Recently we reported the isolation and structure elucidation of three new coumarins, named clauslactones-K (1), -L (2) and -M (3), from the twigs and leaves of Indonesian Clausena excavata (Rutaceae) which have been used as folk medicine for treatment of dermatopathy in Sumatra. Further investigation resulted in the isolation of four additional new coumarins and we now wish to report the structural elucidation of these compounds.

Clauslactone-N (4) was obtained as a colorless oil, $[\alpha]_D^{15} +58.6^\circ$ (CHCl$_3$). The molecular formula was determined as C$_{20}$H$_{22}$O$_7$ by high resolution (HR)-MS which showed a molecular ion at $m/z$ 374.1314. The UV absorption [258 (sh), 318 nm], IR bands (1727, 1609 cm$^{-1}$) and 1H-NMR signals [d 7.63, 6.27 (each 1H, d, $J = 5.9$ Hz), 7.15, 6.86 (each 1H, d, $J = 5.8$ Hz)] indicated the existence of a 7, 8-disubstituted coumarin skeleton. The resonances at d 4.71 (2H, d, $J = 6.2$ Hz), 5.62 (1H, t, $J = 9.2$ Hz), 7.15, 6.86 (each 1H, d, $J = 8.8$ Hz) characteristic to H-4, H-3, H-5 and H-6 in the 1H-NMR spectrum and d 66.0 (t), 123.0 (d), 136.3 (s), 17.2 (q) and 44.9 (t) in the 13C-NMR spectrum were assigned to the signals of the partial structure $-O-CH_2-CH_5-C(CH_3)-CH_2-$. The signals due to a-methyl [d 1.51 (3H, s)], b-methylene [d 1.79 (1H, dd, $J = 13.6$, 5.5 Hz), 2.44 (1H, dd, $J = 13.6$, 5.5 Hz)], g-methine proton [d 4.79 (1H, m)] on the lactone ring were also observed. To confirm the structure of the side chain, the $^2J$ and $^3J$ connectivities were examined by 1H-detected heteronuclear multiple bond connectivity (HMBC) experiments (Fig. 1). The signal of a methyl group at $\delta_{H}$ 1.51 (17-Me) showed cross peaks with the carbon signals at $\delta_{C}$ 42.5 (C-16), 73.7 (C-17) and 177.5 (C-18). The proton signal at $\delta_{H}$ 2.44 (H$_3$-16) showed cross peaks with the carbon signals at $\delta_{C}$ 177.5 (C-18) and 73.7 (C-17). The proton signal at $\delta_{H}$ 1.79 (H$_3$-16) showed cross peaks with the carbon signals at $\delta_{C}$ 44.9 (C-14), 76.2 (C-15) and 24.2 (17-Me). The proton signals at $\delta_{H}$ 2.37 and 2.50 (H-14) showed cross peaks with the carbon signals at $\delta_{C}$ 177.5 (C-18) and 73.7 (C-17). These connectivities established the structure of the side chain. In the differential nuclear Overhauser effect (NOE) experiments, irradiation of the methylene signal at $\delta_{H}$ 4.71 showed 12% increments on the signal at $\delta_{H}$ 6.86 (H-6) suggesting the location of the methoxy group at C-8. When the methyl signal at $\delta_{H}$ 1.82 was irradiated, 3%, 4% and 7% enhancement were observed for the signals at $\delta_{H}$ 4.71 (H-11), 4.79 (H-15) and 2.44 (H$_3$-16). Irradiation of the methyl signal at $\delta_{H}$ 1.51 (17-Me) showed a 5% increment to the signal at $\delta_{H}$ 1.79 (H$_3$-16). These results supported the E regiochemistry between C-12 and C-13, and the structure around the lactone ring was assigned as shown in structure 4.

Clauslactone-O (5) was isolated as a colorless oil, $[\alpha]_D^{15} +24.3^\circ$ (CHCl$_3$), and the molecular formula was determined as C$_{19}$H$_{20}$O$_6$ from HR-MS analysis. The UV, IR absorptions and the signal patterns in the $^1$H- and $^13$C-NMR spectrum of clauslactone-N (4) are shown in Fig. 1.

