New Cytotoxic Laurene-, Cuparene-, and Laurokamurene-Type Sesquiterpenes from the Red Alga *Laurencia obtusa*

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Three new sesquiterpene alcohols, laur-2-ene-3,12-diol (**1**), cuparene-3,12-diol (**2**), and 8,11-dihydro-1-methoxylaurokamuren-12-ol (**3**), along with one known diterpene, kahukuen-10-ol (**4**) have been isolated from the organic extract of the red alga *Laurencia obtusa*. The chemical structures were elucidated on the basis of spectroscopic analysis. The cytotoxicity of the isolated compounds were evaluated against three cancer cell lines, *i.e.*, KB, HepG2, and MCF-7. Compound **4** exhibited a wide range of cytotoxic activity against KB, HepG2, and MCF-7 cell lines with IC_{50} of 0.100, 0.057, and 0.054 μM, respectively. In addition, **1** showed moderate activities towards KB and MCF-7 cell lines with IC_{50} values of 0.171 and 0.184 μM, respectively and **2** exhibited a moderate activity against KB cell line at a concentration of 0.213 μg/ml. On the other hand, compound **3** exhibited no cytotoxic activity against any of the three cell lines.

Introduction. – Marine algae growing along the Saudi Red Sea coast constitute our favorite source to search for new bioactive natural products, specifically the genus *Laurencia* (Rhodomelaceae, Ceramiales). Species of the genus *Laurencia* are known to produce several secondary metabolites with noticeable biological properties, such as antimicrobial, antitumor, insecticidal, and cytotoxic activities [1–4].

In addition to halogenated and non-halogenated sesquiterpenes and acetogenins, diterpenes are wonderful examples of the uniqueness of the genus *Laurencia* to provide several diterpenoid skeletons with open, and mono-, bi-, tri-, and even tetracyclic structures [5–10], which are mainly associated with halogen substituent(s).

As a part of our systematic endeavors to isolate bioactive compounds from the Saudi Red Sea organisms, we investigated constituents of *L. obtusa* collected off the coast of Jeddah, Saudi Arabia. These efforts resulted in the isolation and characterization of three new sesquiterpenes **1**–**3**, along with a known diterpenoid **4**. Among these natural products, **3** possesses a rearranged laurane-type C-atom skeleton, which is rare in nature. Moreover, metabolite **3** represents the second example of a sesquiterpene with a cyclohexadiene ring instead of an aromatic one [1].

Results and Discussion. – The $CHCl_3$-soluble fraction from the crude extract of dried and powdered algal material was fractionated and purified by conventional chromatographic techniques, employing aluminium oxide and *Sephadex LH-20* columns, and preparative TLC to furnish alcohols **1**–**3**, together with the known diterpene **4** (*Fig. 1*).

Compound **1** was obtained as colorless solid. The molecular formula, $C_{15}H_{20}O_2$ (corresponding to six degrees of unsaturation), was deduced from a combination of its ^{13}C-NMR data (*Table 1*) and HR-ESI-MS (*m/z* 232.1453). The EI-MS of **1** showed a molecular ion peak at *m/z* 232, and two peaks at *m/z* 214 and 217, corresponding to the fragment ions $C_{15}H_{18}O^+$ and $C_{14}H_{17}O_2^+$, arising from the elimination of H_2O or of a Me group, respectively. Moreover, the peaks at *m/z* 107, 91, and 77 evidenced the presence of an aromatic subunit. The IR spectrum displayed two absorption bands at 3300 and 3180 cm^{-1}, which in combination with ^{13}C-NMR signals at δ(C) 79.3 and 65.1 established the presence of two OH functions (*Table 1*).

The presence of a substituted benzene ring was deduced from the UV absorption spectrum, with maxima (λ_{max}) at 212, 273, and 279 nm, supported by IR absorption at 1510 cm^{-1}. Moreover, the IR band at 1633 cm^{-1} was attributed to an isolated C=C bond.

