A Cycloartane Incorporating a Fused Tetrahydrofuran Ring and a Cytotoxic Lactam from *Monocarpia marginalis*

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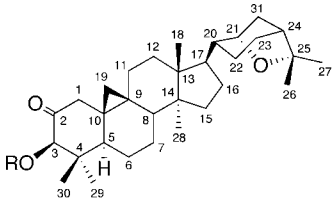
A new cycloartane, monocarpinine (**1**), incorporating a fused tetrahydrofuranyl ring, and a cytotoxic tetracyclic lactam, monomarginine (**2**), were isolated from a stem bark extract of the Malayan species *Monocarpia marginalis*. The structures of these compounds were determined using NMR and MS analysis. Monomarginine (**2**) showed appreciable cytotoxicity toward human KB (both drug-sensitive and drug-resistant) and Jurkat cells.

The genus *Monocarpia* (Annonaceae)[1] is represented by only one species, namely, *M. marginalis*,[2,3] which in Malaysia is found mainly in primary rainforest, usually on hillsides and undulating country up to 2500 feet.[3] There has been one previous study of the plant, which yielded a new cycloartane triterpene and a nitrogenous pigment from the stem bark extract.[4,5] As part of our search for new biologically active natural products,[6,7] we carried out preliminary screening of a bark sample of this species collected from a different location from the earlier study, which showed cytotoxic activity against KB cells. We now report the results of a detailed chemical study, which has resulted in the isolation of new constituents, including a new cycloartane triterpene (**1**), characterized by the presence of an additional tetrahydrofuran ring, as well as a new cytotoxic tetracyclic lactam (**2**).



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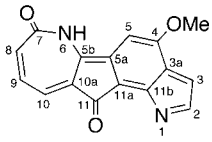
5 R = Ac



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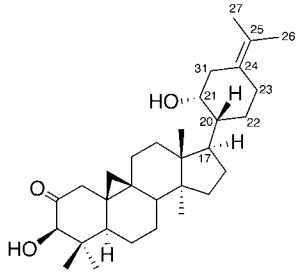

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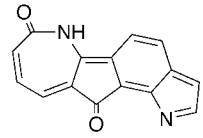

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Repeated fractionation of the hexane as well as the chloroform extracts of the ground stem bark material eventually yielded four constituents, viz., monocarpinine (**1**) and monomarginine (**2**), in addition to the previously known monocarpine (**3**) and monomargine (**4**). Monocarpinine (**1**) was obtained as an amorphous, white solid, mp 182−183 °C, $[\alpha]_D$ +2 (*c* 1.61, $CHCl_3$). The IR spectrum showed bands due to OH (3477 cm^{-1}) and ketone (1714 cm^{-1}) functions. The EIMS of **1** showed a molecular ion at *m/z* 468, which analyzed for $C_{31}H_{48}O_3$. Fragments due to loss of H_2O (*m/z* 450) as well as the side chain (*m/z* 329) were observed in the mass spectrum, in common with that in the case of **3**.[4] The ^{13}C NMR spectrum (Table 1) showed a total of 31 resonances, comprising six methyls, 11 methylenes, seven methines, and seven quaternary carbon atoms, in agreement with the molecular formula. The observed quaternary carbon resonance at δ 211.0 was consistent with the presence of a ketone, while the resonances at δ 76.8, 83.1, and 82.0 were due to two oxymethines and an oxygenated quaternary carbon atom, respectively. The ^1H NMR spectrum (Table 1) showed the presence of six tertiary methyls at δ 0.68, 0.93, 0.95, 1.12, 1.18, and 1.37, a pair of upfield doublets at δ 0.46 and 0.61, another pair of doublets at δ 2.06 and 2.65, a singlet due to an oxymethine at δ 3.89, and another downfield doublet due to another oxymethine at δ 4.31. The spectrum was somewhat similar to that of the cycloartanol triterpene monocarpine (**3**),[4] with the characteristically high-field doublets at δ 0.46 and 0.61, due to the nonequivalent hydrogens (H-19α and H-19β) of the cyclopropyl methylene group.[8] The affinity with **3** was further reinforced by the presence of the characteristically deshielded H-3, due to proximity to the neighboring carbonyl group at C-2 and the presence of β-OH directly attached to C-3, as well as the pair of deshielded doublets at δ 2.06 and 2.65, due to H-1β and H-1α, respectively. The stereochemical distinction of the H-1 signals was based on NOEs (NOEs observed for H-1α/H-3, H-3/H-5). There were, however, several notable differences in the NMR data of **1** when compared with that of **3**. First, two tertiary methyl signals at δ 1.69 and 1.71 in **3**, which were assigned to the two methyls (Me-27, Me-26) attached to the double bond, were shifted upfield to δ 1.18 and 1.37, respectively, in **1**, while the shifts of the other four methyls were essentially unchanged. Furthermore, in compound **3**, the H-21 signal was a broad singlet at δ 4.07, whereas in compound **1**, H-21 was seen as a doublet at δ 4.31. In addition, the signals of the methylene H-31 in **1** were shifted to δ 1.38 and 2.33, compared to those of **3** at δ 2.84 and 1.94, respectively. These observations indicated that a major change has occurred involving the side chain portion of the triterpene molecule in **1**. This conclusion was further supported by the notable absence of the side chain double bond in **1** compared with **3**, as shown by the NMR data. The ^{13}C NMR spectrum of the two compounds were in fact essentially similar except for the absence of the olefinic carbons at δ 126.8 and 125.9 due to C-24 and C-25 of **3**, which in the spectrum of **1** have been replaced by signals at δ 42.2 and 82.0, respectively. Acetylation of monocarpinine (**1**) gave the monoacetate derivative **5**, with the characteristic methyl signal of the acetyl group seen at δ 2.19. The corresponding carbon signals due to the acetyl function were observed at δ 170.5

