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MURRAYANOLIDE, AN UNUSUAL C_{21} TETRACYCLIC TERPENOID LACTONE FROM THE MARINE BRYOZOAN *DENDROBEANIA MURRAYANA*[1]

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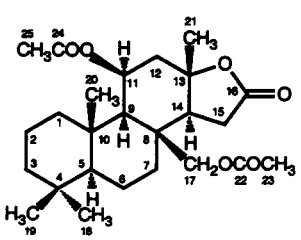
ABSTRACT.—A novel tetracyclic terpenoid lactone possessing an unusual C_{21} skeleton was isolated from a marine bryozoan and the structure was established by spectral methods.

Bryozoans are a source of a variety of chemical structures including the macrocyclic bryostatins (1) as well as simple monoterpenes and halogenated alkaloids, often possessing pendant terpenoid chains (2–4). In our search for bioactive compounds from marine organisms we have isolated an unusual C_{21} tetracyclic terpenoid lactone, murrayanolide [**1**], from an extract of the bryozoan *Dendrobeania murrayana* (Johnston) (Buguliidae), collected off the east coast of Canada.

The CH_2Cl_2 fraction obtained from a MeOH extract of freshly thawed bryozoan (0.5 kg) was purified following a sequence of Si gel, gel permeation, and C_{18} reversed-phase hplc to yield **1** as a colorless, optically active, crystalline solid (6 mg). The molecular formula $C_{25}H_{38}O_6$ was established on the basis of the hreims [M^+ 434.2681, ΔM +1.5 mmu], and requires seven degrees of unsaturation in the molecule. The combined ^1H- and ^{13}C-nmr data, together with DEPT and HMQC experiments (Table 1), suggested the presence of four tertiary methyl groups, two acetate groups, eight methylenes, four methines, four quaternary carbons, and a third carbonyl group. The characteristic ir absorption at 1778 cm^{-1} indicated a γ-lactone ring, which, together with the two acetate groups, accounted for four of the seven degrees of unsaturation in the molecule. With the absence of any olefinic carbon resonances, the remaining three degrees of unsaturation must be due to rings.



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Four separate spin-systems, **a**–**d** (see Figure 1), were identified by the usual homonuclear coupling data obtained by standard 2D nmr methods (COSY and TOCSY). It is notable that in system **a**, the two methyl resonances at δ 1.00 (H_3-20) and δ 1.50 (H_3-21), assigned to two axial methyl groups (5), showed long-range COSY correlations to the methine proton at δ 1.29 (H-9) and the methylene proton at δ 1.91 (H-12), respectively. The low-field chemical shift for H-11 (δ 5.69) located one of the acetate groups at this position in partial structure **a**. The other partial structures were routinely established as shown (Figure 1).

These partial structures **a**–**d** were linked as shown in Figure 2 through a series of HMBC experiments. For ex-

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TABLE 1. ^{13}C- and ^{1}H-Nmr Chemical Shifts of **1**.

Position	δ_C (ppm) ($CDCl_3$)	Multiplicity (DEPT)	δ_H (ppm) ($CDCl_3$)	Position	δ_C (ppm) ($CDCl_3$)	Multiplicity (DEPT)	δ_H (ppm) ($CDCl_3$)
1	39.5	CH_2	0.96 (Hα, m) 1.60 (Hβ, m)	14	58.2	CH	2.05 (Hα, m)
2	18.3	CH_2	1.48 (Hα, m) 1.63 (Hβ, m)	15	31.8	CH_2	2.52 (Hα, dd) 2.99 (Hβ, dd)
3	41.7	CH_2	1.13 (Hα, m) 1.38 (Hβ, m)	16	176.0	C=O	—
4	33.4	C	—	17	64.7	CH_2-O	4.59 (1H, dd) 4.82 (1H, dd)
5	58.0	CH	0.88 (Hα, dd)	18	33.4	CH_3	0.85 (3H, s)
6	17.9	CH_2	1.66 (Hα, m) 1.53 (Hβ, m)	19	21.2	CH_3	0.82 (3H, s)
7	36.8	CH_2	1.11 (Hα, m) 2.03 (Hβ, m)	20	17.1	CH_3	1.00 (3H, s)
8	40.7	C	—	21	21.4	CH_3	1.50 (3H, d)
9	62.2	CH	1.29 (Hα, d)	22	170.4	C=O	—
10	37.9	C	—	23	21.0	CH_3C=O	2.11 (3H, s)
11	68.8	CH-O	5.69 (Hα, dt)	24	169.3	C=O	—
12	43.5	CH_2	1.91 (Hα, dd) 2.33 (Hβ, dd)	25	21.7	CH_3C=O	2.10 (3H, s)
13	84.0	C-O	—				