The ^{1}H-, ^{13}C-, HSQC, and DEPT-NMR spectra of **1** indicated the presence of 15 C-atoms, including two Me groups (δ(H)/δ(C) 1.41/31.5 and 1.54/30.5), four CH_2 groups including one O-bearing (δ(H)/δ(C) 4.69/65.1), an exocyclic CH= group (δ(H)/δ(C) 5.05 and 5.52/108.9), four sp^2 CH groups (δ(C) 126.5 and 127.0) and five quaternary C-atoms (δ(C) 50.6, 79.3, 138.4, 147.9, and 166.1). In addition, the ^{1}H-NMR spectrum showed a characteristic A_2B_2 spin coupling system due to a 1,4-disubstituted benzene ring (δ(H) 7.37 and 7.31 (*d*, $J = 8.4$, each 2 H)). From the previous evidences, the structure should be composed of a 1,4-disubstituted benzene ring, an exocyclic C=C bond, primary and quaternary OH groups, and one more ring (to fulfill six degrees of unsaturation). The ^{1}H,^{1}H-COSY spectrum supported the presence of an 1,4-disubstituted benzene ring through the ^{1}H,^{1}H-spin system observed between the H-atoms of H–C(7) and H–C(8), H–C(10) and H–C(11) in addition to the long-range correlation

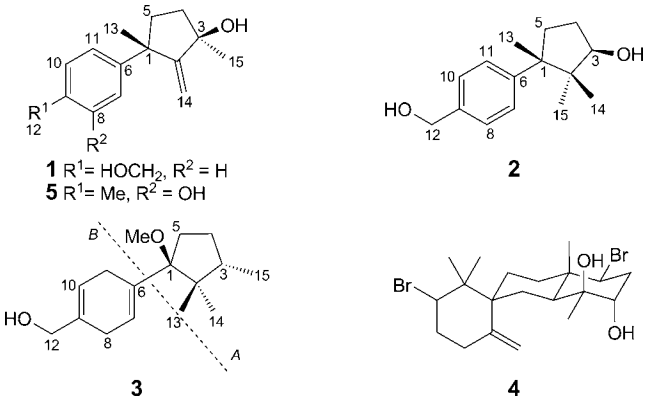


Fig. 1. *Compounds* **1**–**5** *isolated from* L. obtusa

Table 1. *^{1}H- and ^{13}C-NMR* (at 600 and 150 MHz, resp., in $CDCl_3$) *Spectral Data of* **1**–**3**. Atom numbering as indicated in *Fig. 1.*

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	–	50.6	–	47.5	–	87.0
2		166.1	–	48.8	–	47.8
3	–	79.3	4.08 (*dd*, *J* = 10.2, 9.0)	61.9	2.16 (*ddq*, *J* = 9.0, 6.6, 1.8)	41.0
4	2.11 (*ddd*, *J* = 12.6, 6.6, 6.0, H_a), 2.03 (*ddd*, *J* = 13.2, 9.0, 1.8, H_b)	39.1	2.56 (*dddd*, *J* = 15.0, 9.6, 9.0, 5.4, H_a), 2.26 (*dddd*, *J* = 15.0, 10.2, 10.2, 4.8, H_b)	33.7	1.96 (*dddd*, *J* = 13.2, 9.6, 5.4, 2.0, H_a), 1.25 (*dddd*, *J* = 13.2, 12.0, 9.0, 3.6, H_b)	28.2
5	1.86 (*ddd*, *J* = 12.6, 6.6, 6.0, H_a), 1.65 (*ddd*, *J* = 13.2, 6.6, 1.8, H_b)	39.3	2.33 (*ddd*, 13.2, 9.6, 4.8, H_a), 2.01 (*ddd*, 13.2, 10.2, 5.4, H_b)	36.6	2.31 (*ddd*, *J* = 13.8, 12.0, 5.4, H_a), 1.96 (*ddd*, *J* = 13.8, 9.6, 3.6, H_b)	34.9
6	–	147.9	–	146.2	–	137.7
7	7.37 (*d*, *J* = 8.4)	127.0	7.30 (*d*, *J* = 8.4)	126.2	5.72 (br. *s*)	120.7
8	7.31 (*d*, *J* = 8.4)	126.5	7.24 (*d*, *J* = 8.4)	127.7	2.78–2.81 (*m*)	27.5
9	–	138.4	–	138.6	–	134.1
10	7.31 (*d*, *J* = 8.4)	126.5	7.24 (*d*, *J* = 8.4)	127.7	5.86 (br. *s*)	120.8
11	7.37 (*d*, *J* = 8.4)	127.0	7.30 (*d*, *J* = 8.4)	126.2	2.85–2.88 (*m*)	28.1
12	4.69 (*s*)	65.1	4.69 (*s*)	64.9	4.06 (*s*)	66.8
13	1.54 (*s*)	30.5	1.47 (br. *s*)	25.1	0.60 (*s*)	19.1
14	5.52, 5.05 (*s*)	108.9	1.13 (*s*)	21.0	0.95 (*s*)	19.8
15	1.41 (br. *s*)	31.5	0.65 (br. *s*)	22.6	0.87 (*d*, *J* = 6.6)	14.6
MeO	–		–		3.49 (*s*)	50.9

