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# Coumarins from Malaysian *Micromelum minutum*

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**Abstract**

In a continuation of our study of the Rutaceae, detailed chemical investigation on *Micromelum minutum* (Rutaceae) collected from Sepilok, Sabah, Malaysia gave four new coumarins. The structures of the coumarins have been fully characterised by spectroscopic methods as 3″,4″-dihydrocapnolactone **1**, 2′,3′-epoxyisocapnolactone **2**, 8-hydroxyisocapnolactone-2′,3′-diol **3** and 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol **4**.
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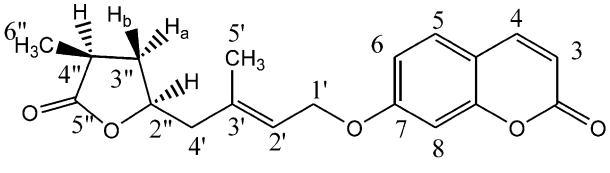
*Keywords:* *Micromelum minutum*; Rutaceae; Coumarins; 3″,4″-Dihydrocapnolactone; 2′,3′-Epoxyisocapnolactone; 8-Hydroxyisocapnolactone-2′,3′-diol; 8-Hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol

## 1. Introduction

*Micromelum minutum* (G. Forst.) Wight & Arn (Rutaceae) is a small to medium-sized tree or shrub commonly found in the forests and limestone areas in Peninsular Malaysia, Sabah and Sarawak (Jones, 1995). The leaves are traditionally used in the treatment of fever and giddiness and a poultice of the boiled roots is used for ague (Burkill, 1935). Leaves and stems of *Micromelum* species are known to contain numerous coumarins, particularly 6- and 8-prenylated coumarins (Cassady et al., 1979; Das et al., 1984; Tantishaiyakul et al., 1986; Kong et al., 1988). Previous studies have yielded different coumarins from two separate collections of this species from Peninsular Malaysia (Rahmani et al., 1993, 1994). A sample collected from one location gave micromelin and a new dihydrocinnamic acid derivative of micromelin, while another sample from a different location gave two new tetracyclic coumarins, microminutinin and 6-methoxymicrominutinin. In continuation of this work, we wish to report the isolation and structural elucidation of four new coumarins, 3″,4″-dihydrocapnolactone **1**, an isomer of oxycapnolactone in which the furan double bond has migrated from 3″/4″ to 4″/6″ (2′,3′-epoxyisocapnolactone) **2**, 8-hydroxyisocapnolactone-2′,3′-diol **3** and 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol **4** from the chloroform extract of the leaves of a collection from Sepilok, Sabah, Malaysia.

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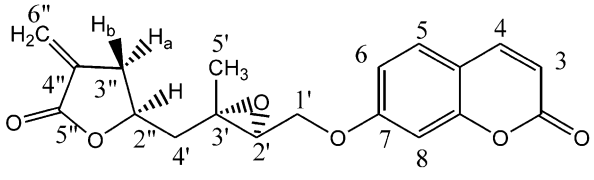


**2**

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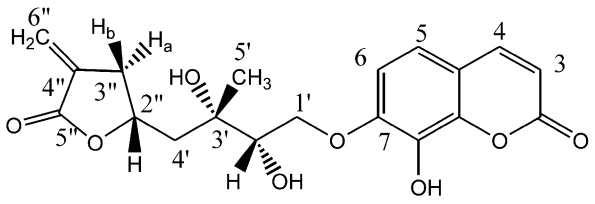

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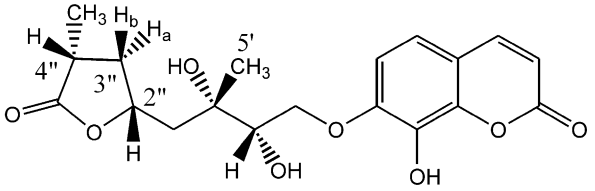

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## 2. Results and discussion

Leaves were successively extracted with petroleum ether, chloroform and methanol to give three dark viscous semisolid materials after solvent removal. The crude chloroform extract was separated by silica gel column chromatography to furnish four new coumarins (**1**–**4**) whose structures were characterised by detailed NMR investigations including $^{1}H$ and $^{13}C$ NMR, HSQC, COSY, HMBC and NOESY experiments. All these coumarins could be considered as umbelliferone derivatives with a $C_{10}$ side chain (De Silva et al., 1980). In each coumarin, the presence of a long-range correlation between H-1 and C-7 indicated the side chain to be attached at C-7. Table 1 and Table 2 lists $^{1}H$ and $^{13}C$ NMR spectral data of compounds (**1**–**4**).