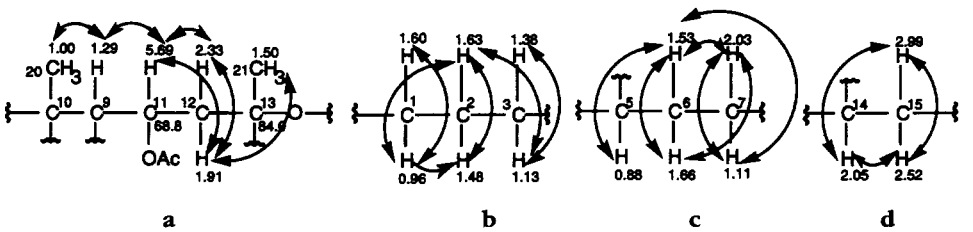


FIGURE 1. Partial structures of **1** elucidated by a 2D COSY nmr experiment.

ample, correlations of the quaternary C-10 (δ 37.9) with H-2 (δ 1.63) and C-20 (δ 17.1) with H-5 (δ 0.88) linked C-10 of partial structure **a** with C-1 of partial structure **b** and C-5 of partial structure **c**. Additional correlations between C-5 (δ 58.0) and H-1 (δ 1.60) further substantiated the linkage of **c** with **b**. A correlation to only one of the geminal protons is not unexpected considering the Karplus

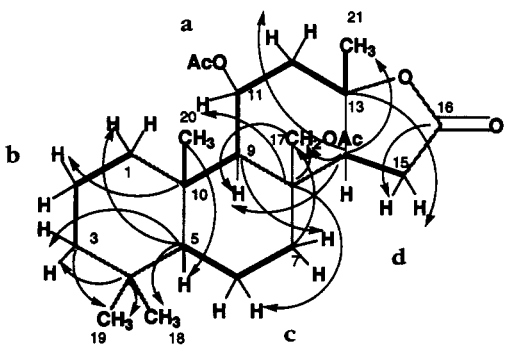


FIGURE 2. Some key long-range C/H correlations for connecting the partial structures of **1** by HMBC experiments.

variation of the $^3J_{CH}$ with respect to the H-C-C-C angle (6) and the observations reported here were consistent the proposed stereochemistry of **1** established by other methods (see below). Finally, the first ring was established by the additional correlation of C-4 (δ 33.4) with H_2-3 (δ 1.13, 1.38) and H_3-19 (δ 0.82), C-19 (δ 21.2) with H_3-18 (δ 0.85), C-3 (δ 41.7) with H_3-19, and C-5 with H_3-18 and H-3 (δ 1.38).

The second ring was established by the HMBC data which showed that the isolated quaternary carbon C-8, bearing an acetoxymethylene group, was connected to C-9 of partial structure **a** and to C-7 of partial structure **c**. The key correlations were C-8 (δ 40.7) with H-9, H-11, H-17 (δ 4.82), H-6 (δ 1.66) and H-7 (δ 2.03), and C-17 (δ 64.7) with H-9. In addition, C-8 was also linked to C-14 of partial structure **d** by the correlations of C-8 with H-14 (δ 2.05) and C-14 (δ 58.2) with H-9, H-12 (δ 2.33), H-15 (δ 2.99), H_2-17 and H_3-21. Furthermore, the oxygen-bearing quaternary carbon C-13 (δ 84.0) was seen clearly to be correlated to H_3-21, H-11, and H-12 (δ 1.91). Finally the γ-lactone ring was linked to the third ring by the HMBC correlations of both C-13 and the γ-lactone carbonyl carbon C-16 (δ 176.0) with the methylene protons H_2-15 (δ 2.52, 2.99). This combined evidence established the tetracyclic terpenoid lactone structure [**1**] for murrayanolide.