[a]) All assignments are based on 1D and 2D recordings (HMBC, HSQC, COSY).

between H–C(8) and CH_2(12). This allowed us to deduce the presence of 1,4-disubstituted benzene ring together with a 1,2,3-trimethylcyclopentenyl partial structure (*Fig. 1*). Furthermore, the ^{1}H,^{1}H-COSY spectrum established an aliphatic H-atom sequence. The coupling between the CH_2 H-atoms resonating at δ(H) 2.03 and 2.11 with other CH_2 H-atoms at 1.86 and 1.65 evidenced the connectivity H–C(2)/H–C(3).

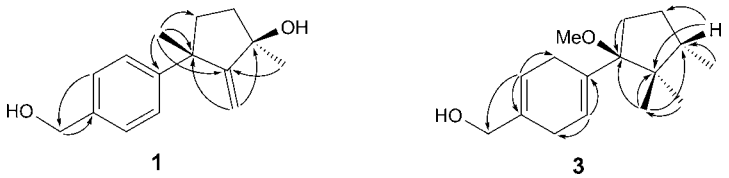
The spectral data of compound **1** resemble those reported for laur-2-en-3-ol (**5**) [11], except the presence of an additional OH function. The absence of an aromatic Me group in compound **1**, compared to **5**, and the presence of a $HOCH_2$ group, together with HMBCs between H–C(12) and C(9) in addition to C(8) and C(10) confirmed the location of the carbinol group in the *para*-position to the other substitution (*Fig. 2*).

Moreover, the HMBCs between Me(13) (δ(H) 1.54) with C(1), C(2), C(5), and C(6) confirmed the structure of **1**. The downfield shift of the Me(13), δ(H) 1.54, in case of **1** compared to δ(H) 1.44 reported by *Sun et al.* [11] could be attributed to the anisotropic effect of the benzene ring and to the 1,3-diaxial effect of the OH group. Thus, in view of the above-mentioned data and discussion, compound **1** was identified as laur-2-ene-3,12-diol.

The relative configuration of C(1) and C(3) was proposed on the basis of the NOESY experiments. The weak NOE observed between Me(13) and Me(15) suggested the cofacial orientation of Me(13) and of the OH group to C(3), which led to the downfield of the chemical-shift value of Me(13). Accordingly, the relative configuration of **1** was deduced as (1*R**,3*S**).

Compound **2** was obtained as colorless solid. The molecular formula $C_{15}H_{22}O_2$ (corresponding to five degrees of unsaturation), was deduced from a combination of its ^{13}C-NMR (*Table 1*) and HR-ESI-MS data (*m*/*z* 234.1620). The EI-MS of **2** showed a molecular-ion peak at *m*/*z* 234, the peak at *m*/*z* 219, corresponding to $C_{14}H_{19}O_2$, arose from the loss of Me, and that at *m*/*z* 201, corresponding to the fragment $C_{14}H_{17}O^+$, arose from the elimination of Me and H_2O.

