New Terpenylated Dihydrochalcone Derivatives Isolated from *Mitrella kentii*

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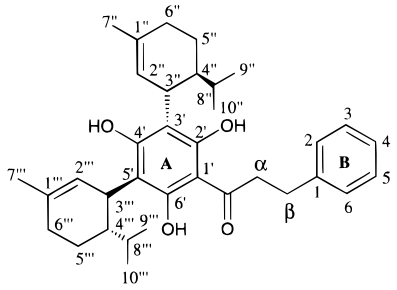
From the EtOH extract of the stem bark of *Mitrella kentii* (Annonaceae), two new terpenylated dihydrochalcones were isolated, namely (−)-neolinderatin (**1**) and (−)-linderatin (**2**), together with known compounds 2′,6′-dihydroxy-4′-methoxydihydrochalcone and (+)-catechin. Their structures were elucidated by means of combined analytical methods including HREIMS, DC, 1D, and 2D NMR spectroscopies.

Mitrella kentii is an annonaceous species of climber growing in different Indonesian areas, especially distributed in the Malaysian Peninsula and in the islands of Sumatra, Borneo, and New Guinea.[1] Previous chemical studies on this species allowed to characterize different isoquinoline alkaloids.[2,3] In a continuation to our search for biologically active compounds from the Malaysian flora,[4] an ethanolic extract of the bark of this plant was selected for phytochemical investigations due to its significant cytotoxic activity against KB cells.[5] This study led to the isolation of two new dihydrochalcone derivatives, (−)-neolinderatin (**1**) and (−)-linderatin (**2**), together with known compounds 2′,6′-dihydroxy-4′-methoxydihydrochalcone and (+)-catechin.

The molecular formula $C_{35}H_{46}O_4$ of **1** was established by high-resolution mass measurement (HREIMS) of the molecular ion (*m*/*z* 530.3380), and the typical fragment ions at *m*/*z* 133, 105, 91, and 77 suggested the presence of a *β*-propiophenone moiety in the molecule.[6] The corresponding keto function was characterized on the IR spectrum by an absorption at 1612 cm^{-1}.

As shown in Table 1, a strongly chelated hydroxyl (OH-6′) was located on the ^1H NMR spectrum of **1** as a deshielded singlet at δ_H 14.02 ppm.[7,8] Another two exchangeable protons were in evidence at δ_H 6.82 and 6.91 ppm together with signals for an ethylene group at δ_H 3.00 (t, 2H, J = 8.0 Hz) and 3.39 ppm (t, 2H, J = 8.0 Hz). These elements were in agreement with the presence of a dihydrochalcone fragment in the molecule of **1**. HMBC data were then used to identify this fragment, and $^1H-^{13}C$ shift correlations by long-range coupling constants showed that the dihydrochalcone was



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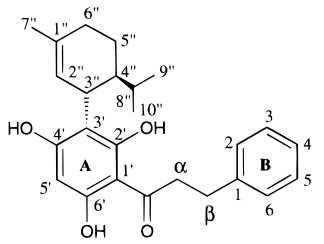

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substituted with hydroxyl groups at the C-2′, C-4′, and C-6′ positions of ring A (Figure 1).

Further examination of the ^{13}C NMR, HMQC, and HMBC data suggested that the 3′ and 5′ substituents of ring A were identical. Indeed, elements of symmetry were in evidence in the ^1H and ^{13}C NMR spectra of **1** since two olefinic protons (δ_H 5.50, δ_C 124.6 and 125.0 ppm), two allylic methyls (δ_H 1.78, δ_C 23.7 and 23.8 ppm) and two isopropylic methyls (δ_H 0.81, δ_C 16.5 and 16.6; 21.6 and 21.7 ppm) could be characterized through heteronuclear correlations. Substraction of the contributions of the 2′,4′,6′-trihydroxydihydrochalcone moiety from the molecular formula of **1** thus gave the same subformula $C_{10}H_{17}$ for 3′ and 5′ substituents. The

