THREE NEW COUMARINS FROM *CLAUSENA EXCAVATA*

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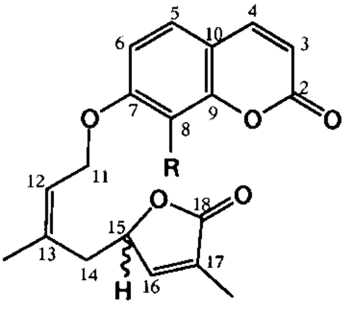
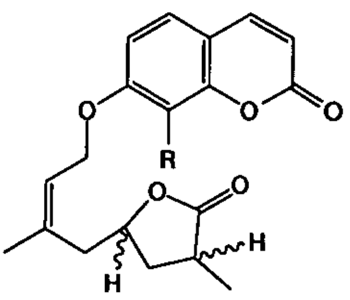
Abstracts ——Three new lactone coumarins named clauslactone-K (**1**), -L (**3**) and -M (**4**) were isolated from the twigs and leaves of *Clausena excavata*, collected in Sumatra, Indonesia and their structures were elucidated by spectroscopic analysis.

The aerial and underground parts of *Clausena* species have been extremely studied and afforded many coumarins and carbazole alkaloids.[1] On the constituents of *Clausena excavata* (Rutaceae), many compounds have been isolated from stem bark,[2] root bark[3] and leaves.[4] The twigs and leaves of this plant have traditionally been used as a folk medicine (called as "Tonok-tonok") for treatment of dermatopathy in Semelako, Bengkulu Province, Indonesia. In the course of chemical study of Indonesian medicinal plants, we have investigated the chemical constituents and have isolated three new coumarins named clauslactone-K (**1**), -L (**3**) and -M (**4**). This paper deals with the isolation and structure elucidation of these new coumarins.

Clauslactone-K (**1**) was obtained as colorless oil, $[\alpha]_D$ -57.1° ($CHCl_3$). The molecular formula of **1** was assigned as $C_{20}H_{20}O_6$ based on HR-MS. The UV [205, 250, 258 (sh), 318 nm] spectrum suggested the presence of 7,8-dioxygenated coumarin nucleus.[5] The IR bands at 1753, 1728 and 1608 cm^{-1} indicated the existence of α,β-unsaturated γ-lactone and α,β-unsaturated δ-lactone groups.[5] The ^{1}H-NMR spectrum showed characteristic signals due to H-4, H-3 [δ 7.61, 6.26 (each 1H, d, J=9.4 Hz)], H-5 and H-6



(1) R=OCH_3
(2) R=H



(3) R=OCH_3
(4) R=H

[δ 7.15, 6.85 (each 1H, d, J=8.6 Hz)], a methoxyl group [δ 3.99 (3H, s)]. In the NOE differential spectrum, irradiation of the methoxy signal showed no increments on any aromatic proton signals indicating the location of this group at C-8. A pair of doublet at δ 4.71 (2H, J=6.0 Hz) and 5.63 (1H, J=6.0 Hz) was assumed to the signals of -OCH_2-CH=C- group. The signals assignable to β-proton, α-methyl and γ-methine protons of the α,β-unsaturated γ-lactone group at δ 7.00 (1H, t, J=1.7 Hz), 1.91 (3H, d, J=1.7 Hz) and 5.00 (1H, m), methylene signal at δ 2.41 (2H, m) and olefinic methyl signal at δ 1.84 (3H, s) were also observed. These signal patterns were in accord to those of capnolactone (2),[6] and the differences were the appearance of a methoxy signal and AB type aromatic proton signals in 1. Confirmation of the structure of the side chain was performed by HMBC experiment (Figure 1). The ^{2}J and ^{3}J connectivities were observed between H-11 (δ 4.71)/ C-7 (δ 154.7), C-12 (δ 123.4), C-13 (δ 136.0); H-12 (δ 5.63)/ 13-Me (δ 17.5); H-14 (δ 2.45) / C-12, C-13, C-15 (δ 79.5), C-16 (δ 148.1). The above results established the existence of -O-CH_2-CH=C(CH_3)-CH_2- group between coumarin nucleus and α,β-unsaturated γ-lactone moiety. Based on the above results, the structure of clauslactone-K was assigned as 1 except for the stereochemistry at C-15.

