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Lactonic Carbazole Alkaloids from the Root Bark of *Clausena excavata*

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Examination of the extract from the root bark of *Clausena excavata*, yielded four new lactonic carbazole alkaloids, clausevatine-D (1), -E (2), -F (3), -G (4), as well as the known clausamine-A (5). Their structures were elucidated by spectroscopic analyses.

Key words *Clausena excavata*; Rutaceae: lactonic carbazole alkaloid

Clausena (*C.*) *excavata* (Rutaceae) is a wild shrub that is used as a folk medicine in the treatment of snakebites, abdominal pain and as a detoxificant.[1)] In continuation of our investigations on carbazole alkaloids from the Rutaceae plants of Taiwan,[2)] the acetone extract of the root bark of *C. excavata* was subjected to repeated chromatography on silica gel to give four new lactonic carbazole alkaloids, clausevatine-D (**1**), -E (**2**), -F (**3**) and -G (**4**), and the known alkaloid, clausamine-A (**5**).[3)] We report herein the structural elucidation of these new compounds by spectroscopic analyses.

Clausevatine-D (**1**) exhibited the molecular formula $C_{18}H_{17}NO_4$, from the pseudo molecular ion at *m/z* 312 ($M^+ + 1$) in the FAB-MS spectrum. The UV (λ_{max} 202, 223, 241, 251, 272, 278 (sh), 325, 340 nm) and IR (ν_{max} 3370, 1700 cm^{-1}) spectra were similar to those of clausamine-A (**5**) and suggested a 1-hydroxy-3-carbonyloxycarbazole alkaloid derivative.[3)] Thus, the down field D_2O exchangeable aromatic hydroxyl at δ 9.14 in the ^{1}H-NMR spectrum was assigned to the proton at C-1. Four mutually coupled ^{1}H signals in the aromatic region at δ 7.27 (td, J=7.7, 1.0 Hz, H-6), 7.47 (td, J=7.7, 1.0 Hz, H-7), 7.68 (dd, J=7.7, 1.0 Hz, H-8) and 8.21 (dd, J=7.7, 1.0 Hz, H-5) revealed an unsubstituted A-ring in the carbazole skeleton (Table 1). A lone aromatic singlet at δ 7.55 showed that only one C-ring carbon had no substituent. The location of this unsubstituted carbon was proven to be C-2 due to the presence of long range ^{1}H–^{13}C interactions between H-2 (δ 7.55) and C-1 (δ 142.9) and C=O (δ 166.4) in the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) experiment, as well as the absence of a nuclear Overhauser effect (NOE) between H-2 and H-5 in the nuclear Overhauser and exchange spectroscopy (NOESY) experiment (Figs. 1 and 2). In addition, the presence of the $-CH_2CH(OR)-$ fragment was supported by two benzylic signals at δ 3.42 (dd, J=16.5, 12.6 Hz) and 3.78 (dd, J=16.5, 3.4 Hz) which coupled with an oxygenated methine signal at δ 4.44 (dd, J=12.6, 3.4 Hz). Thus, a lactone ring fused to ring C, using the bond between C-3 and C-4 was established. It was further confirmed by the existence of NOE between H-1′ (δ 3.42 and 3.78) and H-5 (δ 8.21). Finally, a $(CH_3)_2C(OH)-$ (2-hydroxy-2-methylethyl) side chain attached to C-2′ was obtained from the remaining ^{1}H-NMR signals at δ 1.43 (s, 2×Me), 3.97 (br s, OH) and ^{13}C-NMR signals at δ 26.8 (2×Me), 71.3 (a quaternary carbon). In spite of the geminal coupling between the two benzylic protons, larger axial–axial coupling (12.6 Hz) and the smaller axial–equatorial coupling (3.4 Hz) of H-2′ with two H-1′, as well as the presence of NOE between H-2′ and only the equatorial H-1′ (δ 3.78) indicated this 2-hydroxy-2-methylethyl substituent should be located in an equatorial position. The full assignment of the ^{1}H-NMR and ^{13}C-NMR signals were confirmed by ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), HMBC and NOESY spectra (Figs. 1 and 2). These results let us to propose that clausevatine-D possessed the structure of **1** with an equatorial 2-hydroxy-2-methylethyl group.