Compound **1,** white needles, m.p. 59–62 °C had the molecular formula $C_{19}H_{20}O_5$ by FAB-MS because of the M + Na ion peak at 351.1195 (Calcd. for $C_{19}H_{20}O_5Na$: 351.1208). The UV spectrum had maxima at 289.8, 298.0 (sh), and 321.0 characteristic of a coumarin nucleus oxygenated at the C-7 position. IR bands at 1762 and 1732 $cm^{-1}$ indicated the presence of γ- and δ-lactone groups. An intense band at 1618 $cm^{-1}$ was characteristic of conjugated C–C double bonds. The NMR data indicated that **1** was an epimer of clauslactone-M, a colourless oil isolated from the twigs and leaves of *Clausena excavata* Burm. f. (Nakamura et al., 1998). The relative stereochemistry of the chiral centres could be established by examining the various cross peaks in the NOESY spectrum. Correlations between the $H_2$-1′ and $H_3$-5′ and between H-2′ and H-4′a/b indicate that the C-2′, C-3′-double bond was *E*. The H-2″ signal showed correlations with H-4″, H-3″a and H-5′ signals, while correlations were also observed between $H_3$-6″ and H-3″b signals which suggested *S* configurations for both C-2″ and C-4″. Hence **1** was (2′*E*, 2″*S*, 4″*S*) - 7 - [4′ - (4″ - methyl - 5″ - oxo - 2″ - tetrahydrofuranyl) - 3′ - methyl - 2′ - butenyloxy]coumarin or 3″,4″-dihydrocapnolactone.

Compound **2**, white needles, m.p. 103–105 °C, of composition $C_{19}H_{18}O_6$ because of the $M+Na^+$ ion peak at 365.0995 (Calcd. for $C_{19}H_{18}O_6Na$: 365.1001). UV (289.8, 298.0 (sh) and 319.5 nm) and IR (1753 and 1711 $cm^{-1}$) spectra indicated the presence of oxygenated coumarin nucleus, with γ- and δ-lactone groups. The $^{1}H$ NMR and $^{13}C$ NMR data for the coumarin nucleus were almost identical with those of **1**. In the side-chain, olefinic H-2′ in **1** was replaced by an oxymethine at δ 3.21 (1H, *dd*, *J* = 6.4, 3.4 Hz) typical of an epoxide. The C-5′ methyl signal was also shifted to higher field at δ 1.49 indicating the absence of an adjacent double bond. The terminus of the side-chain was presumably a γ-lactone as in **1**, but the methyl at C-4″ was replaced by an exomethylene group whose protons were represented by two triplets δ 6.27 (H-6″a) and 5.68 (H-6″b), while the signals due to H-2″, H-3″a and H-3″b appeared at δ 4.78 (1H, *m*), 3.16 (1H, *qt*) and 2.68 (1H, *dddd*), respectively. Selected C–H long-range correlations provided the carbon framework of **2** as shown in Fig. 1. Detailed examination of the NOESY spectral data allowed assignment of the relative configuration as (2′*R*,3′*S*,2″*S*)-7-4′-(4″-methylene-5″-oxo-2″-tetrahydrofuranyl)-3′-methyl-2′,3′-epoxybutoxycoumarin or 2′,3′-epoxyisocapnolactone.

Compound **3,** white needles, m.p. 72–73 °C, $C_{19}H_{20}O_8$ (EI-MS) had UV and IR spectra similar to that of **1** and **2**, except for the appearance of a broad strong band at 3424 $cm^{-1}$ which suggested the presence of hydroxyl groups. The presence of a 7,8,-dioxygenated coumarin nucleus was demonstrated by two pairs of doublets at δ 6.27 and 7.64 (each 1H, *J* = 9.5 Hz) and at δ 6.98 and 6.85 (each 1H, *J* = 8.6 Hz) due to H-3, H-4, H-5 and H-6, respectively. The presence of a 2′,3′-diol subunit was indicated by shifts of the H-2′, C-2′ and C-3′ signals to lower magnetic fields at δ 4.17, 78.0 and 72.2 compared with **2** and by the loss of water from the molecular ion. The terminal portion of the side chain corresponded to that of **2** but in the NOESY spectrum, the cross peaks between H-2′ and $H_3$-5′ signals clearly indicated the existence of the *erythro* form of the 2′,3′ diol with *R* and *S* configurations at C-2′ and C-3′, respectively. The H-2″ signal showed cross correlations with the $H_3$-5′ and H-2′ signals, which suggested the *R* configuration at C-2″. Thus, compound **3** was determined to be (2′*R*,3′*S*,2″*S*)-8-hydroxy-7-4′-(4″-methylene-5″-oxo-2″-tetrahydrofuranyl)-3′-methyl-2′,3′-dihydroxybutoxycoumarin or 8-hydroxyisocapnolactone-2′,3′-diol.