Clauslactone-L (3), $[\alpha]_D$ +195.7° ($CHCl_3$), was isolated as colorless oil. The molecular formula $C_{20}H_{22}O_6$ was analyzed by HR-MS. The UV [206, 249, 257 (sh), 318 nm] and IR (1764, 1729, 1606 cm^{-1}) spectra suggested the presence of 7,8-dioxygenated coumarin skeleton, γ-lactone and α,β-unsaturated δ-lactone nuclei.[5] The characteristic signals due to H-4, H-3, H-5 and H-6 of coumarin skeleton were observed at δ 7.62, 6.27 (each 1H, d, J=9.5 Hz), δ 7.15 and 6.86 (each

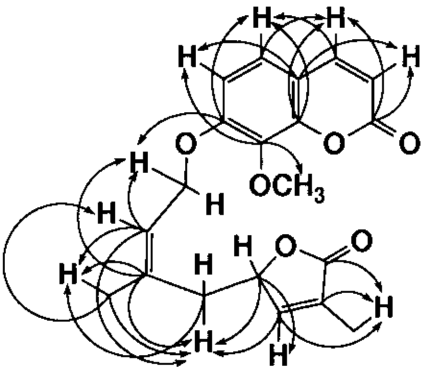


Figure 1. Correlations Observed in the HMBC Spectrum of Clauslactone-K (1)

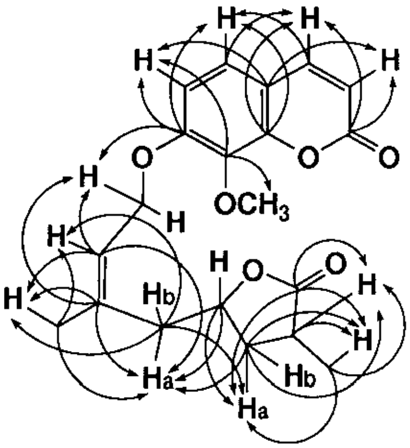


Figure 2. Correlations Observed in the HMBC Spectrum of Clauslactone-L (3)

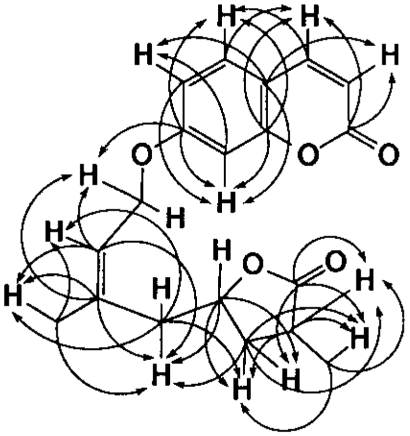


Figure 3. Correlations Observed in the HMBC Spectrum of Clauslactone-M (4)

1H, d, J=8.8 Hz). Though irradiation of the methoxy signal at δ 3.99 showed no increment on any aromatic protons, irradiation of the methylene signal at δ 4.71 (H-11) showed 9% increment of the signal at δ 6.86 (H-6). The above NOE results established the location of the methoxy group at C-8 and indicated the presence of alkoxy group at C-7. By means of the ^{1}H-^{1}H COSY spectrum, it was possible to characterize the spin systems and to assign all proton resonances. The signals at δ 4.71 (2H, d, J=6.2 Hz), 5.62 (1H, t, J=6.2 Hz) and 1.82 (3H, s), coupled by observation of cross peaks between δ 4.71 (H-11) and 5.62 (H-12), indicated the presence of partial structure -O-CH$_2$-CH=C(CH$_3$)-. The methylene protons at δ 2.50 (H-14a) and 2.39 (H-14b) showed cross peaks to methine proton at δ 4.50 (H-15) which exhibited coupling to methylene signals at δ 1.54 (H-16b) and 2.48 (H-16a). The methylene protons at C-16 showed cross peaks to methine protons at δ 2.67 (H-17) and 4.50 (H-15). The methine proton at δ 2.67 (H-17) showed coupling to methylene protons [δ 1.54 (H-16b) and 2.48 (H-16a)] and a methyl group [δ 1.27 (17-Me)]. These results suggested the presence of -CH$_2$-CH(O)-CH$_2$-CH(CH$_3$)- group. The connection pattern of these groups was clarified by HMBC experiments (Figure 2). Three particularly important observations arose from this study. First, the H-14a signal (δ 2.50) exhibited ^2J and ^3J correlations with C-12 (δ 122.7),

