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# Four Sesquiterpenes from the South African Nudibranch *Leminda millecra*

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***Abstract***: Extracts of the South African nudibranch *Leminda millecra* were found to contain the new sesquiterpenes millecrone A (**1**), millecrone B (**2**), millecrol A (**3**), and millecrol B (**4**). Sesquiterpenes**1**–**4** are typical of metabolites found in soft corals and investigation of the digestive gland of *L. millecra* revealed the presence of spicules from *Alcyonium foliatum*, *A. valdiviae*, and *Capnella thyrsoidea*.

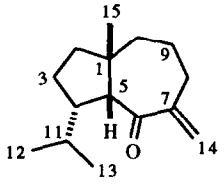
*Leminda millecra*, which is endemic to South Africa, is a translucent white nudibranch with an expanded, blue-edged mantle. Little is known about the biology or diet of this rare nudibranch which was first described in 1985.[1] Four specimens of *L. millecra* were collected at Coffee Bay, Republic of Transkei, in April, 1992. A preliminary $^1$H NMR spectrum of the acetone extract of the animals prompted us to study the chemistry of *L. millecra*. Our investigation indicated that the interesting $^1$H NMR signals were associated with the sesquiterpenes millecrone A (**1**), millecrone B (**2**), millecrol A (**3**), and millecrol B (**4**).

The acetone extract of *L. millecra* was concentrated and chromatographed on silica gel using a heptane/dichloromethane/ethyl acetate solvent gradient to yield fractions enriched in sesquiterpenes, as well as one fraction which contained peroxy-sterols, as determined by examination of the $^1$H NMR spectrum. Further silica gel chromatography of the sesquiterpene fractions resulted in the purification of millecrone A (**1**, 13.6 mg, 3.4 mg/animal), millecrone B (**2**, 3.2 mg, 0.8 mg/animal), millecrol A (**3**, 2.9 mg, 0.7 mg/animal), and millecrol B (**4**, 4.4 mg, 1.1 mg/animal).

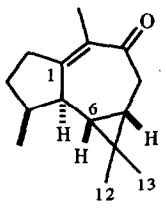
Millecrone A (**1**) was isolated as a colorless oil. The molecular formula, $C_{15}H_{24}O$, required four sites of unsaturation. A band in the infrared spectrum at 1692 cm$^{-1}$ indicated that the molecule contained an $\alpha,\beta$-unsaturated ketone. Resonances in the $^{13}$C NMR spectrum at $\delta$ 202.1 (s), 146.0 (s), and 121.6 (t) confirmed the presence of the ketone conjugated to an exocyclic double bond. The $^{13}$C NMR spectrum contained no evidence for additional unsaturated functionalities and thus we concluded that **1** was bicyclic.

The COSY spectrum of **1** contained correlations from the doublets at $\delta$ 0.72 (3 H, $J$ = 6.5 Hz, $CH_3$-12) and 0.86 (3 H, $J$ = 6.5 Hz, $CH_3$-13) to a single proton resonance at 1.35 (H-11) which was further coupled to a proton signal at 2.65 (H-4), implying the presence of an isopropyl group. A sharp singlet at $\delta$ 1.11 ($CH_3$-15) had HMBC correlations to carbon signals at $\delta$ 35.4 (t), 41.9 (t), 46.1 (s), and 62.5 (d) which permitted assignment of the fully substituted carbon at 46.1 (C-1) and the methine at 62.5 (C-5, $\delta_H$ = 2.48) to the ring junction. HMBC correlations observed from the proton resonance at $\delta$ 2.65 (H-4) to carbon signals at 62.5 (C-5) and 202.1 (C-6) resulted in the location of both the isopropyl group on ring A and the ketone on ring B

adjacent to C-5. Additional HMBC correlations from proton resonances at δ 2.65 (H-4) and 2.48 (H-5) to a carbon signal at 28.0 ($\delta_H$ = 1.76 and 1.41) located a methylene at C-3. HMBC correlations from the proton resonances at δ 1.76 and 1.41 to the carbon resonances at 46.1 (C-1) and 62.5 (C-5) could only be accommodated if ring A were five-membered. The remaining proton and carbon resonances were assigned by analysis of the spectral data (Table I).