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Table 1. ^{1}H and ^{13}C NMR Data (δ) for **1** and **5** (400 MHz, $CDCl_3$)[a]

	1		**5**	
position	^{13}C	^{1}H	^{13}C	^{1}H
1β	46.6	2.06 br d (13)	47.7	2.00 br d (13)
1α		2.65 br d (13)		2.68 br d (13)
2	211.0		204.1	
3	83.1	3.89 br s	84.4	4.97 br s
4	45.8		45.2	
5	46.4	1.90 m	47.3	1.90 m
6	21.1	0.84 m	20.7	0.85 m
		1.70 m		1.70 m
7	25.4	1.05 m	25.4	1.05 m
		1.58 m		1.58 m
8	48.6	1.92 m	48.7	1.90 m
9	19.5		19.6	
10	28.0		27.7	
11	26.5	1.06 m	25.7	1.08 m
		1.90 m		1.90 m
12	32.7	1.68 m	32.8	1.70 m
		1.98 m		1.80 m
13	45.1		45.1	
14	48.5		48.5	
15	35.3	1.22 m	35.3	1.25 m
		1.30 m		1.30 m
16	26.6	1.30 m	26.6	1.30 m
		1.90 m		1.90 m
17	47.6	1.56 m	48.6	1.55 m
18	19.6	0.95 s	19.8	0.95 s
19α	28.9	0.46 d (4.8)	28.8	0.50 d (4.8)
19β		0.61 d (4.8)		0.62 d (4.8)
20	45.1	1.46 m	43.9	1.45 m
21	76.8	4.31 d (7)	76.8	4.31 d (7)
22	27.3	1.20 m	27.4	1.20 m
		1.78 m		1.78 m
23	25.6	1.44 m	25.6	1.45 m
		1.58 m		1.55 m
24	42.2	1.80 m	42.2	1.80 m
25	82.0		82.0	
26	30.3	1.18 s	30.4	1.18 s
27	22.3	1.37 s	21.0	1.37 s
28	19.2	0.93 s	19.2	0.92 s
29	25.3	1.12 s	22.3	1.04 s
30	15.0	0.68 s	16.2	0.83 s
31β	38.1	1.38 m	38.2	1.38 m
31α		2.33 dddd (11, 7, 4.5, 2.5)		2.33 dddd (11, 7, 4.5, 2.5)
O*C*OCH$_3$			170.5	
OCO*C*H$_3$			77.2	2.19 s

[a] Assignments based on COSY, HMQC, HMBC, and NOE data.

and 77.2 in the ^{13}C NMR spectrum. This confirmed the occurrence of only one hydroxyl group, i.e., that at C-3, compared with monocarpine (**3**), with two OH groups, the other OH attached to C-21 in the side chain ring.

