Structures of Clausamine-A, -B, -C, Three Novel Carbazole Alkaloids from *Clausena anisata*[1)]

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Three novel carbazole alkaloids, named clausamine-A (1), -B (2), and -C (3) were isolated from *Clausena anisata* (Rutaceae) collected in Thailand, and their structures were elucidated by means of spectroscopic analyses. All these novel carbazoles have 1-oxygenated 3,4-disubstituted structures with a lactone moiety. This is the first report of the isolation of lactonic carbazole alkaloids from a natural source.

Key words *Clausena anisata*; carbazole alkaloid; clausamine; lactone; Rutaceae

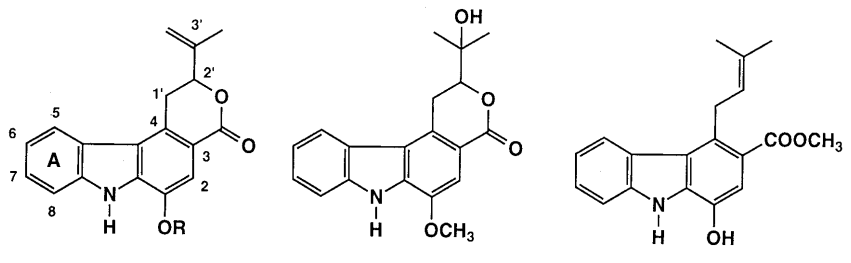
Our phytochemical studies of the constituents of the plants of the genus *Clausena* have resulted in the isolation of various coumarins and carbazole alkaloids.[2,3)] In this paper we wish to describe the isolation and structure elucidation of three novel carbazole alkaloids, clausamine-A (**1**), -B (**2**), and -C (**3**), from the branches of *Clausena anisata* (WILLD.) OLIV. collected in Thailand.

Results and Discussion

The dried branches of *C. anisata* were extracted with acetone at room temperature. The acetone extract was fractionated by a combination of silica gel column chromatography and preparative TLC to give three novel carbazole alkaloids, along with known carbazoles.

Structure of Clausamine-A (1) Clausamine-A (**1**) was obtained as a pale yellow powder, $[\alpha]_D$ 0°. The molecular formula was determined as $C_{18}H_{15}NO_3$ by high-resolution (HR)-MS. The UV spectrum [λ_{max}: 204, 222, 238, 248, 269, 278 (sh), 310 (sh), 322, 335 nm] was similar to that of clausine-F (**4**),[4)] which had previously been isolated by us from this plant. The ^{1}H-, ^{13}C-NMR (acetone-d_6) and IR spectra suggested the presence of an imino group [δ_H 10.94 (1H, br s), ν_{max} 3462 cm^{-1}], a hydroxyl group [ν_{max} 3329 (br) cm^{-1}], and a lactone carbonyl group [δ_C 166.03 (s), ν_{max} 1697 cm^{-1}]. The ^{1}H-NMR spectrum revealed a set of four-spin protons at δ_H 8.19 (1H, d, J=8.1 Hz, H-5), 7.25 (1H, t, J=8.1 Hz, H-6), 7.45 (1H, t, J=8.1 Hz, H-7), and 7.66 (1H, d, J=8.1 Hz, H-8), as well as a lone singlet at δ_H 7.55 (1H, s, H-2). These spectral data suggested the presence of a 1-hydroxy-3-substituted carbazole skeleton having no substituent in the A-ring.[5–7)] Further, the ^{1}H- and ^{13}C-NMR spectra showed signals assignable to a vinyl methyl group [δ_H 1.98 (3H, s); δ_C 18.57 (q)], a vinyl methylene [δ_H 5.26 and 5.07; δ_C 113.58], a methine [δ_H 5.12 (1H, dd, J=3.7, 11.4 Hz); δ_C 81.18 (d)] on a carbon bearing an oxygen atom, a methylene [δ_H 3.71 (1H, dd, J=3.7, 16.5 Hz), 3.52 (1H, dd, J=11.4, 16.5 Hz); δ_C 29.80 (t)], and a quaternary vinyl carbon [δ_C 144.82 (s)] in addition to signals due to the carbazole nucleus. In ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectroscopy (Fig. 1), C–H three- bond correlations were detected between the carbon signal at δ_C 81.18 (C-2′) and a vinyl methyl group at δ_H 1.98 (3′-CH_3), and between the carbon signal at δ_C 81.18 (C-2′) and methylene protons at δ_H 5.26, 5.07 (3′-CH_2). This led to the following structure of the side chain: –CH_2CH(OR)–CCH_3(=CH_2). The presence of a lactone carbonyl group at C-3 was suggested by a significant C–H three-bond correlation between a carbonyl carbon at δ_C 166.03 (C=O) and a lone singlet at δ_H 7.55 (H-2), which was also related to the quarternary carbon (C-9a, δ_C 134.40) bearing a nitrogen atom. Further, the presence of a C_5 unit at C-4 in this compound was indicated by the following results of HMBC and nuclear Overhauser effect (NOE) experiments: a) the observations of C–H three- or two-bond correlation between a lone singlet (H-2, δ_H 7.55) and a carbon at δ_C 128.54 (C-4), which was also related to the methylene protons (H-1′, δ_H 3.71, 3.52); b) the methylene protons (H-1′, δ_H 3.71, 3.52) showed long-range correlations with the carbon signals at δ_C 117.05 (C-4a); c) an NOE enhancement between the H-5 (δ 8.19) and H-1′ (δ 3.71) signals was observed.