1

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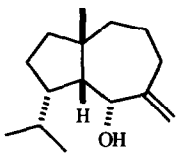


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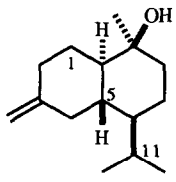


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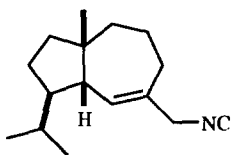


5

Irradiation of the proton resonance at δ 1.11 ($CH_3$-15) resulted in NOE enhancement of the signals at 2.65 (4%, H-4), 2.48 (4%, H-5), 1.88 (5%, H-9β), 1.47 (H-10β) and 1.35 (H-2β). Consequently, the isopropyl group was assigned α to ring A and trans to H-5 which was in turn cis to the methyl group at C-1. A molecular model of **1** predicted $J_{4,5}$ = 6.8 Hz which was in good agreement with the observed value of 6 Hz. Examination of the literature revealed that two metabolites, exemplified by **5**, with the same carbon skeleton as **1** have been isolated from the marine sponge *Acanthella acuta*.[2] The $^1H$ and $^{13}C$ NMR data for **1** agreed well with the corresponding data for **5**. Significant differences were observed between the chemical shifts for C-4 (δ 45.7 in **1**; 50.5 in **5**) and H-4 (2.65 in **1**; 1.76 in **5**) but these were consistent with the differences in substitution and stereochemistry at the C-4 center.[3]

Millecrol A (**3**) was a colorless oil of molecular formula $C_{15}H_{26}O$. The infrared spectrum contained a broad band at 3500–3300 $cm^{-1}$ diagnostic of an hydroxyl functionality but lacked bands in the carbonyl region. The $^1H$ and $^{13}C$ NMR spectra of **3** contained some signals that were similar to those observed for millecrone A (**1**). COSY correlations were observed from the exocyclic methylene proton resonances at δ 4.78 (br s, H-14) and 4.74 (br s, H-14, $\delta_C$ = 109.8) to a proton resonance at 4.28 (br s, H-6, $\delta_C$ = 78.7). The proton resonance at 4.28 had, in turn, a COSY correlation to a signal at 1.40 (H-5, $\delta_C$ = 57.1) which was assigned to the ring junction methine on the basis of an HMBC correlation from the carbon signal at 57.1 (C-5) to the methyl singlet at 0.91 ($CH_3$-15). Consequently, the hydroxyl functionality was located at C-6. Irradiation of the proton resonance at δ 1.40 (H-5) caused NOE enhancements of signals at 0.91 (4%,

$CH_3$-15), 1.88 (7%, H-3), and 4.28 (4%, H-6) indicating that **3** had the same stereochemistry as **1** and that the hydroxyl group was in the α configuration. Oxidation of **3** with manganese dioxide resulted in a 46% yield of **1**, which confirmed that the stereochemistry about C-1, C-4, and C-5 was the same in both metabolites.[3]

Table I. $^1H$ and $^{13}C$ NMR Data for millecrone A (**1**) and millecrol A (**3**)

| Carbon no. | millecrone A (**1**) | | millecrol A (**3**) | |
|---|---|---|---|---|
| | δ H (mult.) | δ C (mult.) | δ H (mult.) | δ C (mult.) |
| 1 | – | 46.1 (s) | – | 44.3 (s) |
| 2 | 1.35 (m) | 41.9 (t) | 1.33 | 42.6 (t) |
| | 1.31 (m) | | 1.32 | |
| 3 | 1.76 (m) | 28.0 (t) | – | 26.4 (t) |
| | 1.41 (m) | | | |
| 4 | 2.65 (m) | 45.7 (d) | 1.88 (m) | 50.3 (d) |
| 5 | 2.48 (d, $J$ = 6 Hz) | 62.5 (d) | 1.40 | 57.1 (d) |
| 6 | – | 202.1 (s) | 4.28 (br s) | 78.7 (d) |
| 7 | – | 146.0 (s) | – | 153.5 (s) |
| 8 | 2.71 (m) | 29.7 (t) | 2.40 (ddd) | 34.8 (t) |
| | 2.47 (m) | | 2.22 (m) | |
| 9 | 1.88 (m) | 23.8 (t) | – | 24.2(t) |
| | 1.58 (m) | | | |
| 10 | 1.47 (m) | 35.4 (t) | – | 39.8(t) |
| | 1.27 (m) | | | |
| 11 | 1.35 (m) | 34.1 (d) | 1.66 | 32.0 (d) |
| 12 | 0.72 (d, $J$ = 6.5 Hz) | 21.6 (q) | 0.86 (d, $J$ = 6.5 Hz) | 18.8 (q) |
| 13 | 0.86 (d, $J$ = 6.5 Hz) | 20.8 (q) | 0.93 (d, $J$ = 7 Hz) | 22.5 (q) |
| 14 | 5.92 (br s) | 121.6 (t) | 4.78 (br s) | 109.8 (t) |
| | 5.18 (br s) | | 4.74 (br s) | |
| 15 | 1.11 (s) | 25.3 (q) | 0.91 (s) | 29.7 (q) |