The ^{1}H-, ^{13}C-, HSQC, and DEPT-NMR spectra of **2** showed signals of three Me groups at δ(H)/δ(C) 1.47/25.1, 1.13/21.0, and 0.65/22.6, and a CH–O group (δ(H)/δ(C) 4.08 (*dd*, *J* = 10.2, 9.0, 1 H)/61.9. In addition, the ^{1}H,^{1}H-COSY spectrum evidenced the following H-atom sequence; the coupling between H-atom resonating at δ(H) 4.08 and the CH_2 H-atoms with signals at 2.56 and 2.26, as well as these signals and the CH_2 signals at δ(H) 2.33 and 2.01 established the connectivity H–C(3)/CH_2(4)/CH_2(5). The remaining signals were almost identical to those of the 1,4-disubstituted benzene moiety of **1**. Hence, **2** differs from **1** only in the aliphatic part, which was unambiguously trimethylcyclopentanol. The methylation pattern was deduced from the HMBC



1 **3**

Fig. 2. *Key HMBCs for compounds* **1** *and* **3**

spectrum as 1,2,2 fashion, *i.e.*, the two Me groups resonating at δ(H)/δ(C) 1.13/21.0 (C(14)) and 0.65/22.6 (C(15)) were both at C(2), while the third one resonating at δ(H)/δ(C) 1.47/25.1 was attached to C(1). Therefore, **2** had a cuparene C-atom skeleton, O-bearing in positions 3 and 12. Thus, in view of the above-mentioned data and discussion, compound **2** was identified as cuparene-3,12-diol. The relative configuration was proposed on the basis of the NOESY experiments. The strong NOE observed between Me(13) and Me(14), and the weak correlation with H–C(3) resonating δ(H) 4.08, suggested the cofacial orientation of Me(13), Me(14), and the OH group at C(3), and thus, the relative configuration of **2** was deduced as (1*R**,3*S**).

Compound **3** was isolated as optically active colorless oil. The GC/MS exhibited a molecular-ion peak at *m*/*z* 250 (M^+). The peaks at *m*/*z* 219 and 201 originated from the loss of MeO and MeO + H_2O, respectively. The molecular formula of $C_{16}H_{26}O_2$ (corresponding to four degrees of unsaturation), was deduced from a combination of its ^{13}C-NMR (*Table 1*) and HR-ESI-MS data (*m*/*z* 250.1923). Compound **3** displayed absorption bands for a OH group (3380 cm^{-1}) and a C=C bond (1630 cm^{-1}), and no absorptions due to an aromatic ring were detected. Again, the absence of an aromatic ring was supported by checking the UV spectrum.

The ^{1}H-, ^{13}C-, and DEPT-NMR spectra of **3** indicated the presence of 16 C-atoms (*Table 1*), including four Me (one O-bearing resonating at δ(H)/δ(C) 3.49/50.9; two quaternary resonating at 0.95/19.8 and 0.60/19.1, and a Me group with a *doublet* signal at 0.87/14.6), five CH_2 (one O-bearing resonating at δ(H)/δ(C) 4.06/66.8), and three CH groups (two sp^2 CH resonating at δ(H)/δ(C) 5.86/120.8 and 5.72/120.7, and one sp^3 with a signal at 2.16/41.0), and four quaternary C-atoms (including one O-bearing resonating at δ(C) 87.0; two sp C-atoms with signals at 137.7 and 134.1; along with one sp^3 C-type hybrid at 47.8). Since compound **3** had four degrees of unsaturation with four signals due to olefinic C-atoms (*i.e.*, two C=C bonds), it possessed a bicyclic C-atom skeleton. After association of all C- and H-atoms from the HSQC spectrum, both ^{1}H,^{1}H-COSY and HMB correlations played a crucial role in identifying the structure of compound **3**. ^{1}H,^{1}H-COSY Correlations indicated the presence of three spin systems including Me–CH–CH_2–CH_2 (C(15) to C(5)), CH_2–CH (C(8) to C(7)), and CH_2–CH=C–CH_2 (C(11) to C(12)). HMBCs (*Fig. 2*) from Me(13) and Me(14) to C(2), C(1), and C(3), and to C(14) and C(13), respectively, confirmed the position of the geminal dimethyl groups at C(2), and the secondary Me group should be at C(3). Furthermore, the HMBC from H–C(5) to C(1), C(4), and C(2) led to deduction of the structure of the moiety *A* (*Fig. 1*) as 1-methoxy-2,2,3-trimethylcyclopentyl partial structure. After having established the moiety *A*, there remained two sp^3 CH_2, two sp^2 CH, two quaternary sp, and one O-bearing CH_2 C-atoms to complete the structure. HMBCs from H–C(10) (δ(H) 5.86) to C(9), C(11), C(12), and C(6), and the correlations from H–C(7) to C(6), C(8), C(11), and C(1) led us to deduce the rest of the structure as 1,4-disubstituted cyclohexa-1,4-diene (*Fig. 1*). The positions of the C=C bonds were further confirmed to be 1,4 from the absence of any absorption at *ca.* 260 nm in the UV spectrum (*i.e.*, no apparent C=C bond conjugation) [12]. The strong NOE observed between Me(13) and Me(15), and the correlation of H–C(3) with Me(14), together with the absence of correlation of H–C(3) and the MeO signal, suggested the cofacial orientation of Me(13), Me(15), and the MeO group attached to C(1). Hence, the relative configuration of **3** was deduced as (1*R**,3*R**).

