flash chromatography on silica gel (hexane EtOAc step gradient). The fraction eluted with 15% EtOAc in hexane was further purified by preparative TLC (1 : 1 hexane ether, $R_f \cong 0.5$) to yield **19** (5 mg) as a light yellow oil.

Compound **19** HRMS, obs. 363.2890, $C_{21}H_{37}N_3O_2$ cal. 363.2886; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.91 (t, J = 7 Hz, 3H), 0.95 (t, J = 7 Hz, 3H), 1.56 (d, J = 7 Hz, 3H), 2.01 (q, J = 7 Hz, 2H), 5.21 (q, J = 7 Hz, 1H) ppm; MS, *m/z* 363 (20), 348 (3), 334 (10), 281 (10), 238 (20), 86 (100).

Reduction of **20**. A soln of **20** (20 mg) in 0.5 ml of toluene was stirred at RT for 8 hr in the presence of excess DIBAL. Addition of 5 ml of EtOH, followed by filtration of the mixture and evaporation of the solvents, resulted in one major product. Preparative TLC (1 : 4, EtOAc hexane $R_f \cong 0.3$) provided 13 mg (85% yield) of white crystalline **21**.

Compound **21** m.p. 126–127° (hexane-Et_2O) (lit. 125–126°);[23] $[\alpha]_D = -9°$ ($c = 0.1$, $CHCl_3$), (lit. −9°);[23] HRMS, obs. 290.2623, $C_{20}H_{34}O$ cal. 290.2609; IR ($CHCl_3$) 3600–3300, 2920, 2850, 1450, 1390 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.84 (s, 3H), 0.87 (s, 3H), 0.90 (s, 3H), 1.78 (s, 3H), 2.06 (dt, J = 4 and 13 Hz, 1H), 3.72 (dd, J = 6 and 12 Hz, 1H), 3.85 (dd, J = 4 and 12 Hz, 1H), 5.50 (bs, 1H); MS, *m/z* 290 (40), 275 (5), 260 (5), 245 (5), 192 (100), 177 (95), 95 (40), 81 (60), 69 (85).

Reduction of **24** *and* **25**. Separate solns of **24** (15 mg) and **25** (6 mg) were treated with DIBAL and subsequently purified in a manner identical to the reduction of **20**. This produced 8 mg (77% yield) and 3 mg (72% yield) respectively of white crystalline **21**, which was identical in all respects to the reduction product of **20**.

Reduction of **26**. A soln of **26** (6 mg) was treated with DIBAL and subsequently purified in a manner identical to the reduction of **20**. This produced 2.6 mg (50% yield) of white crystalline **27**.

Compound **27** m.p. 94–95° (lit. 95 96°);[26] $[\alpha]_D = -20°$ ($c = 0.08$, $CHCl_3$) (lit. −20°);[26] HRMS, obs. 222.1984, $C_{15}H_{26}O$ cal. 222.1984; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.837 (s, 3H), 0.844 (s, 3H), 0.867 (s, 3H), 1.76 (s, 3H), 3.73 (dd, J = 13 and 7.2 Hz, 1H), 3.84 (dd, J = 13 and 5 Hz, 1H) ppm; MS, *m/z* 222 (10), 191 (10), 124 (45), 109 (75), 85 (75), 83 (100), 69 (60). Reduction product **27** was identical to authentic drimenol by ^{1}H-NMR, MS, TLC (ethyl acetate hexane (1 : 4)), $R_f \cong 0.3$ on Polygram 0.25 mm silica gel plates and GC (co-injection of **27** and authentic drimenol gave a single peak: initial temperature 140°, rate 10°/min, RT = 6.75 min on 3% OV-17).

^{14}*C Incorporation studies*. A total of 22 specimens of *A. montereyensis* and 13 specimens of *A. ohdneri* were employed in the incorporation studies. 2 μCi of [2-^{14}C] RS-mevalonic acid dibenzylethylenediamine salt (New England Nuclear; 45 mCi/mmol) in (1 : 1) ethanol sterile seawater (0.05 ml) was directly injected into the digestive gland of each animal by means of a syringe. After injection, the nudibranchs were placed in a running seawater aquarium for 24 hr and then extracted with methanol in the normal manner. The extracts were fractionated and the glyceride metabolites purified by HPLC as previously described for both species. Compounds **20** and **26** obtained from *A. montereyensis*, were reduced to alcohols **21** and **27** respectively by DIBAL in toluene. The alcohols were purified by HPLC (Partisil-10, 8% EtOAc hexane) and crystallized from ether-hexane prior to ^{14}C counting. Compound **12**, obtained from *A. ohdneri* was reduced to farnesol and then converted to its 3,5-dinitrobenzoate derivative **35**. Compound **35** was crystallized from ether-hexane prior to counting. The samples (0.5–4 mg) were dissolved in 10 ml Aquasol (NEN) scintillation counting fluid. Radioactive counts were determined in a Unilux III liquid scintillation system. Quenching was corrected by external standardization.