Analysis of the COSY and HMQC data confirmed the presence of common fragments constituting the tetracyclic core of the triterpene skeleton, indicating that the difference between the two compounds resides in the side chain portion. The COSY spectrum of **1** did not show any coupling between H-20 and H-21, unlike that of monocarpine (**3**). In the ^{1}H NMR spectrum of **1**, H-21 was seen as a doublet with $J = 7$ Hz. The COSY spectrum, as well as the observation of H-31α at δ 2.33 as a dddd with $J = 11$, 7, 4.5, and 2.5 Hz, indicated that the 7 Hz coupling was between H-21 and H-31α (the assignment of the signal at δ 2.33 to H-31α is supported by the long-range $J_{31\alpha-23}$ (*W*) coupling of 2.5 Hz). This, in turn, suggested that the dihedral angle between H-21 and H-20, as well as between H-21 and H-31β, is 90°, implying that H-20 and H-31β are both axial, and H-21 equatorial, in a six-membered side chain ring.

There are three oxygenated carbons in **1**, and the oxymethine at δ 83.1 was assigned to the hydroxyl bearing C-3 from the HMQC spectrum. Since only one oxygen atom remains, as indicated by

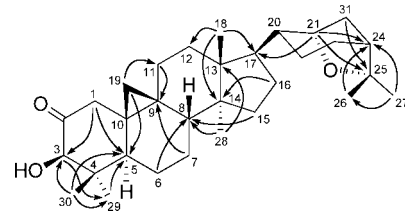

Figure 1. Selected HMBCs of **1**.

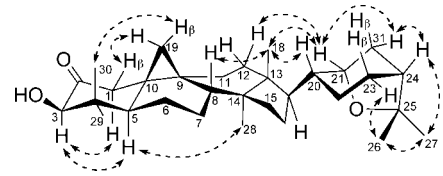

Figure 2. Seleceted NOEs of **1**.

the molecular formula, and acetylation has confirmed the presence of only one alcohol function, the remaining oxygen must be part of an ether linkage bridging the two oxygenated carbons at δ 76.8 (C-21) and 82.0 (C-25), constituting part of an oxabicyclo[3.2.1]octane fragment. This conclusion is also consistent with formation of another ring, as required by the molecular formula.

The spectroscopic data at this point allowed the proposed structure as shown in **1** to be assembled. Confirmation of the structure was provided by the HMBC spectrum (Figure 1), which showed the following three-bond correlations: from H-21, H-31 to C-25; H-21 to C-17; H-26, H-27 to C-24; and H-27 to C-26. These observations provided firm support for the proposed structure of monocarpinine as shown in **1**. Monocarpinine therefore represents a new cycloartane triterpene with a novel side chain fragment incorporating fused cyclohexyl and tetrahydrofuranyl rings.

Finally, the structure is compatible with the NOE data (Figure 2), which were in agreement with the overall relative configuration shown. The NOESY spectrum showed NOEs between H-18 and H-20, H-21 and between H-21 and H-12, which allowed the assignment of the relative configurations of C-17 and C-20 as *R* and *R*, respectively. The relative configuration of C-21 was deduced to be *R*, from the presence of an equatorially oriented H-21 as described earlier, as well as on the assumption that **1** is biogenetically related to **3**. The observed H-18/H-20, H-21 NOEs also implied a preferred conformation about the C-17/C-20 bond, in which the C-18/C-13 bond bisects the C-20/C-21 bond, or where H-20 and H-17 are *trans*-diaxial. Examination of models confirmed that this arrangement is the one with the least steric congestion.

Monomarginine (**2**) was obtained as a red, amorphous solid. The IR spectrum showed bands due to secondary amide (3434 cm^{-1}) and conjugated ketone and lactam carbonyl functions (1639 and 1606 cm^{-1}, respectively), while the UV spectrum showed absorption maxima at 231 and 478 nm, which is somewhat similar to that of monomargine (**4**).[5] The EIMS of monomarginine (**2**) showed a molecular ion at *m*/*z* 278, which analyzed for $C_{16}H_{10}N_2O_3$. The molecular formula of **2** indicates 13 degrees of unsaturation, which was the same as that of monomargine (**4**). The ^{13}C NMR showed a total of 16 resonances, comprising one methoxy, six sp^2 methines, and nine quaternary carbon atoms, in agreement with the molecular formula. The observed quaternary carbon resonances at δ 186.9 and 164.3 are consistent with the presence of conjugated ketone and lactam functionalities, respectively. The ^{1}H NMR spectrum showed the presence of eight signals, seven of which were found in the low-field region. The spectrum bore some similarities to that of monomargine (**4**), with the characteristically sharp singlet at δ 12.88 assigned to an amide NH. The ^{1}H NMR spectrum showed two vicinally coupled AX doublets at δ 9.09 and 8.17, with $J =$ 5.6 Hz. The signals due to three hydrogens with coupling behavior