On the basis of these spectral data, coupled with MS fragmentations (Fig. 2), we assigned the structure **1** to



1, R = H
2, R = CH_3

3

4

Chart 1

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clausamine-A.

Structure of Clausamine-B (2) Clausamine-B (**2**) was isolated as a colorless oil, $[\alpha]_D$ 0°. The molecular formula

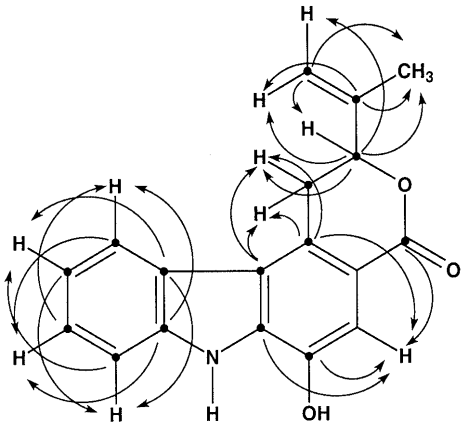

Fig. 1. C–H Three-Bond Long-Range Correlations in the HMBC Spectrum of Clausamine-A (**1**) in Acetone-d_6

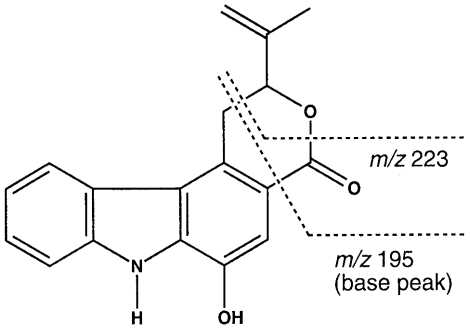

Fig. 2. MS Fragmentations of Clausamine-A (**1**)

$C_{19}H_{17}NO_3$, a difference of CH_2 compared with **1**, was established by HR-MS. The UV spectrum (see Experimental) was similar to that of **1**. The ^{1}H-NMR spectrum (Table 1) also showed a similar signal pattern to that of **1**, except for the additional 3H singlet at δ 4.07. In NOE experiments, irradiation of the methoxy group at δ 4.07 resulted in a 20% area increase of the signal at δ 7.66 (H-2). These spectral data, together with the results of the HMBC spectrum (Fig. 3), indicated the presence of a methoxy group at C-1 on **2**, instead of a hydroxyl group on **1**. These spectral data let us to assign the structure **2** to clausamine-B.