The FAB-MS peaks at *m/s* 328 ($M^+ + 1$) indicated that clausevatine-E (**2**) and -F (**3**) were isomers, with a molecular formula of $C_{18}H_{17}NO_5$. By comparison of the ^{1}H-NMR spectra of **2** and **3** with that of **1**, an aliphatic hydroxyl signal replacing one of the benzylic protons was observed. Furthermore, the UV, IR and ^{1}H-NMR spectra of **2** were almost the same as those of **3** which inferred that these two molecules

Table 1. ^{1}H-NMR Spectral Data for Compounds **1**—**4** (Acetone-d_6, δ, multiplicity, J, Hz)

	1	2	3	4
1-OH	9.14 br s	9.35 br s	9.44 br s	9.60 br s
H-2	7.55 s	7.55 s	7.55 s	7.62 s
H-5	8.21 dd (7.7, 1.0)	8.31 d (8.0)	8.34 d (8.0)	8.35 d (8.0)
H-6	7.27 td (7.7, 1.0)	7.28 t (8.0)	7.27 t (8.0)	7.34 t (8.0)
H-7	7.47 td (7.7, 1.0)	7.48 t (8.0)	7.46 t (8.0)	7.49 t (8.0)
H-8	7.68 dd (7.7, 1.0)	7.67 d (8.0)	7.66 d (8.0)	7.73 d (8.0)
NH	10.87 br s	10.87 br s	10.94 br s	11.06 br s
H-1′	3.42 dd (16.5, 12.6) (ax.) 3.78 dd (16.5, 3.4) (eq.)	5.84 dd (4.0, 1.9) (eq.)	5.87 dd (6.7, 1.3) (eq.)	7.65 s
1′-OH	—	5.35 d (4.0)	4.92 d (6.7)	—
H-2′	4.44 dd (12.6, 3.4) (ax.)	4.38 d (1.9) (ax.)	4.65 d (1.3) (eq.)	—
3′-OH	3.97 br s	4.71 s	4.10 s	4.70 s
2×3′-Me	1.43 s	1.53, 1.61 s	0.92, 1.35 s	1.61 s

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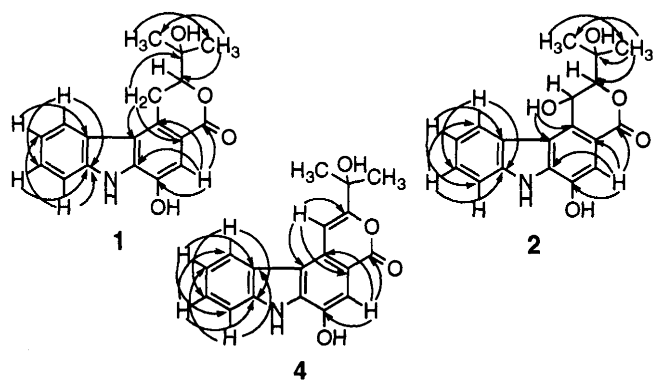
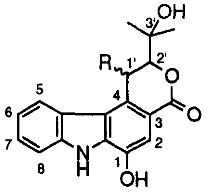
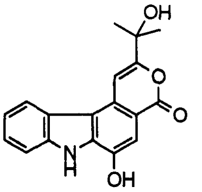


Fig. 1. The HMBC spectral data of **1**, **2** and **4**



1: R = H
2: R = *cis*-OH
3: R = *trans*-OH



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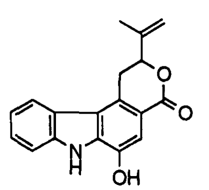

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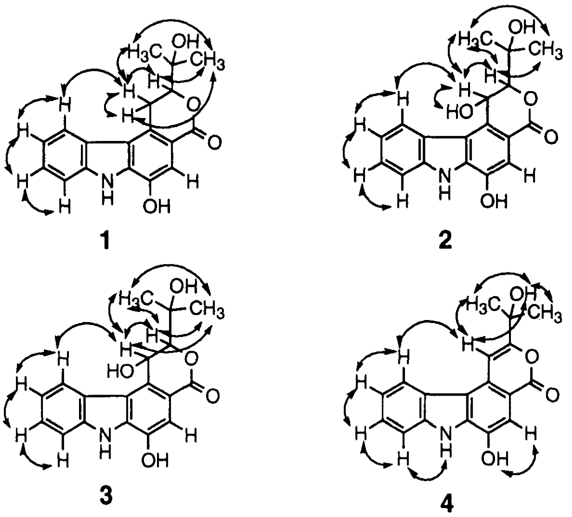