Chart 1. Structures of Coumarins Isolated from Clausena excavata

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From these data, the structure of clauslactone-Q was deduced due to aromatic protons in the 1H-NMR spectrum. The regiochemistry between C-12 and C-13 was determined as E by differential NOE experiment (see Experimental). Thus, the structure of clauslactone-O was assumed to be 5 and rigorous confirmation of the structure was carried out by HMBC experiments (Fig. 2).

Clauslactone-P (6) was obtained as colorless cubes, mp 126–128 °C, [ε]D 9.6° (CHCl3). The molecular formula was determined as C20H20O7 by HR-MS, which showed a molecular ion at m/z 372.1182. The IR and UV absorptions suggested the presence of a 7,8-dioxogenated coumarin skeleton. The 1H-NMR spectrum of 6 showed characteristic signals at δ 7.61 (1H, d, J = 9.8 Hz), 7.15 (1H, d, J = 8.6 Hz) and 6.85 (1H, d, J = 8.6 Hz) due to H-4, H-3, H-5 and H-6 of coumarin skeleton. The signals at δ 4.71 (2H, d, J = 2.2 Hz), 5.11 (1H, ddt, J = 1.7, 1.7, 5.6 Hz) and 2.45 (1H, dd, J = 7.7, 14.1 Hz) indicated the presence of the partial structure –O–CH2–CH2–. The presence of a 2-hydroxymethyl 4-substituted α,β-unsaturated γ-lactone moiety was suggested by the signals at δ 7.25 (1H, dd, J = 3.0, 1.7 Hz), 5.11 (1H, ddt, J = 1.7, 1.7, 5.6 Hz), 4.43 (2H, t, J = 1.7 Hz) in the 1H-NMR spectrum and the signals at δ 172.1 (s), 148.5 (d), 134.0 (s), 80.4 (d), 57.2 (t) in the 13C-NMR spectrum. In the differential NOE experiment (acetone-d6), irradiation at δ 1.87 (13-Me) gave 4% enhancement at δ 4.79 (H-11), therefore the regiochemistry between C-12 and C-13 was determined as E. From the above data, we assigned the structure 6 to clauslactone-P.

Clauslactone-Q (7) was isolated as colorless cubes, mp 168–170 °C, [ε]D 9.3° (CHCl3). The HR-MS showed a molecular ion at m/z 342.1108 consistent with a molecular formula C19H19O6. The IR and UV absorptions, 1H- and 13C-NMR signal patterns of 7 resembled those of 6 (see Table 1). The remarkable differences were the lack of a methoxy signal and the appearance of ABC signals at δ 7.62 (1H, d, J = 8.5 Hz), 7.00 (1H, d, J = 2.6 Hz) and 6.95 (1H, dd, J = 8.5, 2.6 Hz) due to aromatic protons on the coumarin skeleton. From these data, the structure of clauslactone-Q was deduced as 7.

In the previous paper, we described the regiochemistry...
around C-12 and C-13 of the side chains of clauslactones-K (1), -L (2) and -M (3) as Z. Upon further examination by differential NOE, irradiation of the 13-methyl signals gave 4% NOE for the 11-methylene signals (see Experimental). This result revealed the E geometry around C-12 and C-13, therefore the structure should be corrected as shown in this paper.

**Experimental**

Melting points were measured on a Yanagimoto micromelting point hot-stage apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. 1H- and 13C-NMR, NOE and HMBC spectra were recorded on an A-400 or A-600 (JEOL) spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. Electron impact (EI)- and HR-MS were taken with a JMS-HX 110 spectrometer. UV spectra were recorded on a Shimadzu UV 160A in CHCl3. Preparative TLC was done on Kieselgel 60 F254 (Merck).

**Isolation** The eluates2) of the silica gel column chromatography of the acetone and MeOH extract obtained from dried twigs and leaves (2.5 kg) were subjected to repeated silica gel column and preparative thin-layer chromatography (solvent; acetone–CH2Cl2 eluate (17.82 g)). Clauslactone-P (6) (3.1 mg) were isolated from the acetone–CH2Cl2 eluate (17.82 g).