Millecrone B (**2**) was isolated as a colorless oil of molecular formula $C_{15}H_{22}O$. Although the $^1H$ NMR contained no resonances downfield of δ 2.80, a band in the infrared spectrum at 1651 $cm^{-1}$ and signals in the $^{13}C$ NMR spectrum at δ 201, 166 and 130 indicated that **2** contained a fully substituted α,β-unsaturated ketone. The $^{13}C$ NMR spectrum contained no other signals below 50 ppm, suggesting that millecrone B was tricyclic. The COSY spectrum contained a correlation from a resonance at δ 0.80 (t, 1 H, $J$ = 10 Hz, H-6, $\delta_C$ = 31.3) to the signal at 0.69 (m, 1 H, H-7). HMBC correlations were observed from the carbon resonance at δ 31.3 to two signals at 1.16 (br s, 3 H, $CH_3$-12) and 1.04 (br s, 3 H, $CH_3$-13) appropriate for a 1,1-dimethyl substituted cyclopropane ring. The proton resonance at δ 0.69 (H-7) had a COSY correlation to a pair of methylene proton resonances at 2.78 (H-8') and 2.34 (H-8). The C-8 methylene signals, in turn, showed HMBC correlations to carbon signals at δ 201 and 130 which required that C-8 be adjacent to the α,β-unsaturated ketone. HMBC correlations from a methyl resonance at δ 1.79 (br s, $CH_3$-15) to carbon signals at 201, 166, and 130 allowed the assignment of the methyl group at C-10. The COSY spectrum contained correlations from the proton resonance at δ 0.80 (H-6) to a methine signal at 2.61 (m, 1 H, H-5) which was in turn correlated to a proton resonance at 2.19 (m, 1 H, H-4). The resonance at δ 2.19 (H-4) had additional COSY correlations to the methyl signal at 1.02 (d, 3 H, $CH_3$-14, $J$ = 7 Hz) and to a pair of methylene resonances at 1.84 (m, 1 H, H-3') and 1.39 (m, 1 H, H-3). HMBC correlations from the H-3' proton resonance at δ 1.84 to carbon signals at 166 (C-1), 45.2 (C-5), and 15.4 ($CH_3$-14) established the location of methyl-14 at C-4 of a five-membered ring.

Table II. $^1H$ and $^{13}C$ NMR Data for millecrone B (**2**) and millecrol B (**4**)

| Carbon no. | Millecrone B (2) | | Millecrol B (4) | |
|---|---|---|---|---|
| | δ H (mult.) | δ C (mult.) | δ H (mult.) | δ C (mult.) |
| 1 | – | 166[a] (s) | 1.42 (m) | 19.2 (t) |
| | | | 1.24 (ddd, $J$ = 5, 13 Hz) | |
| 2 | 2.64 (m) | 34.1 (t) | 2.36 (m) | 34.6 (t) |
| | 2.32 (m) | | 1.97 (m) | |
| 3 | 1.84 (m) | 32.3 (t) | – | 149.1 (s) |
| | 1.39 (m) | | | |
| 4 | 2.19 (m) | 37.2 (d) | 2.54 (dd, $J$ = 4, 13 Hz) | 38.5 (t) |
| | | | 1.54 (dd, $J$ = 12, 13 Hz) | |
| 5 | 2.61 (m) | 45.2 (d) | 1.31 (ddd, $J$ = 4, 11 Hz) | 40.5 (d) |
| 6 | 0.80 (t, $J$ = 10 Hz) | 31.3 (d) | 1.06 (m) | 48.2 (d) |
| 7 | 0.69 (m) | 22.8 (d) | 1.41 (m) | 27.0 (t) |
| | | | 1.00 (m) | |
| 8 | 2.78 (dd, $J$ = 15, 5 Hz) | 41.9 (t) | 1.72 (m) | 40.5 (t) |
| | 2.34 (m) | | 1.41 (m) | |
| 9 | – | 201[a] (s) | – | 70.7 (s) |
| 10 | – | 130[a] (s) | 1.09 (m) | 50.1(d) |
| 11 | – | 25.6 (s) | 1.98 (m) | 26.2 (d) |
| 12 | 1.04 (br s) | 28.2 (q) | 0.73 (d, $J$ = 6.5 Hz) | 15.0 (q) |
| 13 | 1.16 (br s) | 16.0 (q) | 0.89 (d, $J$ = 7.0 Hz) | 21.5 (q) |
| 14 | 1.02 (d, $J$ = 7 Hz) | 15.4 (q) | 4.58 (br s) | 106.8 (t) |
| 15 | 1.79 (br s) | 15.0 (q) | 1.17 (s) | 28.7 (q) |