Table 1. ^{13}C- and ^{1}H-NMR spectral data of clauslactone-L (3) and -M (4).

	3		4	
position	δ_C	δ_H	δ_C	δ_H
2	160.5		161.2	
3	113.6	6.27 (d, 9.5)	113.1	6.26 (d, 9.5)
4	143.6	7.62 (d, 9.5)	143.4	7.64 (d, 9.5)
5	122.6	7.15 (d, 8.8)	128.8	7.37 (d, 8.8)
6	110.3	6.86 (d, 8.8)	113.1	6.85 (dd, 8.8, 2.2)
7	154.7		161.9	
8	136.8		101.6	6.81 (d, 2.2)
8-OMe	61.4	3.99 (3H, s)		
9	148.2		155.8	
10	113.9		112.6	
11	65.9	4.71 (2H, d, 6.2)	65.1	4.62 (2H, d, 6.2)
12	122.7	5.62 (t, 6.2)	122.3	5.59 (t, 6.2)
13	136.7		136.9	
13-Me	17.3	1.82 (3H, s)	17.3	1.83 (3H,s)
14	45.1	2.39 (m) 2.50 (m)	45.2	2.37 (m) 2.53 (m)
15	76.7	4.50 (m)	76.3	4.50 (m)
16	37.2	1.54 (m) 2.48 (m)	37.2	1.54 (m) 2.48 (m)
17	35.7	2.67 (m)	35.7	2.68 (m)
17-Me	15.0	1.27 (3H, d, 7.3)	15.0	1.28 (3H, d,7.0)
18	179.2		179.2	

13-Me (δ 17.3), C-16 (δ 37.2), C-13 (δ 136.7) and C-15 (δ 76.7). Second, the H-16b signal (δ 1.54) revealed ^2J and ^3J correlations with C-15 (δ 76.7), C-17 (δ 35.7) and 17-Me (δ 15.0). Third, the H-17 signal (δ 2.67) exhibited ^2J correlations with C-18 (δ 179.2), C-16 (δ 37.2) and 17-Me (δ 15.0). From the above results, the structure **3** was assigned to clauslactone-L.

Clauslactone-M (**4**) was obtained as colorless oil, $[\alpha]_D$ +118.3° ($CHCl_3$). The molecular formula $C_{19}H_{20}O_5$ was determined by HR-MS which showed molecular ion at m/z 328.1305. The IR and UV spectra were similar to those of umbelliferone. The ^{1}H-NMR spectrum of **4** showed resemble signal patterns to that of **3** (Table 1), the notable differences were the disappearance of the methoxyl signal and the change of coupling pattern of aromatic proton signals, suggesting that clauslactone-M was demethoxylated compound of **3**. The estimated structure was confirmed unambiguously by HMBC experiments as shown in Figure 3.

Both clauslactones-L (3) and -M (4) were considered to be isolated as a sole diastereomer and the stereochemistry of these coumarins remains to be determined. Further structure elucidations of other isolates are now in progress.