This metabolite, identified as 8,11-dihydro-1-methoxylaurokamuren-12-ol, is a new laurokamurene-type sesquiterpene, with a 2,2,3-methylation pattern, along with a MeO group at C(1).

The isolated compounds were tested for their cytotoxic activities against three human cancer cell lines, *i.e.*, KB (epidermoid nasopharynx carcinoma), HepG2 (hepatocellular carcinoma), and breast cancer (MCF-7), using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Kahukuen-10-ol (**4**) revealed a wide range of cytotoxic activity against all tested cell lines with IC_{50} values [μM] comparable with those of 5-FU. However, compound **1** showed only moderate activity towards KB and MCF-7 cell lines (IC_{50} 0.171 and 0.184 μM, resp.). Compound **2** exhibited moderate activity against KB cell line at a concentration of 0.213 μM. On the other hand, compound **3** exhibited no cytotoxic activity against any of the tested cell lines ($IC_{50} > 0.5$ μM) (*Table 2*).

Interestingly, compound **4** displayed an equipotent effect on both HepG2 and MCF-7, cell lines and seems to be a promising potential drug against cancer, especially hepatoma, as shown in *Figs. 3* and *4*.

Table 2. *Cytotoxic Activities of Compounds* **1**–**4** *against Human Tumor Cell Lines*[a])

Compound	*In vitro* cytotoxicity IC_{50} [μM]		
	KB	HepG2	MCF-7
1	0.171 ± 0.005	> 0.500	0.184 ± 0.006
2	0.213 ± 0.005	> 0.500	> 0.500
3	> 0.500	> 0.500	> 0.500
4	0.100 ± 0.003	0.057 ± 0.002	0.054 ± 0.004
5-Fluorouracil	0.095 ± 0.009	0.101 ± 0.010	0.109 ± 0.016

[a]) KB, HepG2, and MCF-7 cells were the drug-sensitive human oral carcinoma, human hepatocellular carcinoma cells, and human breast adenocarcinoma cells, respectively.

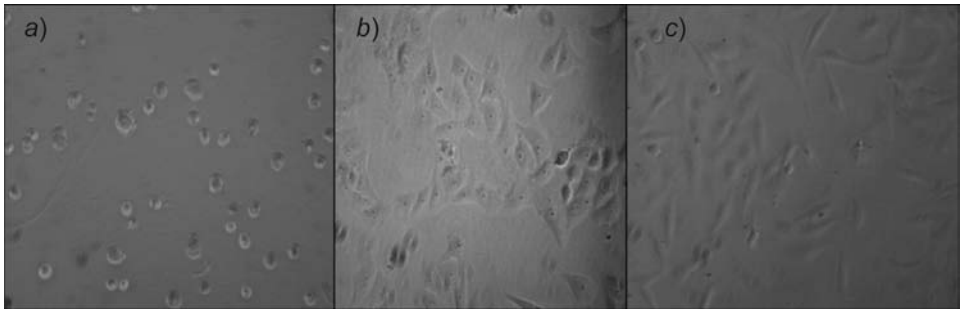


Fig. 3. *Dose-dependent cytotoxicity assay.* Dose-dependent cytotoxicity effect over cell viability of MCF-7. *a*) Compound **4** (IC_{50} 0.054 μM), *b*) Compound **1** (IC_{50} 0.184 μM), and *c*) DMSO (negative control).