The relative stereochemistry of **1** was derived from a combination of ^{1}H- and ^{13}C-nmr chemical shift and 2-D NOESY data (which showed the same positive cross-peaks when recorded with mixing times of 100 or 300 msec), as well as molecular modeling using SYBYL software (7). The ^{13}C-nmr chemical shifts of ring junction methyl groups have proven particularly useful in identifying cis- versus trans-ring fusion (5). Thus, the ^{13}C-nmr chemical shifts for C-18, C-19, C-20, and C-21 are consistent with an array of all trans-fused cyclohexyl rings. This was further supported by several NOESY correlations (Figure 3). In particular, both axial methyl groups H_3-20 and H_3-21 showed strong correlations with the acetoxymethylene protons H_2-17 and the acetyl protons H_3-25, while H_3-20 also displayed a correlation with the axial methyl hydrogens H_3-19. Conversely, all the ring junction protons H-5, H-9, and H-14, as well as H-11 and the other geminal methyl protons (H_3-18) clearly showed spatial correlations to one another but not to the axial methyl groups, and were thus concluded to be on the opposite face of the molecule. This conclusion was confirmed by molecular modeling using SYBYL software and ConGen routines, which use high temperature molecular dynamics to search configurational space for the relative stereochemistry most compatible with the distance restraints derived from NOESY data (7). This procedure converged rapidly on the configuration 5*S*, 8*S*, 9*S*, 10*R*, 11*S*, 13*S*,

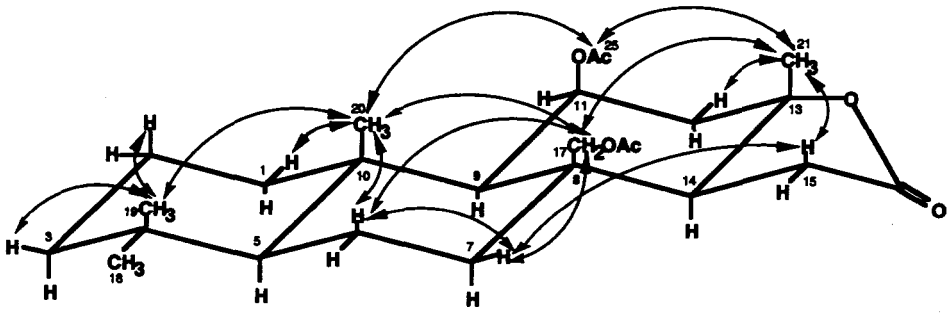


FIGURE 3. Configuration of the tetracyclic ring system of **1** from the NOESY experiment.

14*S* (or the enantiomer), shown in Figure 4 in the conformation which best agreed with the NOESY constraints.

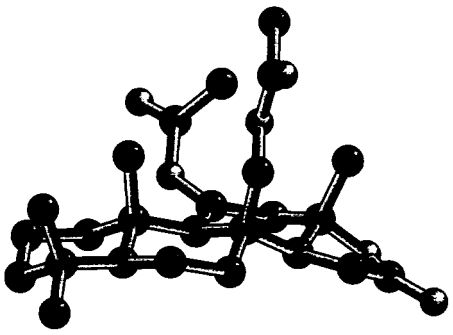
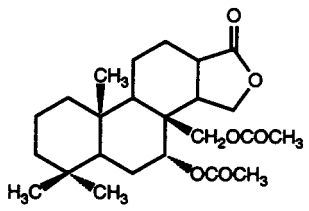


FIGURE 4. Most favored conformer of the murrayanolide configuration derived by SYBYL ConGen molecular modelling.

To the best of our knowledge, **1** is the first C_{21} tetracyclic terpenoid lactone from a bryozoan and a marked departure from the alkaloids (albeit often bearing a terpenoid C_5 side-chain) and simpler diterpenes more commonly isolated from these organisms (2,3). Few C_{21}–C_{23} terpenes bearing a general similarity to structure **1** have been reported from marine organisms (8). Some relevant examples are the C_{20} spongian group (9) and various analogues, the related lactone 16-oxospongian-7,17-diyl diacetate [**2**] also isolated from a sponge (10), as well as the C_{23} terpene luteone [**3**] isolated from a mollusk (11). Compound **1** exhibited moderate inhibitory activity (54% inhibition at 25 μg/ml) against metalloprotease collagenase IV.