Compound **4** was the minor component of the chloroform extract and isolated as a colourless semisolid compound. The molecular ion peak could not be observed in the EI mass spectrum, however, a prominent fragment ion occurred at *m*/*z* 360, 2 MU more than

Table 1
$^1H$, $^{13}C$ NMR, HMBC, COSY and NOESY spectral data of (**1**) and (**2**)

| H/C | **1** | | | | | **2** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC | COSY | NOESY | $\delta_H$ | $\delta_C$ | HMBC | COSY | NOESY |
| 2 | | 161.4 | | | | | 161.3 | | | |
| 3 | 6.25 (1H, *d*, 9.5) | 113.4 | C-2, 10 | H-4 | | 6.28 (1H, *d*, 9.5) | 113.8 | C-2, 10 | H-4 | |
| 4 | 7.64 (1H, *d*, 9.5) | 143.6 | C-2, 9, 5 | H-3 | H-5 | 7.64 (1H, *d*, 9.5) | 143.5 | C-10, 2, 5, 9 | H-3 | H-5 |
| 5 | 7.37 (1H, *d*, 8.4) | 129.0 | C-6, 10, 4, 7, 9 | H-6 | H-4, 8 | 7.40 (1H, *d*, 8.6) | 129.1 | C-10, 4, 7, 9 | H-6 | H-4, 8 |
| 6 | 6.85 (1H, *dd*, 8.4; 2.4) | 113.4 | C-7, 8, 10 | H-5 | H-1′ | 6.90 (1H, *dd*, 8.6; 2.3) | 113.1 | C-7, 8,10 | H-5 | H-1′a, 1′b |
| 7 | | 162.1 | | | | | 161.8 | | | |
| 8 | 6.81 (1H, *d*, 2.4) | 101.9 | C-7, 9, 6, 10 | | H-1′, 5 | 6.87 (1H, *d*, 2.3) | 102.1 | C-7, 9, 6, 10 | | H-1′a, 1′b, 5 |
| 9 | | 156.1 | | | | | 156.0 | | | |
| 10 | | 113.3 | | | | | 113.2 | | | |
| 1′ | 4.62 (2H, *d*, 6.3) | 65.3 | C-2′, 7, 3′ | H-2′ | H-6, 8, 5′ | a) 4.36 (1H, *dd*, 11.2; 3.5) | 67.5 | C-2′, 3′, 7 | H-1′b | H-6, 8, 5′ |
| | | | | | | b) 4.08 (1H, *dd*, 11.2; 6.5) | | C-2′, 3′, 7 | H-1′a H-2′ | H-6, 8, 5′ |
| 2′ | 5.59 (1H, *m*) | 122.5 | C-1′, 4′, 5′ | H-1′ | H-4′a, 4′b | 3.21, (1H, *dd*, 6.4; 3.4) | 61.3 | | H-1′b | H-4′ |
| 3′ | | 137.2 | | | | | 58.5 | | | |
| 4′ | a) 2.52 (1H, *m*) | 45.4 | C-3′, 2″,2′, 5′, 3″ | H-2″, 4′b | H-2′ | 1.93 (2H, *m*) | 45.4 | C-3′, 2″, 2′, 5′, 3″ | H-2″ | H-2′ |
| | b) 2.39 (1H, *m*) | | C-3′, 2″,2′, 5′, 3″ | H-2″, 4′a | H-2′ | | | | | |
| 5′ | 1.83 (3H, *s*) | 17.5 | C-3′, 2′, 4′ | | H-1′, 2″ | 1.49 (3H, *s*) | 17.2 | C-3′, 4′ | | H-1′a, 1′b |
| 2″ | 4.50 (1H, *m*) | 76.9 | | H-3″a, 3″b, 4′a, 4′b | H-3″a, 4″, 5′ | 4.78 (1H, *m*) | 74.5 | | H-4′, 3″a, 3″b | H-5′, 3″a, 4′ |
| 3″ | a) 2.50 (1H, *m*) | 37.5 | C-2″, 4″,5″ | H-2″, 4″, 3″b | H-2″ | a) 3.16 (1H, *qt*) | 34.2 | C-2″, 4″, 5″, 6″ | H-2″, H-3″b | H-6″a |
| | b) 1.56 (1H, *dd*, 12.3; 1.8) | | C-2″, 4″,6″, 4′ | H-2″, 4″, 3″b | H-6″ | b) 2.68 (1H, *dddd*) | | C-2″, 4″, 5″, 6″ | H-2″, H-3″a | H-6″b, 4′ |
| 4″ | 2.68 (1H, *m*) | 36.0 | C-3″, 5″, 6″ | H-3″a, 3″b, 6″ | H-2″ | | 134.0 | | | |
| 5″ | | 179.4 | | | | | 170.0 | | | |
| 6″ | 1.27 (3H, *d*, 7.1) | 15.3 | C-4″,3″, 5″ | H-4″ | H-3″b | a) 6.27 (1H, *t*, 2.8) b) 5.68 (1H, *t*, 2.8) | 122.9 | C-4″, 3″, 5″ | | H-3″a H-3″b |