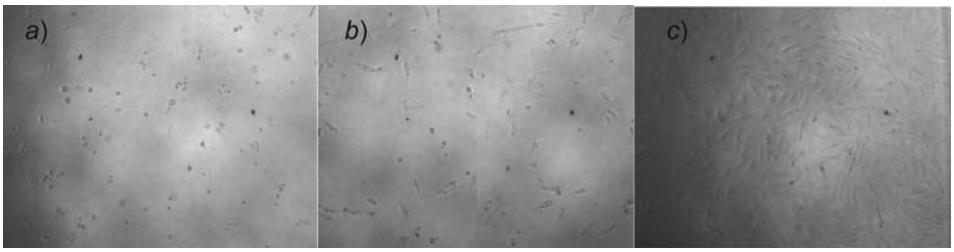


Fig. 4. *Dose-dependent cytotoxicity assay.* Dose-dependent cytotoxicity effect over cell viability of HepG2. *a*) Compound **4** (IC_{50} 0.057 μM), *b*) Compound **1** ($IC_{50} > 100$ μM), and *c*) DMSO (negative control). The images were acquired by Gx microscopes (*GXMGXD202 Inverted Microscope*); (10 × eyepiece).

Experimental part

General. TLC: Silica gel (SiO_2; *Kieselgel 60 F254*) of 0.25-mm layer thickness. Optical rotations: *ATAGO POLAX-L 2* polarimeter. 1D- and 2D-NMR: *Bruker AVANCE III WM* at 600 MHz, and ^{13}C-NMR at 150 MHz; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *Shimadzu-QP 2010*; in *m*/*z*. GC/MS: *RTX-1* column (30 m, 0.25 mm) apparatus; in *m*/*z*.

The red alga *Laurencia obtusa* was collected in June 2011, off the Saudi Arabia Red Sea Coast at Jeddah. Voucher sample (JAD 03060) was deposited with the Marine Chemistry Department, King Abdulaziz University, Jeddah, Saudi Arabia.

Extraction and Isolation. The algal material was washed with H_2O and dried in the shade at r.t. The dried material of the red alga *L. obtusa* (200 g) was exhaustively extracted with equal volumes of CH_2Cl_2/MeOH (2 × 6 l; 24 h for each batch) at r.t.

The residue (18.5 g) was partitioned between Et_2O and H_2O, the org. layer was homogenized with small amounts of aluminum oxide and of $CHCl_3$, and poured onto the top of aluminum oxide column (260 g; 60 × 5 cm), employing a gradient elution from hexane to Et_2O, and from CH_2Cl_2 to MeOH (∅ = 50, $L = 100$ cm, *ca.* 25 ml each); 150 fractions were collected. Similar fractions were pooled together, according to TLC pattern, into nine *Pools P-A* – *P-H*, employing *UV254* lamp and/or 50% H_2SO_4 in EtOH as spraying reagent for detection of spots. All compounds were purified by prep. TLC and re-purified by employing *Sephadex LH-20* with MeOH/$CHCl_3$ 9 : 1.

P-A (hexane/Et_2O 9 : 1; 80 mg) was purified by prep. TLC/aluminium oxide with hexane/Et_2O 9.5 : 0.5 to yield 2,10-dibromo-3-chloro-α-chamigrene (27 mg). *P-B* (hexane/Et_2O 8.5 : 0.5; 50 mg) was purified by prep. TLC-/aluminium oxide with hexane/Et_2O 9.5 : 0.5 to give (12*E*)-*cis*-maneonene-A (20 mg). *P-C* (hexane/Et_2O 7.5 : 0.5; 150 mg) was purified by prep. TLC/aluminium oxide with hexane/Et_2O 8 : 2 to yield (12*Z*)-*cis*-maneonene-D (81 mg). *P-D* (hexane/Et_2O 7.5 : 2.5; 10 mg) was purified by prep. TLC/aluminium oxide with hexane/Et_2O 9 : 1 to afford (12*E*)-*cis*-maneonene-E (5 mg). *P-E* (hexane/Et_2O 7 : 3; 30 mg) was purified by prep. TLC/aluminium oxide with hexane/Et_2O 7 : 3 to give (12*Z*)-*trans*-maneonene-C (12 mg). *P-F* (hexane/Et_2O 1 : 1; 30 mg) was purified by prep. TLC/aluminium oxide with hexane/AcOEt 8 : 2 to furnish 12-hydroxyisolaurene (22 mg). *P-G* (hexane/Et_2O 1 : 1; 50 mg) was purified by prep. TLC/aluminium oxide with hexane/AcOEt 7 : 3 to yield compound **2** (8 mg). *P-H* (CH_2Cl_2/AcOEt 1 : 1) was purified by prep. TLC/aluminium oxide with benzene/AcOEt/MeOH 5 : 5 : 0.1 led to isolation of **3** (3 mg) and **4** (5 mg). *P-I* (CH_2Cl_2/AcOEt 1 : 1) was purified by prep. TLC/SiO_2 with hexane/AcOEt 6 : 4 to afford **1** (2.7 mg).