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Table 1. ^{1}H NMR Data for (−)-Neolinderatin (**1**) and (−)-Linderatin (**2**) in $CDCl_3$

proton	1	2
H-ar	7.32 (m)	7.30 (m)
H-α	3.39 (t, J = 8 Hz)	3.41 (t, J = 8 Hz)
H-β	3.00 (t, J = 8 Hz)	3.02 (t, J = 8 Hz)
OH-2′	6.91 (s)	
OH-4′	6.82 (s)	
OH-6′	14.02 (s)	14.02 (s)
H-5′		5.92 (s)
H-2″	5.50 (br s)	5.49 (br s)
H-2‴	5.50 (br s)	
H-3″	4.00 (d, J = 9 Hz)[a]	3.90 (m)
H-3‴	3.85 (d, J = 9 Hz)[a]	
H-8″	1.50 (m)	1.50 (m)
H-8‴	1.50 (m)	
Me-7″	1.78 (s)	1.78 (s)
Me-7‴	1.78 (s)	
Me-9″	0.81 (m)	0.86 (d, J = 6.6 Hz)
Me-9‴	0.81 (m)	
Me-10″	0.81 (m)	0.86 (d, J = 6.6 Hz)
Me-10‴	0.81 (m)	

[a]Assignments may be interchanged.

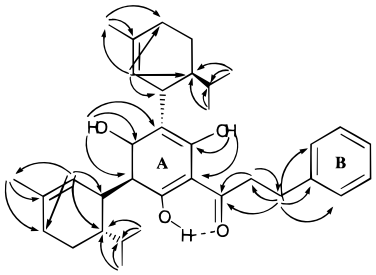

Figure 1. HMBC (J = 6 Hz) correlations for (−)-neolinderatin (**1**).

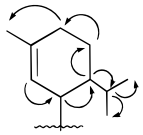

Figure 2. HOHAHA correlations for the *p*-menthene substituents of (−)-neolinderatin (**1**).

substructures were finally established from a 2D-HOHAHA experiment with a spin-locking time of 90 ms. Indeed, examination of the magnetization transfers exhibited on a 2D contour plot revealed, through spin−spin connectivities, the spin systems of two *p*-menthene moieties (Figure 2). Hence, in the HREIMS spectrum of **1**, the fragment ions observed at m/z 487 (29%) and 460 (94%) could be attributed to the loss of one isopropyl group and to a retro Diels−Alder rearrangement of one terpenyl unit, respectively. These evidences thus allowed us to identify **1** as a trihydroxydihydrochalcone derivative substituted with two *p*-menthenyl at C-3′ and C-5′. Total assignments of the proton and the carbon NMR lines were then completed through HMQC and HMBC data (Figure 1, Tables 1 and 2). This structure corresponded to the one previously described for (+)-neolinderatin, a dihydrochalcone derivative isolated from a lauraceous species, namely *Lindera umbellata*.[9−11] The structure and absolute configuration of (+)-neolinderatin have already been firmly established through total synthesis. This allowed us to compare different spectral data of **1** with those obtained with an authentic sample of (+)-neolinderatin. In this instance, the relative stereochemistry at [C-3″, C-4″] and [C-3‴, C-4‴] was determined to be *trans* for both compound by comparison of the values of their vicinal coupling

Table 2. ^{13}C NMR Data for (−)-Neolinderatin (**1**) and (−)-Linderatin (**2**) in $CDCl_3$

carbon	1	2
C-1	142.1	141.8
C-2	128.4	128.5
C-3	128.6	128.9
C-4	125.8	125.9
C-5	128.6	128.9
C-6	128.3	128.5
C-1′	105.0	104.6
C-2′	157.4	162.1
C-3′	108.1	109.8
C-4′	160.3	158.2
C-5′	108.6	95.6
C-6′	162.3	164.6
C=O	205.1	204.8
C-α	46.3	45.7
C-β	30.8	30.7
C-1″	141.1[a]	140.7
C-2″	125.0[b]	124.8
C-3″	34.8[c]	34.9
C-4″	43.6	43.6
C-5″	22.4[d]	23.7
C-6″	30.7	31.8
C-7″	23.8[e]	23.7
C-8″	28.2[f]	30.7
C-9″	16.6[g]	16.4
C-10″	21.7[h]	21.0
C-1‴	140.6[a]	
C-2‴	124.6[b]	
C-3‴	34.6[c]	
C-4‴	43.6	
C-5‴	22.3[d]	
C-6‴	30.7	
C-7‴	23.7[e]	
C-8‴	27.9[f]	
C-9‴	16.5[g]	
C-10‴	21.6[h]	