Reduction of **12**. A soln of **12** (52 mg) was treated with DIBAL and subsequently purified in a manner identical to the reduction of **20**. Final purification by HPLC (Partisil-10, 8% EtOAc in hexane) provided 26 mg of farnesol as a colourless oil.

3,5-Dinitrobenzoate derivative of farnesol. To a soln of **34** (26 mg; 0.117 mmol) in 1.0 ml pyridine was added 4-dimethylaminopyridine (20 mg, 0.164 mmol) and 3,5-dinitrobenzoyl chloride (31 mg, 0.135 mmol). After stirring at RT for 12 hr, 5 ml methanol was added and the mixture was stirred for an additional 2 hr. The mixture was filtered through a plug (1 × 1 cm dia.) of silica gel using $CHCl_3$ as eluant. Removal of the solvent under reduced pressure and preparative TLC (25% EtOAc in hexane) provided 39 mg of **35** m.p. 77–79°; ^{1}H-NMR (80 MHz, $CDCl_3$): 1.58 (s, 6H), 1.64 (s, 3H), 1.79 (s, 3H), 1.90–2.19 (m, 8H), 4.93 (d, J = 8 Hz, 2H), 5.04 (m, 2H), 5.45 (t, J = 8 Hz, 1H), 9.21 (m, 3H); MS, *m/z* 416 (20), 401 (5), 195 (50), 136 (60), 93 (50), 81 (170), 69 (100).

Fish antifeedant bioassay. Antifeedant activity was assessed by observing the feeding response of the tide pool sculpin *Oligocottus maculosus* toward food pellets (Wardely Shrimp Pellets) treated with varying concentrations of dorid metabolites. The sculpins were collected intertidally at Barkley Sound, British Columbia, and were starved for 24 hr prior to testing. Test compounds were applied to food pellets with acetone which was evaporated at room temp. Control pellets were treated with solvent only. Single pellets were randomly added to a group of 40 fish (5–8 cm in length) in a 10 gallon aquarium and the feeding response was observed for up to 1 hr. The fish exhibited voracious feeding behaviour toward control pellets and pellets treated with inactive compounds. These pellets were aggressively attacked by groups of fish and consumed within 5–10 min. Indigestible hard parts in the pellets were ignored. Test compounds that were active caused a marked cessation of feeding. After initial inspections, the fish avoided the active pellets which remained uneaten after 1 hr. The feeding ability of the fish was verified by feeding them untreated pellets both before and after each test. In all cases the untreated pellets were rapidly consumed.

Acknowledgements—We wish to acknowledge financial support from the Natural Sciences and Engineering Research Council. Financial support from U.B.C. in the form of a graduate fellowship to KG is gratefully acknowledged. The authors wish to thank Mike LeBlanc, Sandra Millen and the staff of the Bamfield Marine Station for assistance with collecting and identifying nudibranchs.

REFERENCES AND NOTES

[1a]G. R. Schulte and P. J. Scheuer, *Tetrahedron* **38**, 1857 (1982); [b]J. E. Thompson, R. P. Walker, S. J. Wratten and D. J. Faulkner, *Ibid.* **38**, 1865 (1982); [c]G. Cimino, S. De Stephano, S. De Rosa, G. Sodano and G. Villani, *Bull. Soc. Chim. Belg.* **89**, 1069 (1980).

[2a]R. E. Johannes, *The Veliger* **5**, 104 (1963); [b]T. E. Thompson, *J. Mar. Biol. Ass. U.K.* **39**, 123 (1960).

[3]B. J. Burreson, P. J. Scheuer, J. S. Finer and J. Clardy, *J. Am. Chem. Soc.* **97**, 4763 (1975).

[4a]G. Cimino, S. De Rosa, S. De Stephano and G. Sodano, *Comp. Biochem. Physiol. B* **73**, 471 (1982); [b]B. Carté and D. J. Faulkner, *J. Org. Chem.* **48**, 2315 (1983).