Laur-2-ene-3,12-diol (= *(1*R*,3*S*)-3-[4-(Hydroxymethyl)phenyl]-1,3-dimethyl-2-methylidenecyclopentanol*; **1**). Yield: 2.7 mg (0.0014%). Solid. M.p. 72–73°. R_f (hexane/AcOEt 6 : 4) 0.3; violet appearance under UV, while brown spot developed upon spraying with 50% H_2SO_4 in EtOH. $[\alpha]_D = +13.4$ ($c = 0.27$, $CHCl_3$). UV (MeOH) 212 (4.27), 273 (3.67), 279 (3.44). IR (KBr): 3300, 3180, 3045, 2925, 2861, 1633, 1510, 1020, 950, 935, 898, 813. ^{1}H- and ^{13}C- NMR: see *Table 1*. EI-MS (70 eV): 232, 217 ($[M - Me]^+$), 214 ($[M - H_2O]^+$), 107, 91, 77. HR-ESI-MS: 232.1453 (M^+, $C_{15}H_{20}O_2^+$; calc. 232.1463).

Cuparene-3,12-diol (=*(1R*,3S*)-3-[4-(Hydroxymethyl)phenyl]-2,2,3-trimethylcyclopentanol*; **2**). Yield: 8 mg (0.0040%). Colorless solid. M.p. 87–89°. R_f (benzene/AcOEt/MeOH 5:5:0.1) 0.65; violet appearance under UV, while yellow-orange spot developed upon spraying with 50% H_2SO_4 in EtOH. $[\alpha]_D = +61.8$ ($c = 0.01$, $CHCl_3$). UV (MeOH): 208 (4.33), 277 (3.45). IR (KBr): 3349, 3182, 3055, 2971, 2876, 1512, 1459, 1389, 1368, 1284, 1222, 1151, 1120, 1076, 1014, 834, 752. ^{1}H- and ^{13}C-NMR: see *Table 1*. GC-MS: 234 (100, M^+, $C_{15}H_{22}O_2^+$), 219 (15, $[M-Me]^+$), 201 ($[M-H_2O+Me]^+$), 189 (10), 175 (10), 150 (15), 132 (15). HR-ESI-MS: 234.1620 (M^+, $C_{15}H_{22}O_2^+$; calc. 234.1629).

8,11-Dihydro-1-methoxylaurokamuren-12-ol (=*{4-[(1R*,3R*)-1-Methoxy-2,2,3-trimethylcyclopentyl]cyclohexa-1,4-dien-1-yl}methanol*; **3**). Yield: 3 mg (0.0015%). Colorless oil. R_f (hexane/AcOEt 7:3) 0.47, grey spot developed upon spraying with 50% H_2SO_4 in EtOH. $[\alpha]_D = +75.6$ ($c = 0.3$, $CHCl_3$). IR (film): 3380, 3015, 2924, 2820, 1630. ^{1}H- and ^{13}C- NMR: see *Table 1*. GC/MS: 250 (10, M^+, $C_{16}H_{26}O_2^+$), 219 (100, $[M-MeO]^+$), 201 (30, $[M-H_2O+MeO]^+$), 191 (10), 178 (30), 160 (15), 150 (45), 132 (50). HR-ESI-MS: 250.1923 (M^+, $C_{16}H_{26}O_2^+$; calc. 250.1933).