corresponding to an AMX spin system were seen at δ 8.10 (dd, J = 8, 1 Hz), 7.64 (t, J = 8 Hz), and 7.10 (dd, J = 8, 1 Hz). All these features are strongly reminiscent of monomargine (**4**), except for a missing pair of coupled doublets due to H-4 and H-5, at δ 7.60 and 8.85, respectively. In its place, a 1-H aromatic singlet and an additional 3-H singlet due to a methoxy group were observed at δ 8.31 and 4.17, respectively. The AX doublets at δ 9.09 and 8.17 could therefore be assigned to H-2 and H-3, respectively, while the AMX signals at δ 8.10, 7.64, and 7.10 are due to H-8, H-9, and H-10, respectively. The isolated aromatic singlet at δ 8.31 is due to H-4 or H-5. Likewise, comparison with the ^{13}C NMR spectrum of monomargine (**4**) showed a close correspondence of the NMR data except for the attachment of a methoxy group at either C-4 or C-5.[5]

The structure is consistent with the 2D COSY and HMQC data, which confirmed the presence of NCHCH and (C=O)CHCHCH fragments. The HMBC data permitted assignment of the site of methoxy substitution at C-4 (three-bond correlations from H-5 to C-3a, C-5b, C-11a and from OMe to C-4 (δ 150.0)). Additional confirmation of C-4 methoxy substitution was provided by the observed reciprocal NOEs observed between NH and H-5, as well as between H-5 and C(4)-OMe. Monomarginine (**2**) is therefore the 4-methoxy derivative of monomargine (**4**).

The present results show that the same plant collected from different locations possesses different composition of the secondary metabolites. Whereas the previous sample from Johore gave only **3** and **4**,[4,5] the present sample from Kedah gave two additional new constituents, i.e., **1** and **2**, in addition to **3** and **4**, with the latter two compounds obtained as the minor constituents.

While the two terpenoid compounds **1** and **3** were ineffective toward KB cells (IC_{50} > 5 μg/mL), the lactams, monomarginine (**2**) and monomargine (**4**), showed appreciable cytotoxicity toward both drug-sensitive and vincristine-resistant KB (VJ300) cells, as well as Jurkat cells (IC_{50} 4.0, 2.6, and 0.4 μg/mL, respectively, in the case of **2**; IC_{50} 4.7, 3.0, and 0.7 μg/mL, respectively, in the case of **4**).[9]

Experimental Section

General Experimental Procedures. All melting points were uncorrected. Optical rotations were determined on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz, respectively. ESIMS were obtained on a Perkin- Elmer API 100 instrument. Mass spectrometric measurements were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia (Kratos Concept ISQ mass spectrometer). HPLC was carried out using a Waters 600 multisolvent delivery system equipped with a Waters 486 UV detector.

Plant Material. Plant material was collected near Nami in Kedah, Peninsular Malaysia, in May 1995, and voucher specimens (K588) are deposited at the Herbarium, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation. Ground bark material of *M. marginalis* was extracted sequentially with hexanes followed by $CHCl_3$ to give ca. 7.6 and 23 g of crude extract, respectively. The crude mixtures were then chromatographed over silica gel ($CHCl_3$ with increasing MeOH gradient) to furnish semipure fractions, which were then further fractionated by successive centrifugal TLC to give the pure compounds **1**−**4**. Solvent systems used for centrifugal TLC were $CHCl_3$/MeOH, Et_2O/hexanes (1:4), Et_2O/hexanes (1:5), and Et_2O/hexanes (2:3). Final purification of **4** required HPLC (Waters radial compression module with a preparative Nova-Pak HR C_{18} column segment, 6 μm, 25 × 100 mm). The column was eluted with MeCN/H_2O (gradient: MeCN from 40% to 100% over 30 min) at a flow rate of 10 mL/min to afford pure **4**. The yields (g kg^{-1}) of the compounds isolated were as follows: **1** (0.040), **2** (0.023), **3** (0.011), and **4** (0.002).