Structure of Clausamine-C (3) Clausamine-C (**3**) was obtained as a pale yellow oil, $[\alpha]_D$ 0°. The HR-MS analysis indicated the molecular formula to be $C_{19}H_{19}NO_4$. The UV spectrum showed bands at λ_{max} 204, 223, 238, 249, 270, 278 (sh), 312 (sh), 322, 336 nm, which were similar to those of **1** and **2**. The IR spectrum exhibited bands at ν_{max} 3464, 3400 (br), and 1703 cm^{-1} due to imino, hydroxyl, and lactone carbonyl groups, respectively. In the ^{1}H-NMR spectrum, the appearance of a four-spin system in the aromatic proton region (Table 1) indicated that one of the rings on the carbazole nucleus was unsubstituted. The ^{1}H-NMR spectrum revealed a 3H singlet at δ 4.07 (OCH_3) and a lone 1H singlet at δ 7.63, in addition to a broad singlet at δ 8.66 (NH). In the NOE experiment, an NOE enhancement between the 3H singlet (δ 4.07) and the 1H singlet at δ 7.63 was observed. Further, ABC-type signals at δ 3.67 (1H, dd, J=3.3, 16.1 Hz, H-1′), 3.47 (1H, dd, J=12.8, 16.1 Hz, H-1′), and 4.47 (1H, dd, J=3.7, 12.8 Hz, H-2′), and two 3H singlets at δ 1.50 and 1.47 assignable to two methyls attached to a carbon atom bearing an oxygen function were seen. Based on the

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for the Novel Carbazole Alkaloids in $CDCl_3$

	1[a]		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	—	142.86 (s)	—	144.60 (s)	—	144.59 (s)
1-OCH_3	—	—	4.07 (3H, s)	55.94 (q)	4.07 (3H, s)	55.92 (q)
2	7.55 (s)	110.89 (d)	7.66 (s)	106.55 (d)	7.63 (s)	105.40 (d)
3	—	121.56 (s)	—	116.38 (s)	—	119.79 (s)
4	—	128.54 (s)	—	128.70 (s)	—	128.90 (s)
4a	—	117.05 (s)	—	120.27 (s)	—	116.02 (s)
4b	—	124.30 (s)	—	123.43 (s)	—	123.41 (s)
5	8.19 (d, 8.1)	122.93 (d)	8.10 (d, 7.7)	121.95 (d)	8.09 (d, 7.7)	121.95 (d)
6	7.25 (t, 8.1)	120.75 (d)	7.31 (t, 7.7)	120.63 (d)	7.33 (t, 7.7)	120.69 (d)
7	7.45 (t, 8.1)	126.66 (d)	7.49 (t, 7.7)	126.22 (d)	7.50 (t, 7.7)	126.24 (d)
8	7.66 (d, 8.1)	112.66 (d)	7.55 (d, 7.7)	111.58 (d)	7.55 (d, 7.7)	111.58 (d)
8a	—	141.40 (s)	—	139.45 (s)	—	139.45 (s)
9a	—	134.40 (s)	—	133.50 (s)	—	133.65 (s)
NH	10.94 (br s)	—	8.64 (br s)	—	8.66 (br s)	—
C=O	—	166.03 (s)	—	166.58 (s)	—	166.38 (s)
1′	3.71 (dd, 3.7, 16.5) 3.52 (dd, 11.4, 16.5)	29.8 (t)[b]	3.66 (dd, 4.0, 16.5) 3.54 (dd, 11.4, 16.5)	29.70 (t)	3.67 (dd, 3.3, 16.1) 3.47 (dd, 12.8, 16.1)	25.49 (t)
2′	5.12 (dd, 3.7, 11.4)	81.18 (d)	5.10 (dd, 4.0, 11.4)	80.88 (d)	4.47 (dd, 3.3, 12.8)	83.98 (d)
3′	—	144.22 (s)	—	142.28 (s)	—	71.39 (s)
3′-CH_3	1.98 (3H, s)	18.57 (q)	2.00 (3H, s)	18.39 (q)	1.50 (3H, s) 1.47 (3H, s)	25.99 (q) 24.81 (q)
3′-CH_2	5.26 (br s) 5.07 (d, 1.1)	113.58 (t)	5.25 (br s) 5.10 (br s)	114.08 (t)	—	—

Values in (δ_H and δ_C) ppm. The coupling constants (J) in parentheses are in Hz. All signals correspond to 1H, and were observed as singlets, unless otherwise stated. *a*) Spectra were taken in acetone-d_6. *b*) Overlapped with the solvent.