[a] δ C assigned from the HMBC spectrum

Irradiation of the proton resonance at δ 2.19 (H-4) caused NOE enhancement of the signal at 2.61 (7%, H-5) and resulted in assignment of the β configuration to the methyl group. A molecular model indicated that $J_{5,6}$ = 10 Hz was appropriate for a trans relationship between these protons while $J_{6,7}$ = 10 Hz indicated that H-6 and H-7 were located cis on the cyclopropane ring.[3] Analysis of the spectral data was consistent with the proposed structure for **2**. The assigned $^1H$ and $^{13}C$ NMR chemical shifts for **2** were in good agreement with two compounds previously reported in the literature which possess the same carbon skeleton.[2]

Millecrol B (**4**) was isolated as an oil of molecular formula $C_{15}H_{26}O$. The methyl signals at δ 0.73 (d, 3 H, $J$ = 6.5 Hz, $CH_3$-12) and 0.89 (d, 3 H, $J$ = 7.0 Hz, $CH_3$-13), coupled to a methine proton at 1.98 (H-11), implied that an isopropyl group was present. A methyl resonance at δ 1.17 (s, $CH_3$-15) with HMBC correlations to carbon signals at 40.5 (t, C-8), 50.1 (d, C-10), and 70.7 (s, C-9) suggested that both a single hydroxyl functionality and $CH_3$-15 were located on a quaternary carbon (C-9), which was in turn adjacent to a ring junction methine (C-10). The only downfield carbon signals, at δ 106.8 (t, C-14) and 149.1 (s, C-3), were assigned to an exocyclic methylene. The cadinane carbon skeleton accommodated all of the structural features of **4** and the spectral data were in good agreement with a number of related compounds which had been reported previously.[4]

A difference decoupling $^1H$ NMR experiment involving irradiation at δ 2.54 (H-4$_\beta$) permitted us to determine that $J_{4\alpha,5}$ = 11.5 Hz and so H-4$_\alpha$ and H-5 are trans diaxial. The same experiment indicated that $J_{5,10} = J_{5,6}$ = 11 Hz and therefore the ring junction was trans and the isopropyl moiety was in the β configuration. When the $^1H$ NMR spectrum of **4** was taken in pyridine-$d_5$, the H-5 resonance was shifted downfield to δ 1.70 (1.31 in $CDCl_3$) while the H-10 resonance did not shift significantly (1.04 in pyridine-$d_5$; 1.09 in $CDCl_3$). This result was consistent with the trans stereochemistry assigned to the ring junction and

indicated that the hydroxyl functionality had the β configuration.[5] A NOESY correlation from the resonance at δ 2.54 (H-4β) to the methine signal at 1.98 (H-11) was consistent with the β configuration assigned to the isopropyl group.[3]

Although the nudibranchs were collected from sponges, a literature search indicated that *L. millecra* belongs to the family Arminidae which are known to feed on soft corals.[1b] In order to better understand the origin of the compounds isolated from *L. millecra*, we dissected the digestive glands of two animals and dissolved the organic material with bleach. The digestive glands were found to contain spicules from the soft corals *Alcyonium foliatum*, *A. valdiviae*, and *Capnella thyrsoidea*. Although an earlier investigation of the chemistry of *A. valdiviae* yielded only diterpenoid compounds,[6] sesquiterpenes have been reported from other *Alcyonium*[7] and *Capnella* species.[8]

Millecrone A (**1**) inhibited the growth of *Candida albicans* at 50 μg/disk. Millecrone B (**3**) was inactive against *C. albicans* but inhibited the growth of both *Staphylococcus aureus* and *Bacillus subtilis* at 50 μg/disk while millecrol B (**4**) was active against *B. subtilis* at 50 μg/disk.