Monocarpinine (1): white, amorphous solid; mp 182−183 °C; $[\alpha]_D$ +2 (*c* 1.61, $CHCl_3$); IR (dry film) ν_{max} 3477, 1714 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 468 $[M]^+$ (19), 450 (100), 435 (20), 407 (59), 368 (20), 329 (54), 281 (23), 215 (92), 147 (81), 121 (98), 95 (96), 81 (69), 69 (62), 43 (67); HREIMS *m*/*z* 468.3595 (calcd for $C_{31}H_{48}O_3$, 468.3603).

Monomarginine (2): red, amorphous solid; mp 252−254 °C; UV (EtOH) λ_{max} (log ϵ) 204 (4.61), 231 (4.60), 256 (4.44), 309 (3.85), 478 (4.02) nm; IR (dry film) ν_{max} 3434, 1639, 1606 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.88 (1H, s, NH), 9.09 (1H, d, J = 5.6 Hz, H-2), 8.31 (1H, s, H-5), 8.17 (1H, d, J = 5.6 Hz, H-3), 8.10 (1H, dd, J = 8, 1 Hz, H-8), 7.64 (1H, t, J = 8.0 Hz, H-9), 7.10 (1H, dd, J = 8, 1 Hz, H-10), 4.17 (1H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 186.9 (C-11), 164.3 (C-7), 150.0 (C-4), 148.1 (C-2), 147.2 (C-11b), 142.6 (C-5b), 137.8 (C-9), 135.7 (C-10a), 131.5 (C-3a), 127.1 (C-5), 119.8 (C-10), 119.6 (C-11a), 119.2 (C-3), 116.2 (C-8), 116.1 (C-5a), 56.7 (OCH_3); EIMS *m*/*z* 278 $[M]^+$ (65), 263 (6), 250 (18), 235 (100), 219 (6), 207 (63), 191 (5), 179 (18), 164 (7), 152 (27), 125 (20), 111 (7), 99 (10), 84 (13), 71 (16), 57 (20), 40 (63); HREIMS *m*/*z* 278.0690 (calcd for $C_{16}H_{10}N_2O_3$, 278.0691).

Monocarpine (3): white, amorphous solid; mp 198−200 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.07 (1H, br s, H-21), 3.90 (1H, s, H-3), 2.84 (1H, dt, J = 14, 3.5 Hz, H-31β), 2.69 (1H, m, H-23), 2.65 (1H, br d, J = 14 Hz, H-1α), 2.08 (1H, br d, J = 14 Hz, H-1β), 1.98 (1H, m, H-17), 1.94 (1H, m, H-5), 1.94 (1H, m, H-31α), 1.90 (1H, m, H-11), 1.90 (1H, m, H-16), 1.85 (1H, m, H-12), 1.71 (3H, s, H-26), 1.70 (1H, m, H-6), 1.70 (1H, m, H-12), 1.69 (3H, s, H-27), 1.66 (1H, m, H-23), 1.62 (1H, m, H-22), 1.60 (1H, m, H-8), 1.49 (1H, m, H-20), 1.40 (1H, m, H-7), 1.37 (1H, m, H-15), 1.32 (1H, m, H-15), 1.32 (1H, m, H-16), 1.30 (1H, m, H-22), 1.23 (1H, m, H-7), 1.13 (3H, s, H-29), 1.10 (1H, m, H-11), 0.99 (3H, s, H-18), 0.95 (3H, s, H-28), 0.86 (1H, m, H-6), 0.69 (3H, s, H-30), 0.64 (1H, d, J = 4.8 Hz, H-19β), 0.47 (1H, d, J = 4.8 Hz, H-19α).

Acetylation of Monocarpinine (1). A mixture of monocarpinine (**1**) (17.2 mg, 0.037 mmol), acetic anhydride/pyridine (1:1, 2 mL), and DMAP (3.6 mg, 0.029 mmol) was stirred under N_2 at room temperature for 2 h. The mixture was then poured into saturated Na_2CO_3 solution (5 mL) and extracted with CH_2Cl_2. Removal of the solvent, followed by the purification by centrifugal chromatography with ether/hexanes (1:2) as eluent, afforded 11.2 mg (65%) of the monoacetate derivative **5**, as a white, amorphous solid; $[\alpha]_D$ +23 (*c* 0.1, $CHCl_3$); IR (dry film) ν_{max} 1750, 1728 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 511 $[C_{33}H_{50}O_4 + H]$.

Cytotoxicity Assays. The cytotoxicity assay was carried out following a procedure that has been described in detail previously.[10,11]

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