[a−h]Assignments may be interchanged.

constants $J_{3'',4''} = J_{3''',4'''} = 9.0$ Hz, whereas H-2″ and H-2‴ appeared as two overlapped broad singlets at δ_H 5.50 ppm in each ^{1}H NMR spectrum.[12] Furthermore, the ^{1}H and ^{13}C NMR spectra of **1** and (+)-neolinderatin appeared to be perfectly superimposable. However, optical rotations of the methanolic solutions (c = 0.2) of **1** and (+)-neolinderatin were of opposite signs, respectively −40° and +40°, thus suggesting an enantiomeric relationship between these compounds. As expected, the CD curve of **1** was the inverse of that of authentic (+)-neolinderatin (Figure 3a). These observations led to the conclusion that **1** was the (3″R,4″S)-(3‴R,4‴S)-2′,4′,6′-trihydroxy-3′,5′-bis(4-isopropyl-1-methylcyclohex-1-en-3-yl)-dihydrochalcone or (−)-neolinderatin.

The HREIMS spectra of **2** indicated a molecular formula of $C_{25}H_{30}O_4$ (m/z 394.2138) which could simply differ from **1** by the absence of one *p*-menthenyl substitution. Indeed the ^{1}H-NMR spectrum of **2** (Table 1) was almost superimposable with the resonances observed on the ^{1}H-NMR spectrum of **1**. However, six aromatic protons were in evidence as a multiplet centered at δ_H 7.30 ppm (5H, H2-H6) and an isolated singlet at δ_H 5.92 ppm (1H, H-5′). Resonances ascribable to only one *p*-menthenyl group were also located at δ_H 5.49 (bs, 1H, H-2″), 3.90 (bd, $J_{3'',4''}$ = 9.0 Hz, H-3″), 1.78 (s, 3H, H-7″) and 0.86 ppm (d, 6H, $J_{8'',9''} = J_{8'',10''}$ = 7.0 Hz, H-9″ and H-10″).

These elements were in agreement with the structure of the dihydrochalcone (+)-linderatin isolated from the same *L. umbellata*.[9] Nevertheless, as aforementioned, optical rotations for (+)-linderatin and **2** were found to be of opposite signs, respectively +50° and −50° (MeOH,

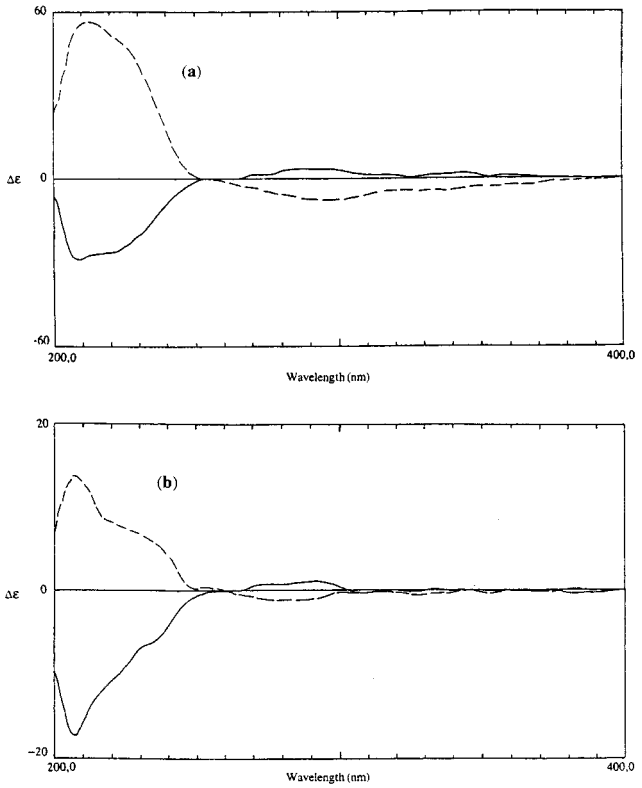

Figure 3. CD spectra of (−)-neolinderatin (**1**), (−)-linderatin (**2**), and the related compounds, (**a**) (−)-neolinderatin (**1**: solid line) and (+)-neolinderatin (dashed line); (**b**) (−)-linderatin (**2**: solid line) and (+)-linderatin (dashed line).