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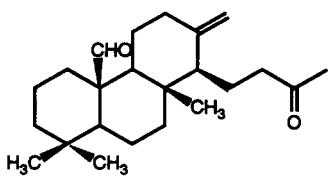

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The biogenesis of **1** and these other marine terpenoids is worthy of brief comment. In the case of luteone [**3**], it has been proposed that the molecule is derived from a degraded sesterterpene, likely a bisnorsesterterpenoid (12). The sponge diterpene **2** could be derived from a carbon skeleton of the kaurane type in which the γ-lactone is formed by oxidation of a methyl group of the terminal isoprene unit prior to incorporation into the lactone ring. Such a pathway is not operable for **1** which possesses an axial methyl group at C-13, and hence a biogenetic route via degradation of a higher terpenoid chain such as that suggested for the biogenesis of **3** is more feasible. Another proposal that **1**, like the quassinoids, could be derived by degradation of a triterpene such as euphol or limonene (12) is unlikely, since the axial methyl group at C-13 in **1** is in the β- configuration whereas the corresponding methyl at C-13 in the limonoid/euphol/quassin series is always found in the opposite configuration (11,13).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp (uncorrected) was determined on a micro hot-stage aparatus. The optical rotation was taken on a Perkin-Elmer 141 polarimeter using a Na lamp operating at 589 nm The ir spectrum was measured with a Bomem Ft-ir spectrometer (model DA 3.02) and the uv spectrum on a Hewlett-Packard 1090 equipped with diode-array detector. Eims was obtained using a VG Analytical ZAB-EQ tandem hybrid mass spectrometer operating at 70 eV. Nmr spectra were recorded in $CDCl_3$ on a Bruker AMX 500 spectrometer equipped with a Bruker X-32 computer using UXNMR software. For comparison purposes, and to recognize any spin-diffusion effects should they occur, the 2D NOESY spectra were recorded with mixing times of 300 and 100 msec. Hplc was carried out on a Waters model 6000A chromatograph equipped with a model 440 absorbance detector and a U6K injector.

ANIMAL MATERIAL.—The marine bryozoan *Dendrobeania murrayana* was collected from Chebucto Head, Nova Scotia at a depth of 25 m in 1985. A voucher specimen has been retained at the Institute for Marine Biosciences, NRCC, Halifax, Nova Scotia.

EXTRACTION AND ISOLATION.—The defrosted bryozoan (519 g) was powdered and extracted exhaustively with MeOH (3×300 ml). The extract was evaporated to remove the MeOH, and then partitioned between equal volumes (500 ml) of CH_2Cl_2 and H_2O. Evaporation of the organic fraction yielded a thick gum (1.95 g) which was fractionated by Si gel flash chromatography using a step gradient of EtOAc (5-50%) in hexane, finishing with a mixture of EtOAc and MeOH (9:1). The fraction eluted with hexane-EtOAc (1:1) was further separated on a Sephadex LH-20 column eluting with MeOH to yield crude **1**. Final purification was accomplished by C_{18} reversed-phase hplc (CSC-ODS2, 5 μm, 20×1 cm) eluting with 65% MeCN. The yield was 0.001% based on wet wt of the organism.

Murrayanolide [**1**].—Colorless powder (6 mg); $[\alpha]^{25}D$ −16.6° (*c*=0.75 $CHCl_3$); mp 185–187°; ir (dry film) ν max 2933, 1778, 1740, 1447, 1389, 1374, 1228, 1024, 915, 731 cm^{-1}; ^1H- and ^{13}C-nmr data, see Table 1; hreims M^+ 434.2681, calcd for $C_{25}H_{38}O_6$, 434.2666.

MOLECULAR MODELING PROCEDURES.—Molecular modeling was carried out on a Silicon Graphics Personal Iris 4D/35 workstation using SYBYL software (Tripos Inc., St. Louis, MO) and ConGen routines (7) for determination of the relative stereochemistry of a molecule of known connectivity but unknown chirality using NOESY data. ConGen begins with the molecule in an arbitrary configuration and submits it to repeated cycles of high temperature (8000° K) molecular dynamics under distance restraints derived by the NOESY data. During each cycle of high temperature dynamics frequent inversions occur at most chiral sites and the distance restraints guide the molecule toward the configuration or configurations consistent with the nOe data. The procedure includes additional inversions to allow for the possibility that some chiral centers may only be rarely inverted by dynamics even at 8000° K. In the case of **1**, ConGen with ten nOe-based distance constraints converged to the *SSSRSSS* or *RRRSRRR* structure within one or two cycles in every one of five runs of 17 to 70 cycles.

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