corresponding signal attributed to the loss of water (*m*/*z* 358) in **3**. The NMR spectral data were similar to those of **3** except for the terminal γ-lactone ring which corresponded to that of **1**; however the configuration at C-4″ differed and could be assigned by the NOESY spectrum which established correlations between $H_3$-6″ and H-3″a, together with the presence of H-2″ cross correlations with $H_3$-5′ and H-4′. Thus, **4** was determined as (2′*R*,3′*S*,2″*R*,4″*R* )-8-hydroxy-7-4′-(4″-methyl-5″-oxo-2″-tetrahydrofuranyl) - 3′ - methyl - 2′,3′ - dihydroxybutoxy-coumarin or 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol.

Three separate Malaysian populations of *Micromelum minutum* have thus afforded different coumarins (Rahmani et al., 1994). Although in all cases the compounds were oxygenated at C-7 with the normal geranyl or prenyl side-chain, while in the current study, two of the compounds were also oxygenated at C-8 (as in **3** and **4**) which has not been previously observed.

## 3. Experimental

### 3.1. General

All melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were recorded using KBr discs on a Perkin Elmer FTIR spectrophotometer model 1275X. The UV spectra were recorded on a Shimadzu UV 160A spectrophotometer in MeOH. The $^1H$ and $^{13}C$ NMR spectra were obtained on a Bruker DRX-500 spectrometer in $CDCl_3$ operating at 500 and 125 MHz, respectively. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal reference.

### 3.2. Plant material

The leaves of *Micromelum minutum* were collected from Sepilok, Sabah, Malaysia and a voucher specimen

Table 2
$^{1}H$, $^{13}C$ NMR, HMBC, COSY and NOESY spectral data of (**3**) and (**4**)