c = 0.2). CD measurement (Figure 3b) and NMR data comparison of each sample then demonstrated that **2** was the enantiomer of (+)-linderatin. Finally **2** was identified as the (3″R,4″S)-2′,4′,6′-trihydroxy-3′-(4-isopropyl-1-methylcyclohex-1-en-3-yl)dihydrochalcone or (−)-linderatin. Two others known compounds, namely 2′,6′-dihydroxy-4′-methoxydihydrochalcone and (+)-catechin, were also isolated fom the same source and were identified by direct comparison with reference compounds.[13]

It may be worth noting that no other compounds directly related to **1** and **2** could be detected in the bark extract of *M. kentii*, whereas the corresponding chalcones and flavanones derivatives of (+)-linderatin and (+)-neolinderatin were present in *L. umbellata*. The four enantiomers have been tested *in vitro* on a non-small-cell bronchopulmonary lung carcinoma (see the Experimental Section). As a matter of fact, this type of cancer represents 80% of all human bronchopulmonary cancers, and its main characteristic is a great chemioresistance to medical treatement.[14] Compounds showing a IC_{50} of ≤5 μg mL^{-1} are considered as significantly active against this cell line. While (+)-linderatin, (+)-neolinderatin, and (−)-neolinderatin were found to be inactive (IC_{50} > 30 μg mL^{-1}), (−)-linderatin exhibited a significant activity toward this type of carcinoma (IC_{50} = 3.8 μg mL^{-1}). This promising activity shoud be further evaluated *in vivo*.

Experimental Section

General Experimental Procedure. NMR spectra were recorded on a JEOL GSX 270 WB spectrometer. IR spectra were obtained on a Perkin-Elmer 580 spectrometer. HREIMS were determined on a Varian Mat 3111 spectromer. UV spectra were recorded on a Hitachi U-2000 spectrophotometer. $[\alpha]_D$ were obtained on a Schmidt−Haensch polarimeter. CD curves were recorded on a Jasco J-600 spectropolarimeter.

Plant Material. The bark of *M. kentii* (Bl.) Miq. were collected in Pahang district in Malaya in January 1991. An herbarium specimen (no. KL4104) is deposited at the Laboratoire de Phanérogamie, MNHN, Paris, and at the University of Malaya, Kuala-lumpur.

Extraction and Isolation. An EtOH extract (21.2 g from 0.4 kg of air-dried powdered stem bark) was solubilized in a methanolic aqueous solution (60%) and partitioned with successively $CHCl_3$ and EtOAc. These extracts were submitted to further purifications using MPLC and preparative TLC on Si gels. Three compounds were isolated from the chloroformic extract: (−)-neolinderatin (**1**) (686 mg), (−)-linderatin (**2**) (203 mg), and 2′,6′-dihydroxy-4′-methoxydihydrochalcone (**3**) (18 mg). (+)-Catechin (**4**) (12 mg) was isolated from the EtOAc extract.

(−)-Neolinderatin (1): amorphous; $[\alpha]_D$ = −40° (*c* 0.2, MeOH); CD (*c* 0.0021, MeOH) λ max 210 nm; IR (KBr) ν max 3505 (O−H), 1612 (C═O) cm^{-1}; UV (MeOH) λ max 337, 290, 226 nm; EIMS *m/z* (%) 530 $[M]^{\bullet+}$ (100), 487 $[M - C_3H_7]^+$ (29), 460 $[M - C_5H_{10}]^{\bullet+}$ (94), 417 $[M - C_5H_{10} - C_3H_7]^+$ (7), 407 $[C_{27}H_{19}O_4]^+$ (8), 390 $[M - C_5H_{10} - C_5H_{10}]^{\bullet+}$ (5), 133 $[C_9H_9O]^+$ (4), 105 $[C_8H_9]^+$ (38), 91 $[C_7H_7]^+$ (99), 43 $[C_3H_7]^+$ (44); ^{1}H-NMR ($CDCl_3$, 270 MHz), see Table 1; ^{13}C-NMR ($CDCl_3$, 67.5 MHz), see Table 2.