Fig. 2. The NOESY spectral data of compounds **1**—**4**

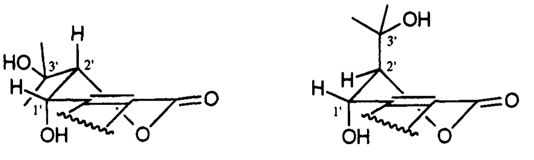

Fig. 3. The lactonic conformations of **2** and **3**

were diastereomers. It is well known that the chemical shift of equatorial protons are more downfield than those of axial protons in a cyclohexane ring.[4] Therefore, the upfield H-2′ at δ4.38 (d, J=1.9 Hz) in **2** is orientated in an axial position whereas the downfield H-2′ at δ4.65 (d, J=1.3 Hz) in **3** is equatorial. Moreover, the two methyls (δ0.92 and 1.35) in the 2-hydroxy-2-methylethyl group of **3**, located in the axial position, were located upfield compared to those in **2** (δ1.53 and 1.61) (Table 1). The small coupling constant between H-2′ and H-1′, together with the NOE enhancement between the benzylic proton (δ5.84 in **2** and 5.87 in **3**) and the two methyls, indicated that H-1′ in both molecules should be located in an equatorial orientation as shown in Fig. 3. Based on the above analysis, clausevation-E possessed the structure **2** with a *cis* configuration, and clausevatine-F was determined to have the structure **3** with a *trans* geometry in the lactone ring.

Clausevatine-G (**4**) was established to have the molecular formula $C_{18}H_{15}NO_4$, two hydrogens less than that of **1**, by the pseudo molecular ion at *m/z* 310 (M^++1) in the FAB-MS spectrum, and the high resolution electron ionization mass spectrometry (HR-EI-MS) for a base ion at *m/z* 291 (M^+-H_2O). Compared to the ^{1}H-NMR spectrum of **1**, the disappearance of aliphatic signals and the appearance of an olefinic singlet at δ7.65 confirmed a double bond in the lactone ring conjugated with the carbazole ring nucleus. On the other hand, the observation of NOEs for H-8 (δ7.73) with the signal at δ11.06 (br s), and H-1 (δ7.62) with the signal at δ9.60 (br s) led to the conclusion that the signal at δ11.06 was for the carbazole NH and the signal at δ9.60 for the 1-OH. Since the common structural feature of a lactone ring fused to a 1-hydroxycarbazole alkaloid existed in compounds **1**—**4**, we assigned the NH and OH signals of the other three compounds shown in Table 1 by the similiarity in ^{1}H-NMR spectra. Consequently, the structure **4** was deduced for clausevatine-G.

Experimental

Melting points were measured on a Yanagimoto MP-S_3 micromelting point apparatus and not corrected. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH solution. The IR spectra were recorded on a Jasco IR Report-100 spectrophotometer as KBr discs. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AC-200, AMX-400 and Varian-400 Unity Plus spectrometers. Chemical shifts are shown in δ values with tetramethylsilane as internal reference. The mass spectra were obtained on a VG 70-250 S spectrometer *via* a direct inlet system. Specific rotations were recorded on a Jasco DIP-370 polarimeter.

Plant Material *C. excavata* was collected from San Dei Men, Pingtung Hsien, Taiwan in June 1989 and verified by Prof. C. S. Kuoh. A specimen of this plant is deposited in the herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation The root bark of *C. excavata* (0.8 kg) was extracted with acetone (3 l×6) at room temperature. The acetone extract (98.3 g) was subjected to chromatography on a silica gel column and eluted with $CHCl_3$–MeOH (25 : 1) to give eight fractions. Fraction 4 was chromatographed on silica gel column and eluted with C_6H_6–Me_2CO *(4 : 1) to* yield **5** (1.0 mg). Fraction 5 was chromatographed on a silica gel column and eluted with $CHCl_3$–MeOH (30 : 1) to afford **1** (10.4 mg) and **4** (1.5 mg). Fraction 6 was chromatographed on a silica gel column and eluted with iso-Pr_2O–MeOH–C_6H_{14} (9 : 1 : 1) to obtain **2** (1.0 mg) and **3** (0.5 mg).