**Clauslactone-N (4):** Colorless oil, [α]D0 +58.6° (c=0.293, CHCl3). HR-MS Calcd for C19H18O6: 342.1108. Found: 342.1103. EI-MS m/z: 342 [M]–, 324, 181, 162 (base peak), 134. UV λmax: 204, 280 (sh), 323 nm. IR νmax cm−1: 3400, 1750, 1720, 1600. 1H- and 13C-NMR see Table 1. Differential NOE: irradiation at δ 4.62—12%, 5% and 5% enhancement at δ 4.79 (H-15) and 2.44 (H A-16); irradiation at δ 4.71—5%, 11% and 4% enhancement at δ 4.69 (H-11); irradiation at δ 4.69—12%, 13% and 9% enhancement at δ 4.71, 7.00 (H-8) and 6.95 (H-6).

**Clauslactone-O (5):** Colorless oil, [α]D0 +24.3° (c=0.0775, CHCl3). HR-MS Calcd for C19H18O6: 344.1259. Found: 344.1266. EI-MS m/z: 344 [M]+, 165, 163, 162 (base peak), 155, 137, 134. UV λmax: 204, 221(sh), 295 (sh), 323 nm. IR νmax cm−1: 3577, 1772, 1730, 1614. 1H- and 13C-NMR see Table 1. Differential NOE: irradiation at δ 4.63—3% and 3% enhancement at δ 4.63 (H-11), 4.81 (H-15) and 2.46 (H16); irradiation at δ 4.63—3% and 3% enhancement at δ 6.85 (H-6) and 6.81 (H-8).

**Clauslactone-P (6):** Colorless cubes, mp 126—128 °C, [α]D24.3° (9.5 Hz), 7.43 (1H, d, J=9.7 Hz), 7.42 (1H, br d, J=1.8 Hz), 7.35 (1H, d, J=8.8 Hz), 7.08 (1H, d, J=8.8 Hz), 6.22 (1H, d, J=9.5 Hz), 5.67 (1H, br t, J=6.2 Hz), 5.20 (1H, ddd, J=1.8, 5.5, 7.7 Hz), 4.79 (2H, d, J=6.2 Hz), 4.26 (2H, br s), 3.90 (1H, dd, J=5.5, 13.9 Hz), 2.41 (1H, dd, J=7.7, 13.9 Hz), 1.87 (3H, s). Differential NOE: irradiation at δ 1.87 (13-Me)—4% enhancement at δ 4.79 (H-11); irradiation at δ 4.79—3% and 15% enhancement at δ 1.87 and 7.08 (H-6); No enhancement was observed at any proton signal on irradiation of the 8-MeO signal at δ 3.90.

Clauslactone-Q (7): Colorless cubes, mp 168—170 °C. [α]D0 −9.3° (c=0.160, CHCl3). HR-MS Calcd for C19H18O6: 342.1103. Found: 342.1108. EI-MS m/z: 342 [M]+, 324, 181, 162 (base peak), 134. UV λmax: 204, 280 (sh), 323 nm. IR νmax cm−1: 3400, 1750, 1720, 1600. 1H- and 13C-NMR see Table 1. Differential NOE: irradiation at δ 1.80 (13-Me)—5% enhancement at δ 4.69 (H-11); irradiation at δ 4.69—12%, 13% and 9% enhancement at δ 1.80, 7.00 (H-8) and 6.95 (H-6).

**Differential NOE of Clauslactone-K (1), -L (2) and -M (3)** Clauslactone-K (1): irradiation at δ 1.84 (13-Me)—4% and 5% enhancement at δ 2.41 (H-14) and 4.71 (H-11); irradiation at δ 1.91 (17-Me)—2% enhancement at δ 7.00 (H-16); Clauslactone-L (2): irradiation at δ 1.82 (13-Me)—4% enhancement at both δ 4.71 (H-11) and 4.50 (H-15); irradiation at δ 4.71—9% and 3% enhancement δ 6.86 (H-6) and 1.82 (13-Me); Clauslactone-M (3): irradiation at δ 1.83 (13-Me)—each 4% enhancement at δ 4.62 (H-11) and 4.50 (H-15); irradiation at δ 4.62—5%, 11% and 4% enhancement at δ 1.83, 6.81 (H-8) and 6.85 (H-6).

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**References and Notes**