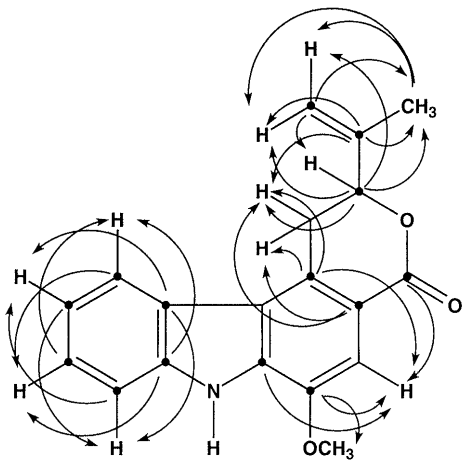

Fig. 3. C–H Three-Bond Long-Range Correlations in the HMBC Spectrum of Clausamine-B (**2**) in $CDCl_3$

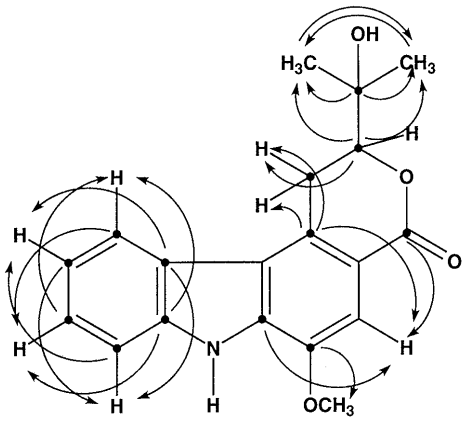

Fig. 4. C–H Three-Bond Long-Range Correlations in the HMBC Spectrum of Clausamine-C (**3**) in $CDCl_3$

aforementioned results, coupled with a significant mass fragment ion at m/z 267 $[M^+ - \cdot C(CH_3)_2\text{–}OH + \cdot H]$ in the EI-MS and the HMBC results shown by arrows in Fig. 4, the structure of clausamine-C was concluded to be **3**.

These alkaloids contain a lactone moiety in the molecule, and are the first examples of lactonic carbazole alkaloids to be found in nature. Other compounds isolated from the acetone extract were characterized as ekeberginine,[8] methyl carbazole-3-carboxylate,[9] clausine-F (**4**),[4] clausine-E,[10] and *O*-demethylmurrayanine,[11] by comparisons of the ^{1}H-NMR, IR, UV, and MS data with those reported in the literature.[4,8–11]

Experimental

^{1}H- and ^{13}C-NMR, NOE, ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), and HMBC (J=8 Hz) spectra were recorded on an A-400 or A-600 (JEOL) spectrometer, in $CDCl_3$. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. All mass spectra were taken under electron impact (EI) conditions, unless otherwise stated, using an M-80 (Hitachi) spectrometer having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (Jasco) in MeOH, IR spectra on an IR-230 (Jasco) in $CHCl_3$, optical rotations on a DIP-370 (Jasco) in $CHCl_3$ at 25 °C, and CD spectra on a J-600 (Jasco) in MeOH. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