## EXPERIMENTAL SECTION

Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrophotometer. A Perkin-Elmer Lambda 3B UV/vis spectrometer was used to measure UV spectra. NMR experiments were recorded on Brüker WP-200 SY and Varian 500 MHz spectrometers. Unless otherwise specified, all NMR data were acquired on samples in $CDCl_3$ and chemical shifts were reported relative to the residual solvent peaks ($CDCl_3$ $\delta_H$ 7.24, $\delta_C$ 77.0; pyridine-$d_5$ $\delta_H$ 7.19, 7.55, 8.71). Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a 10 cm cell. Low resolution mass spectra were recorded on a Hewlett-Packard 5988A spectrometer while high resolution spectra were obtained from the regional facility at UC Riverside. Activated manganese dioxide was purchased from Aldrich immediately prior to use.

**Collection and Extraction of *Leminda millecra*:** Four specimens of the nudibranch *Leminda millecra* (Griffiths, 1985) were collected from the surface of an *Ircinia* sp. sponge at Coffee Bay, Republic of Transkei, in April 1992. The animals were stored in acetone for 12 months. The extract was decanted and the animals were re-extracted with acetone. The extract was concentrated and the residue (800 mg) was purified by short column flash chromatography using normal phase TLC silica gel (5–40 μ mesh) eluted by a solvent gradient of heptane to methylene chloride to ethyl acetate to methanol. The four major metabolites were purified by short column chromatography. The acetone extract yielded the millecrones A (**1**, 13.6 mg, 0.9% dry weight, 4.6 mg/animal) and B (**2**, 3.2 mg, 0.2% dry weight, 0.8 mg/animal) and millecrols A (**3**, 2.9 mg, 0.2% dry weight, 0.7 mg/animal) and B (**4**, 4.4 mg, 0.3% dry weight, 1.1 mg/animal) as well as three minor metabolites present in concentrations too small to permit structure elucidation.

**Millecrone A (1):** oil; UV (heptane) 223 nm (ε 3 500); IR (film) 1692, 1616 $cm^{-1}$; $[\alpha]_D$ +39.5° (*c* 1.36, heptane); $^1H$ NMR (500 MHz, $CDCl_3$) see Table I; $^{13}C$ NMR (50 MHz, $CDCl_3$) see Table I; HREIMS obsd. *m/z* 220.1825, $C_{15}H_{24}O$, $M^+$ requires 220.1827.

**Millecrone B (2):** oil; UV (heptane) 246 nm (ε 5 600); IR (film) 1651 $cm^{-1}$; $[\alpha]_D$ +151.0° (*c* 0.32, heptane); $^1H$ NMR (500 MHz, $CDCl_3$) see Table II; $^{13}C$ NMR (50 MHz, $CDCl_3$) see Table II; HREIMS obsd. *m/z* 218.1670, $C_{15}H_{22}O$, $M^+$ requires 218.1671.

**Millecrol A (3):** oil; IR (film) 3600–3300 (br) $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) see Table I; $^{13}C$ NMR (50 MHz, $CDCl_3$) see Table I; HREIMS obsd. *m/z* 222.1995, $C_{15}H_{26}O$, $M^+$ requires 222.1984.

**Millecrol B (4):** oil; IR (film) 3500–3300 (br) $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) see Table II; $^{13}C$ NMR (50 MHz, $CDCl_3$) see Table II; HREIMS obsd. *m/z* 222.1983, $C_{15}H_{26}O$, $M^+$ requires 222.1984.

**Oxidation of millecrol A (3) to millecrone A (1):** Millecrol A (**3**, 2.4 mg) was dissolved in freshly distilled dichloromethane and activated manganese dioxide (~10 mg) was added to the solution. The reaction was stirred for twenty hours, filtered and concentrated. The product mixture was purified by short column silica chromatography to yield millecrone A (**1**, 1.1 mg, 46% yield).

## ACKNOWLEDGMENTS

The nudibranchs were collected by Dr. Colin Buxton and the staff of the Department of Ichthyology, Rhodes University and were identified by Mary Kay Harper. We would like to acknowledge the contributions of Dr. Mike Davies-Coleman, his colleagues at Rhodes University, and the members of the Coffee Bay community who facilitated our collection of the specimens. Mary Kay Harper suggested and helped perform the dissection of the nudibranchs. The soft coral spicules were identified by Dr. G. Williams, California Academy of Sciences. This research was supported by grants from the National Institutes of Health (CA 49084) and the National Science Foundation (CHE 92–04647).

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