| H/C | **3** | | | | | **4** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC | COSY | NOESY | $\delta_H$ | $\delta_C$ | HMBC | COSY | NOESY |
| 2 | | 160.9 | | | | | 160.9 | | | |
| 3 | 6.27 (1H, *d*, 9.5) | 113.7 | C-2, 10 | H-4 | | 6.27 (1H, *d*, 9.5) | 113.6 | C-2, 10 | H-4 | |
| 4 | 7.64 (1H, *d*, 9.5) | 144.1 | C-3, 10, 2, 5, 9 | H-3 | H-5 | 7.63 (1H, *d*, 9.5) | 144.1 | C-3, 10, 2, 5, 9 | H-3 | H-5 |
| 5 | 6.98 (1H, *d*, 8.6) | 120.0 | C-6, 10, 7, 9 | H-6 | H-4 | 6.99 (1H, *d*, 8.5) | 120.1 | C-6, 10, 7, 9 | H-6 | H-4 |
| 6 | 6.85 (1H, *d*, 8.6) | 113.9 | C-7, 8, 10 | H-5 | | 6.85 (1H, *d*, 8.5) | 113.8 | C-5, 7, 8, 10 | H-5 | H-1′b |
| 7 | | 146.8 | | | | | 146.8 | | | |
| 8 | | 131.5 | | | | | 132.0 | | | |
| 9 | | 144.1 | | | | | 144.1 | | | |
| 10 | | 113.6 | | | | | 113.6 | | | |
| 1′ | a) 4.63 (1H, *dd*, 11.2; 1.9) | 65.3 | C-7 | H-1′b, H-2′ | H-5′ | a) 4.64 (1H, *dd*, 9.2; 1.9) | 65.3 | C-7 | H-1′b, 2′ | H-5′ |
| | b) 4.12 (1H, *m*) | | C-2′ | H-1′a H-2′ | H-5′, 4′b | b) 4.10 (1H, *dd*, 9.2; 1.3) | | C-2′ | H-1′a, 2′ | H-6 |
| 2′ | 4.17 (1H, *m*) | 78.0 | C-1′, 3′, 4′, 5′ | H-1′a, H-1′b | H-5′, 4′b | 4.18 (1H, *dd*, 1.3; 1.9) | 77.8 | C-1′, 3′, 5′ | H-1′a, 1′b | H-4′b, 5′ |
| 3′ | | 72.2 | | | | | 72.2 | | | |
| 4′ | a) 2.33 (1H, *dd*, 15.0; 3.1) | 44.4 | C-3′, 5′, 3″ | H-4′b, H-2″ | H-5′, 2″ | a) 2.32 (1H, *dd*) | 43.8 | C-3′, 5′ | H-4′b, 2′ | H-5′ |
| | b) 1.92 (*m*) | | C-3′, 2′, 2″,3″ | H-1′b H-2″ | H-5′, 2′, 1′b, 2″ | b) 1.86 (1H, *m*) | | C-3′, 2″, 3″ | H-4′a, 2′ | H-2′ |
| 5′ | 1.42 (3H, *s*) | 23.4 | C-3′, 2′, 4′ | | H-1′a, 1′b, 2′ | | | | | |
| 4′a, 4′b, 2″ | 1.42 (3H, *s*) | 23.4 | C-3′, 2′, 4′ | | H-1′a, 2′, 4′a, 2″ | | | | | |
| 2″ | 4.93 (1H, *m*) | 74.4 | | H-4′b, 4′b | | | | | | |
| 3″a, 3″b | H-5′, 2′, 4′a | | | | | | | | | |
| 4′b, 3″a, 3″b | 4.74 (1H, *m*) | 75.1 | | H-4′a, 4′b | | | | | | |
| 3′a, 3′b | H-5′, 4″ | | | | | | | | | |
| 3″ | a) 3.22 (1H, *qt*) | 35.4 | C-4″,5″, 6″ | H-2″, H-3″b | H-2″ | a) 2.65 (1H, *m*) | 38.6 | C-4″ | H-2′, 3′b | H-6″ |
| | b) 2.72 (1H, *m*) | | C-2″, 4″,4′, 6″ | H-2″, H-3″a | H-2″ | b) 1.62 (1H, *m*) | | C-4″ | H-2′, 3′a | H-4″ |
| 4″ | | 133.8 | | | | 2.67 (1H, *m*) | 35.4 | C-3″, 6″,4′, 6″ | H-6′ | |
| 5″ | | 169.8 | | | | | 179.1 | | | |
| 6″ | a) 6.26 (1H, *t*,2.6) | 123.0 | C-4″,3″, 5″ | | | 1.30 (3H, *d*, 6.7) | 15.1 | C-4″, 3″ | H-4′ | H-3″a |
| | b) 5.69 (1H, *t*, 2.6) | | C-3″, 5″ | | | | | | | |

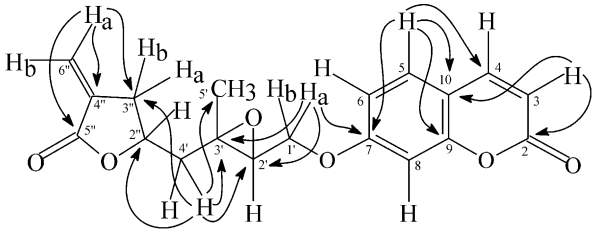


Fig. 1. Selected HMBC correlations of **2**.

was deposited at the Forest Research Centre, Sepilok, Sabah (accession number SAN 142904).