(−)-Linderatin (2): amorphous; $[\alpha]_D$= −50° (*c* 0.2, MeOH); CD (*c* 0.002, MeOH) λ max 210 nm; IR (KBr) ν max 3560 (O−H), 1620 (C═O) cm^{-1}; UV (MeOH) λ max 291, 225 nm; EIMS *m/z* (%) 394 $[M]^{\bullet+}$ (69), 351 $[M - C_3H_7]^+$ (16), 324 $[M - C_5H_{10}]^{\bullet+}$ (69), 309 $[M - C_5H_{10} - CH_3]^+$ (77), 105 $[C_8H_9]^+$ (30), 91 $[C_7H_7]^+$ (100), 77 $[C_6H_5]^+$ (12), 43 $[C_3H_7]^+$ (24); ^{1}H-NMR ($CDCl_3$, 270 MHz), see Table 1; ^{13}C-NMR ($CDCl_3$, 67.5 MHz), see Table 2.

2′,6′-Dihydroxy-4′-methoxydihydrochalcone: colorless plates; mp 174−175°C (crystallized from CH_2Cl_2).

(+)-Catechin: amorphous; $[\alpha]_D$ = +60° (*c* 0.1, MeOH).

Bioassays. Experiments were performed in 96-well microliter plates (2×10^5 cells mL^{-1}). Cell growth was estimated by colorimetric assay based on conversion of tetrazolium dye (MTT) to a blue formazan product using live mitochondria.[15] Eight determinations were performed for each concentration. Control growth was estimated from 16 determinations. Optical density at 570 nm corresponding to solubilized formazan was read for each well on a Titertek Multiskan MKII.

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References and Notes

(1) Sinclair, J. *Garden's. Bull. Singapore*. **1955**, *14*, 364−367.
(2) Leboeuf, M.; Cavé, A.; Bhaumik, P. K.; Mukherjee, B. Mukherjee R., *Phytochemistry*. **1982**, *21*, 2783−2813.
(3) Ellis, J.; Gellert, E.; Summons, R. E. *Aust. J. Chem*. **1972**, *25*, 2735−2736.
(4) Benosman, A.; Richomme, P.; Sévenet, T.; Perromat, G.; Hamid A. Hadi, A.; Bruneton, J. *Phytochemistry*. **1995**, *40*, 1485−1487.
(5) The percentages of cytotoxic activity of the EtOH extract against KB cells were found to be 56, 33, and 17% for concentrations of 10, 5 and 1μg.ml^{-1} respectively.
(6) Kaouadji, M. *Phytochemistry*. **1989**, *28*, 3191−3192.

(7) Hufford, C. D.; Oguntimein, B. O. *Phytochemistry*. **1980**, *19*, 2036−2038.
(8) Hufford, C. D.; Oguntimein B. O. *J. Nat. Prod*. **1982**, *45*, 337−342.
(9) Tanaka, H.; Ichino, K.; Ito, K. *Chem. Pharm. Bull.* 1**984**, *32*, 3747−3750.
(10) Ichino, K.; Tanaka, H.; Ito, K. *Chem. Lett*. **1989**, 363−366.
(11) Ichino, K.; Tanaka, H.; Ito, K. *Tetrahedron*. **1988**, *44*, 3251−3260.
(12) Kane, V. V.; Martin, A. R.; Jaime, C.; Osawa, E. *Tetrahedron*. **1984**, *40*, 2919-2927.
(13) (+)-Catechin was purchased from Sigma Chemical Co. (St. Louis, MO); the dihydrochalcones were previously isolated from *Lindera umbellata* (Tanaka, H.; Ichino, K.; Ito, K. *Phytochemistry* **1984**, *23*, 1198−1199).
(14) Roussakis, C.; Gratas, C.; Audoin, A. F.; Le Boterff, J.; Dabouis, G.; Andre, M. J.; Moyon, E.; Vo, N. H.; Pradal, G.; Verbist, J. F. *Anticancer Research*. **1991**, *11*, 2239−2244.
(15) Mosmann, T. *J. Immunol. Methods*. **1983**, *65*, 55−63. neolinderatin (**1**).

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