Plant Materials The plant materials used in this study, *Clausena anisata* (WILLD.) OLIV., were collected during January–February 1996 in Kanchanaburi province, Thailand. Authentication was achieved by comparison with the herbarium specimen at the Royal Forest Department, Ministry of Agriculture and Cooperative, Thailand. A voucher specimen has been deposited in the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction and Isolation The dried branches (120 g) of *C. anisata* were extracted with acetone (800 ml × 3) at room temperature. The acetone extract (1.02 g) was subjected to silica gel column chromatography eluted with hexane, hexane–acetone (9 : 1, 4 : 1, 3 : 1, 3 : 2, 1 : 1), acetone, $CHCl_3$–MeOH (3 : 1), MeOH, successively, to give 9 fractions. The hexane–acetone (4 : 1) eluate was subjected to preparative silica gel TLC (PTLC) developed with hexane–$CHCl_3$ (3 : 7) to afford ekeberginine (0.3 mg) and methyl carbazole-3-carboxylate (0.5 mg). The hexane–acetone (3 : 1) eluate was subjected to PTLC developed with $CHCl_3$ to afford clausamine-B (**2**) (1.1 mg) and clausine-F (**4**) (27.6 mg). The hexane–acetone (3 : 2) eluate was subjected to PTLC developed with CH_2Cl_2–acetone (9 : 1) to afford six fractions 1—6. Fraction 4 was also subjected to PTLC developed with $CHCl_3$–MeOH (99 : 1) to give clausine-E (3.7 mg). Fraction 5 was subjected to PTLC developed with $CHCl_3$–MeOH (49 : 1) to give clausamine-C (**3**) (1.0 mg) and *O*-demethylmurrayanine (1.6 mg). Fraction 6 was subjected to PTLC developed with iso-Pr_2O–MeOH (19 : 1) to afford clausamine-A (**1**) (1.0 mg).

Clausamine-A (**1**): Pale yellow powder. $[\alpha]_D$ 0° (c=0.072). CD (MeOH, 200—400 nm): no absorption. UV λ_{max} nm: 204, 222, 238, 248, 269, 278 (sh), 310 (sh), 322, 335. IR ν_{max} cm^{-1}: 3462, 3329 (br), 1697, 1589, 1508. EI-MS m/z (%): 293 (M^+, 42), 249 (22), 234 (32), 223 (27), 195 (100), 167 (34), 151 (17). NOE: irradiation of H-5 (δ 8.19) gave 7% NOE at H-1′ (δ 3.71); irradiation of H-1′ (δ 3.71) gave 14% NOE at H-5 (δ 8.19), 25% NOE at H-1″ (δ 3.52) and 8% NOE at H-2″ (δ 5.12). HR-MS Calcd for $C_{18}H_{15}NO_3$: 293.1050. Found: 293.1050.

Clausamine-B (**2**): Colorless oil. $[\alpha]_D$ 0° (c=0.082). CD (MeOH, 200—400 nm): no absorption. UV λ_{max} nm: 205, 222, 237, 248, 269, 278 (sh), 310 (sh), 321, 334. IR ν_{max} cm^{-1}: 3464, 1699, 1603. EI-MS m/z (%): 307 (M^+, 100), 263 (30), 248 (28), 237 (26), 209 (73), 194 (12), 180 (14), 166 (12). NOE: irradiation of 1-OCH_3 (δ 4.07) gave 20% NOE at H-2 (δ 7.66); irradiation of H-5 (δ 8.09) gave 7% NOE at H-1′ (δ 3.66). HR-MS Calcd for $C_{19}H_{17}NO_3$: 307.1207. Found: 307.1209.

Clausamine-C (**3**): Pale yellow oil. $[\alpha]_D$ 0° (c=0.037). CD (MeOH, 200—400 nm): no absorption. UV λ_{max} nm: 204, 223, 238, 249, 270, 278 (sh), 312 (sh), 322, 336. IR ν_{max} cm^{-1}: 3464, 3400 (br), 1703, 1587, 1510. EI-MS m/z (%): 325 (M^+, 57), 307 (11), 292 (7), 278 (7), 267 (58), 252 (7), 250 (8), 238 (100), 224 (9), 222 (16), 210 (21), 206 (7). 195 (42). NOE: irradiation of 1-OCH_3 (δ 4.07) gave 16% NOE at H-2 (δ 7.63); irradiation of H-5 (δ 8.09) gave 8% NOE at H-1′ (δ 3.67). HR-MS Calcd for $C_{19}H_{19}NO_4$: 325.1313. Found: 325.1317.

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References and Notes

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