Kahukuen-10-ol (**4**). Compound **4** was identified by comparison of its spectral data (^{1}H-, ^{13}C-NMR) with those reported in [13].

Cytotoxicity Assay [14]. The cytotoxic activities of the compounds were tested with different mammalian cell lines (KB, HepG2, and MCF-7). The cell lines were obtained from American Type Culture Collection (ATCC). The cells were cultivated at 37° and 10% CO_2 in RPMI-1640 (*Lonza*, *12-702F*) medium supplemented with 10% fetal bovine serum (*FBS*, *Lonza*, Cat. No. 14-801E), 100 IU/ml pencillin, and 100 µg/ml streptomycin (*Lonza*, *17-602E*). Inhibition of proliferation was determined in 96-well plate. Sixty µl of serial dilutions of the test compounds were given to 120 µl of the suspended cells (50,000/ml) in wells of 96-well plates. After 5 d growth, each well was checked visually under the microscope. The metabolic activity of the cells was determined by an MTT assay. After 5 d of incubation at 37 and 10% CO_2, 20 µl of MTT (5 mg/ml PBS, *SERVA*, Cat. No. 20395.01) were added to each well and incubated for 4 h at 37° and 10% CO_2. After removal of the medium, formazan particles were dissolved by adding 100 µl of ^{i}PrOH/HCl. The plates were then incubated for 15 min on a shaker (600 r.p.m/min) to dissolve the formazan crystals. The intensity of the purple color was measured at λ 540 nm by a microplate reader (*ELx800 Absorbance Microplate Reader*, *BioTek*). The cytotoxicity was recorded as the concentrations that led to 50% growth inhibition (IC_{50}).

REFERENCES

[1] W. M. Alarif, S. S. Al-Lihaibi, S-E. N. Ayyad, M. H. Abdel-Rahman, F. A. Badria, *Eur. J. Med. Chem.* **2012**, *55*, 462.
[2] S-E. N. Ayyad, K. O. Al-Footy, W. M. Alarif, T. R. Sobahi, S. A. Basaif, M. S. Makki, A. M. Asiri, A. Y. Al Halwani, A. F. Badria, F. A. Badria, *Chem. Pharm. Bull.* **2011**, *59*, 1294.
[3] W. M. Alarif, S. S. Al-Lihaibi, A. Abdel-Lateff, S.-E. N. Ayyad, *Nat. Prod. Commun.* **2011**, *6*, 1821.
[4] Z. S. Abou-Elnaga, W. M. Alarif, S. S. Al-Lihaibi, *CLEAN-Soil, Air, Water* **2011**, *3*, 787.
[5] M. D. Higgs, D. J. Faulkner, *Phytochemistry* **1982**, *21*, 789.
[6] R. Chatter, M. Kladi, S. Tarhouni, R. Matoug, R. Kharrat, C. Vagias, V. Roussis, *Phytochemistry Lett.* **2009**, *2*, 25.
[7] M. Suzuki, E. Kurosawa, K. Kurata, *Phytochemistry* **1988**, *27*, 1209.
[8] K. Kurata, K. Toniguchi, Y. Agatsuma, M. Suzuki, *Phytochemistry* **1998**, *47*, 363.
[9] N. Y. Ji, X. M. Li, C. M. Cui, B. G. Wang, *Chin. Chem. Lett.* **2007**, *18*, 957.
[10] D. Iliopoullo, N. Mihopoulos, C. Vagias, P. Papazafiri, V. Roussios, *J. Org. Chem*. **2002**, *68* 7667.
[11] J. Sun, D. Shi, M. Ma, S. Li, S. Wang, L. Han, Y. Yang, X. Fan, J. Shi, L. He, *J. Nat. Prod*. **2005**, *68*, 91.
[12] C. S. Vairappan, M. Suzuki, T. Ishii, T. Okino, T. Abe, M. Masuda, *Phytochemistry* **2008**, *69*, 2490.
[13] N.-Y. Ji, X.-M. Li, K. Li, L.-P. Ding, J. B. Gloer, B.-G. Wang, *J. Nat. Prod.* **2007**, *70*, 1901.
[14] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.

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