Clausevatine-D (**1**): Yellow granules, mp 241—244 °C, $[\alpha]_D$ −5.7° (c=0.932, MeOH). UV λ_{max} nm: 202, 223, 241, 251, 272, 278 (sh), 325, 340. IR ν_{max} cm^{-1}: 3370, 1700, 1590. FAB-MS *m/z*: 350 ($[M+K]^+$), 334 ($[M+Na]^+$), 312 ($[M+H]^+$). EI-MS (rel. int.) *m/z*: 311 (M^+, 95), 293 (27), 291 (26), 253 (99), 224 (100), 208 (18), 196 (37). ^{13}C-NMR (acetone-d_6, 100 MHz) δ: 25.3 and 26.8 (2×Me), 26.0 (C-1′), 71.3 (C-3′), 84.8 (C-2′), 110.8 (C-2), 112.7 (C-8), 116.9 (C-4a), 120.7 (C-6), 121.5 (C-4), 122.9 (C-5), 124.4 (C-5a), 126.6 (C-7), 129.0 (C-3), 134.5 (C-1a), 141.4 (C-8a), 142.9 (C-1), 166.4 (C=O).

Clausevatine-E (**2**): Yellowish granules, mp 208—212 °C, $[\alpha]_D$ −92.4°

(c=0.0552, MeOH). HR-EI-MS for $C_{18}H_{13}NO_3$ ($C_{18}H_{17}NO_5$–2×H_2O): Calcd: 291.0895. Found: 291.0893. UV λ_{max} nm: 213 (sh), 222, 240, 251, 271, 282, 315 (sh), 325, 339. IR ν_{max} cm^{-1}: 3350, 1698, 1685, 1585. FAB-MS *m/z*: 328 ($[M+H]^+$). EI-MS (rel. int.) *m/z*: 327 (M+, 1), 309 (3), 291 (22), 251 (88), 223 (100), 195 (47), 167 (17), 139 (33). ^{13}C-NMR (acetone-d_6, 100 MHz) δ: 26.6 and 27.0 (2×Me), 63.3 (C-1′), 72.9 (C-3′), 84.5 (C-2′), 110.1 (C-2), 112.5 (C-8), 116.7 (C-4a), 120.7 (C-6), 121.7 (C-4), 123.3 (C-5), 126.8 (C-7), 129.1 (C-1a), 129.5 (C-5a), 134.5 (C-3), 141.3 (C-8a), 144.3 (C-1), 167.0 (C=O).

Clausevatine-F (**3**): Colorless granules, mp 162—164 °C, $[\alpha]_D$ −199.0° (c=0.0203, MeOH). HR-EI-MS for $C_{15}H_9NO_3$($C_{18}H_{17}NO_5$–$C_3H_8O_2$): Calcd: 251.0582. Found: 251.0583. UV λ_{max} nm: 202, 222, 241, 252, 271, 281, 314 (sh), 326, 339. IR ν_{max} cm^{-1}: 3430, 1695, 1680, 1670. FAB-MS *m/z*: 328 ($[M+H]^+$). EI-MS (rel. int.) *m/z*: 291 ($[M-2\times H_2O]^+$, 11), 251 (65), 223 (100), 195 (44), 167 (18), 139 (35).

Clausevatine-G (**4**): Yellow granules, mp >280° C. HR-EI-MS: Calcd for $C_{18}H_{13}NO_3$($C_{18}H_{15}NO_4$–H_2O) *m/z*: 291.0895. Found: 291.0894. UV λ_{max} nm: 207, 223, 264 (sh), 274, 287, 338, 353. IR ν_{max} cm^{-1}: 3370, 1670, 1580. EI-MS (rel. int.) *m/z*: 291 (M^+-H_2O, 100). 278 (14), 263 (30), 248 (13), 234 (13), 220 (18), 194 (50). FAB-MS *m/z*: 348 ($[M+K]^+$), 332 ($[M+Na]^+$), 310 ($[M+H]^+$). ^{13}C-NMR (atetone-d_6) δ: 29.0 (2×Me), 71.2 (C-3′), 97.2 (C-2), 108.8 (C-1′), 113.1 (C-4, C-8), 121.3 (C-6), 122.5 (C-5), 124.3 (C-5a), 126.6 (C-7), 128.5 (C-4a), 135.4 (C-3), 141.1 (C-8a), 144.2 (C-1a), 163.0 (C-2′), 163.4 (C-1, C=O).

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