[5]G. Cimino, S. De Rosa, S. De Stephano, G. Sodano and G. Villani, *Science* **219**, 1237 (1983).

[6]Sandra Millen, Guide to B.C. nudibranchs. Zoology Department, University of British Columbia, unpublished manuscript.

[7a]J. Hellou, R. J. Anderson and J. E. Thompson, *Tetrahedron* **38**, 1875 (1982); [b]J. Hellou, R. J. Anderson, S. Rafii, E. Arnold and J. Clardy, *Tetrahedron Letters* **22**, 4173 (1981).

[8]R. Kazlauskas, P. F. Murphy and R. J. Wells, *Tetrahedron Letters* 4951 (1978).

[9]G. Cimino, S. De Stephano, A. Guerriero and L. Minale, *Tetrahedron Letters* 3723 (1975).

[10]Y. Ohta, N. H. Andersen and C. B. Liu, *Tetrahedron* **33**, 617 (1977).

[11]J. Nybakken and R. A. Ajeska, *The Veliger* **19**, 19 (1976).

[12]S. W. Ayer and R. J. Andersen, *Experientia* **39**, 255 (1983).

[13] S. W. Ayer, J. Hellou, M. Tischler and R. J. Andersen, *Tetrahedron Letters* **25**, 141 (1984).
[14] S. W. Ayer, R. J. Andersen, C. He and J. Clardy, *J. Org. Chem.* **49**, 2653 (1984).
[15] R. J. Andersen and F. W. Sum, *Tetrahedron Letters* **21**, 797 (1980).
[16] S. W. Ayer and R. J. Andersen, *Tetrahedron Letters* **23**, 1039 (1982).
[17] H. Schildnecht, R. Siewerdt and V. Maschwitz, *Angew. Chem. Int. Ed. Engl.* **5**, 421 (1966).
[18] R. J. Stonard, J. C. Petrovitch and R. J. Andersen, *Steroids* **36**, 2628 (1980).
[19] K. Gustafson and R. J. Andersen, *J. Org. Chem.* **47**, 2167 (1982).
[20] E. Piers, M. J. Chong, K. Gustafson and R. J. Andersen, *Can. J. Chem.* **62**, 1 (1984).
[21] K. Gustafson, R. J. Andersen, M. H. M. Chen, J. Clardy and J. E. Hochlowski, *Tetrahedron Letters* **25**, 11 (1984).
[22] Subsequent to the X-ray analysis, we discovered that J. Hochlowski and D. J. Faulkner had isolated the same compound from specimens of *A. montereyensis* collected in La Jolla, California. They had proposed structure **20** for their compound.
[23a] G. Cimino, D. De Rosa, S. De Stefano and L. Minale, *Tetrahedron* **30**, 645 (1974); [b] D. K. Manh Due, M. Fetizon and M. Kane, *Bull. Soc. Chim. Fr.* 2351 (1975).
[24] R. Kazlauskas, P. T. Murphy and R. J. Wells, *Tetrahedron Letters* 903 (1979).
[25] Kindly provided by R. J. Armstrong, Chemistry Department, University of British Columbia – see Ph.D. thesis (1983).
[26] S. W. Pelletier, S. Lajsic, Y. Ohtsuka and Z. Djarmati, *J. Org. Chem.* **40**, 1607 (1975).
[27] G. R. Pettit and Y. Fujii, *J. Nat. Prod.* **45**, 640 (1982).
[28] J. E. Hochlowski, D. J. Faulkner, G. K. Matsumoto and J. Clardy, *J. Org. Chem.* **48**, 1142 (1983).
[29] D. Castiello, G. Cimino, S. De Rosa, S. De Stephano and G. Sodano, *Tetrahedron Letters* 5047 (1980).
[30] R. P. Walker and D. J. Faulkner, *J. Org. Chem.* **46**, 1475 (1981).
[31] J. E. Hochlowski, D. J. Faulkner, L. Bass and J. Clardy, *J. Org. Chem.* **48**, 2314 (1983).
[32] K. Gustafson and S. W. Ayer, unpublished results.
[33] G. Cimino, S. De Rosa, S. De Stephano and G. Sodano, *Tetrahedron Letters* 1271 (1981).
[34] R. Okuda, P. J. Scheuer, J. E. Hochlowski, R. P. Walker and D. J. Faulkner, *J. Org. Chem.* **48**, 1866 (1983).