### 3.3. Isolation

The dried ground leaves (262.2 g) were extracted successively with petroleum ether, $CHCl_3$ and MeOH. The $CHCl_3$ extract was concentrated to give a dark green viscous residue (15.0 g). The extract was fractionated by vacuum chromatography over silica gel eluting with different mixtures of petroleum ether–chloroform and chloroform–methanol to give 23 fractions. Fraction 16 was further purified by repeated small column chromatography and recrystallized with acetone–petroleum ether to give 3″,4″-dihydrocapnolactone **1** (18.2 mg). Fraction 18 from the original vacuum column was subjected to repeated column chromatography and recrystallized with MeOH to give 2′,3′-epoxy-isocapnolactone **2** (80.6 mg). Fractions 21 and 22, obtained by vacuum chromatography separation, were further fractionated by column chromatography to give 22 fractions. Fractions 5–9 were combined, purified by column chromatography and recrystallized from chloroform and petroleum ether to yield 8-hydroxy-isocapnolactone-2′,3′-diol **3** (134.1 mg). Fractions 19–21 from the above column were combined to afford 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol **4** (4.8 mg).

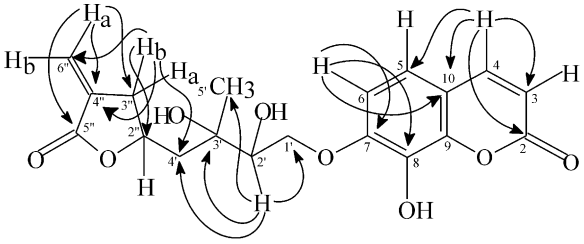

Fig. 2. Selected HMBC correlations of **3**.

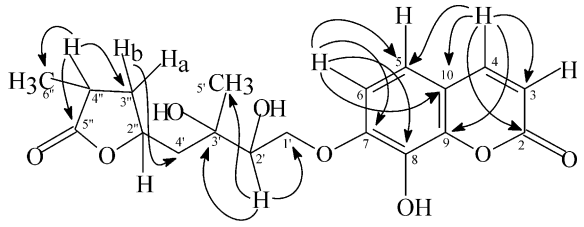

Fig. 3. Selected HMBC correlations of **4**.

3″,4″-Dihydrocapnolactone (**1**): White needles, m.p. 59–62 °C. $^1$H and $^{13}$C NMR ($CDCl_3$)—see Table 1. IR (KBr) cm$^{-1}$: 3050, 2978, 2938, 1762, 1732, 1618, 1460, 1354, 1276, 828. UV $\lambda_{max}$ (MeOH) nm (log $\epsilon$): 289.8 (3.92), 298.0 (sh), 321.0 (4.24). FAB-MS *m*/*z*: 351.1195 ($M+Na^+$) (Calcd. for $C_{19}H_{20}O_5Na$: 351.1208).

2′,3′-Epoxyisocapnolactone (**2**): White needles, m.p 103–105 °C. $^1$H and $^{13}$C NMR ($CDCl_3$)—see Table 1. IR (KBr) cm$^{-1}$: 3097, 3056, 2985, 2925,1753, 1711, 1623, 1453, 1352, 1282, 1245, 831. UV $\lambda_{max}$ (MeOH) nm (log $\epsilon$): 289.8 (3.98), 298.0 (sh), 319.5 (4.29). FAB-MS *m*/*z*: 365.0995 ($M+Na^+$) (Calcd. for $C_{19}H_{18}O_6Na$: 365.1001).

8-Hydroxyisocapnolactone-2′,3′-diol (**3**): White needles, m.p. 72–73 °C. $^1$H and $^{13}$C NMR ($CDCl_3$)—see Table 1 and Fig. 2. UV (MeOH) $\lambda_{max}$ nm (log $\epsilon$): 290.2 (3.89), 298.0 (*sh*), 319.5 (4.19). IR (KBr disc) cm$^{-1}$: 3424 (*br*), 2938, 1756, 1722, 1614, 1572, 1458, 1352, 1278, 830. EIMS *m*/*z* (% intensity): 376 ($M^+$, 0.55), 358 ($M\text{-}H_2O^+$, 14.57), 204 (100.00), 189 (4.70), 176 (8.68), 175 (10.10), 163 (4.35), 147 (18.58), 146 (4.41), 124 (3.84), 109 (3.33), 95 (18.34).

8-Hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol (**4**): Colorless semisolid. $^1$H and $^{13}$C NMR ($CDCl_3$)—see Table 1 and Fig. 3. EIMS *m*/*z* (% intensity): 360 ($M^+$-$H_2O$, 8.24), 274 (4.71), 204 (100.00), 189 (25.88), 175 (63.54), 147 (20.00), 97 (15.29).

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