EXPERIMENTAL

Extraction and Isolation: The twigs and leaves (2.5 kg) of *Clausena excavata* Burm. f. collected at Semelako, Rejang Lebong, Bengkulu Province (Sumatra), Indonesia, were extracted with acetone and MeOH (each 8L x 2) under reflux for 8h. The extract (322.38 g) was chromatographed over silica gel and eluted with toluene, CH_2Cl_2, CH_2Cl_2-acetone, acetone and MeOH, successively. The CH_2Cl_2 eluate (56.47 g) was subjected to repeated silica gel column, preparative thin-layer chromatography [solvent; isopropyl ether, acetone - $CHCl_3$ (1:9); acetone - benzene (3:7), AcOEt - n-hexane (1:1)] to give clauslactone-K (1) (20.3 mg), -L (3) (6.7 mg) and -M (4) (15.1 mg).

Clauslactone-K (1): Colorless oil; $[\alpha]_D$ -57.1° (c= 0.035, $CHCl_3$); HR-MS: m/z 356.1241 (M^+, found), 356.1260 (calcd for $C_{20}H_{20}O_6$); IR ν_{max} ($CHCl_3$) cm^{-1}: 1753, 1728, 1608; UV λ_{max} (MeOH) nm: 205, 250, 258 (sh), 318; EI-MS m/z: 356 (M^+), 192 (base peak), 163, 147; ^{1}H-NMR ($CDCl_3$, δ): 7.61 (1H, d, J= 9.4 Hz, H-4), 7.15 (1H, d, J= 8.6 Hz, H-5), 7.00 (1H, t, J= 1.7 Hz, H-16), 6.85 (1H, d, J= 8.6 Hz, H-6), 6.26 (1H, d, J= 9.4 Hz, H-3), 5.63 (1H, d, J= 6.0 Hz, H-12), 5.00 (1H, m, H-15), 4.71 (2H, d, J= 6.0 Hz, H-11), 3.99 (3H, s, 8-MeO), 2.41 (2H, m, H-14), 1.91 (3H, d, J= 1.7 Hz, 17-Me), 1.84 (3H, s, 13-Me); ^{13}C-NMR ($CDCl_3$, δ): 173.9 (s, C-18), 160.5 (s, C-2),154.7 (s, C-7), 148.2 (s, C-9), 148.1 (d, C-16), 143.6 (d, C-4), 136.8 (s, C-8), 136.0 (s, C-13), 130.4 (s, C-17), 123.4 (d, C-12), 122.7 (d, C-5), 113.9 (s, C-10), 113.7 (d, C-3), 110.2 (d, C-6), 79.5 (d, C-15), 65.9 (t, C-11), 61.5 (q, 8-MeO), 43.2 (t, C-14), 17.5 (q, 13-Me), 10.6 (q, 17-Me).

Clauslactone-L (3): Colorless oil; $[\alpha]_D$ +195.7° (c= 0.138, $CHCl_3$); HR-MS: m/z 358.1393 (M^+, found), 358.1416 (calcd for $C_{20}H_{22}O_6$); IR ν_{max} ($CHCl_3$) cm^{-1}: 1764, 1729, 1606; UV λ_{max} (MeOH) nm: 206, 249, 257 (sh), 318; EI-MS m/z: 358 (M^+), 193, 192 (base peak), 163, 121; ^{1}H-NMR ($CDCl_3$, δ): see Table 1;

differential NOE: irradiation of H-11 (δ 4.71) gave 9% NOE at H-6 (δ 6.86); ^{13}C-NMR ($CDCl_3$, δ): see Table 1.

Clauslactone-M (4): Colorless oil, $[\alpha]_D$ +118.3° (c= 0.0845, $CHCl_3$); HR-MS: m/z 328.1305 (M^+, found), 328.1311 (calcd for $C_{19}H_{20}O_5$); IR ν_{max} ($CHCl_3$) cm^{-1}: 1766, 1730, 1614; UV λ_{max} (MeOH) nm: 206, 220 (sh), 288 (sh), 323; EI-MS m/z: 328 (M^+), 162 (base peak), 149, 134, 121; ^{1}H-NMR ($CDCl_3$, δ): see Table 1; Differential NOE: irradiation of H-11 (δ 4.62) gave 11% NOE at H-8 (δ 6.81); ^{13}C-NMR ($CDCl_3$, δ): see Table 1.

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