A New Phenanthrene Alkaloid, Romucosine I, form *Rollinia mucosa* Baill.

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Bioactivity-directed fractionation led to the isolation of a new *N*-(methoxycarbonyl) phenanthrene alkaloid, romucosine I (**1**), along with three known *N*-(methoxycarbonyl) alkaloids, romucosine C (**2**), tuduranine (**3**), and promucosine (**4**). The structures of these compounds were identified on the basis of spectral data and chemical evidence. A proposed biogenesis about these isolates is also reported in this paper.

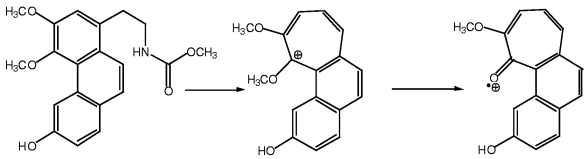
Key words: Rollinia mucosa, Romucosine I, Tuduranine, Phenanthrene Alkaloid

Introduction

The genus *Rollinia* (Annonaceae) is comprised of 65 species [1], and some of them were investigated previously for their chemical constituents and pharmacological activities [1 – 7]. Within the genus, *Rollinia mucosa* has been used as a folk medicine for the treatment of tumors in the West Indies and Indonesia [1], and its alkaloidal extracts were shown to exhibit antimicrobial, antiplatelet, and antifungal activities [2, 8]. In our previous studies, this plant produced aporphine alkaloids, porphyrins, lignans and acetogenins [3, 5, 6, 8 – 10]. In the continuing research on bioactive compounds from this Formosan Annonaceous plant, a new *N*-(methoxycarbonyl) phenanthrene alkaloid, romucosine I (**1**), along with three known *N*-(methoxycarbonyl) alkaloids, romucosine C (**2**), tuduranine (**3**), and promucosine (**4**), was isolated [8, 11]. The structure elucidation of compound **1** was established on the basis of spectroscopical and chemical evidence.

Results and Discussion

Romucosine I (**1**) was isolated as a brown amorphous powder; HREIMS revealed a $[M]^+$ at *m/z* 355.1425 (calcd. 355.1420), corresponding to the molecular formula $C_{20}H_{21}O_5N$. The UV spectrum of **1** showed absorption maxima at 251, 259, 304, and 316 nm, which suggested **1** being a phenanthrene type alkaloid [12]. The IR spectrum of **1** exhibited an absorption band at 1696 cm^{-1}, indicating the presence of a carbonyl group. In the aromatic region of the



355 267 [M-88] 252 [M-88-CH_3]

Fig. 1 Major MS fragments of romucosine I (**1**).

^{1}H NMR spectrum, ABX and AB coupling patterns were also observed. The signals at $\delta = 9.11$ (1H, *d*, $J = 2.4$ Hz), 7.76 (1H, *d*, $J = 8.4$ Hz), and 7.19 (1H, *dd*, $J = 2.4$, 8.4 Hz) revealed a mono-substituted C-ring. The proton signal at $\delta = 9.11$, which falls appreciably downfield and apart from other aromatic hydrogen signals, is characteristic for this type of compounds [12]. The AB pattern resonances at $\delta = 7.74$ (1H, *d*, $J = 9.2$ Hz) and 7.56 (1H, *d*, $J = 9.2$ Hz) agree with the assignments to the H-9 and H-10 of phenanthrene. Furthermore, the aliphatic region exhibited two adjacent methylenes at $\delta = 3.55$ (2H, *q*, $J = 6.8$ Hz) and 3.30 (2H, *t*, $J = 6.8$ Hz), and three methoxyls at $\delta = 4.01$, 3.93, and 3.70. In comparison with the previous data of phenanthrene alkaloids [13], the downfield-shifted coupled methylenes suggest the presence of an electron-withdrawing carbonyl at the nitrogen atom. In the EI-MS, the molecular ion and base peaks were observed at m/z 355 $[M]^+$ and 267 [M-88, base peak]$^+$, respectively. The base peak was elucidated as a typical loss of a *N*-methoxylcarbonyl methylene moiety (Fig. 1) [14].

For an unambiguous assignment of **1**, several chemical reactions were carried out. A known aporphine,

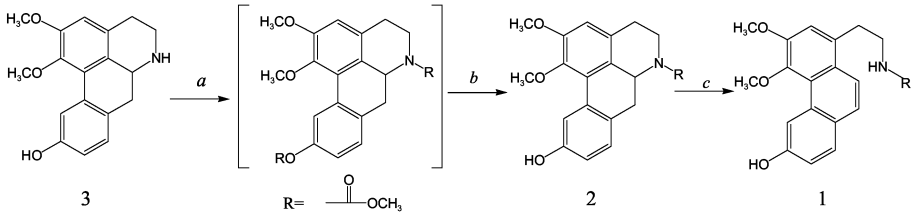


Fig. 2 Procedure of preparing romucosine I (**1**) from tuduranine (**3**).

tuduranine (**3**), was poured into a mixture of dry dichloromethane (CH_2Cl_2, 4 ml) and triethylamine (Et_3N, 10 ml). Methyl chloroformate ($ClCOOCH_3$, 0.5 ml) was added dropwise. After 15 min., the resulting mixture was dried under reduced pressure. The dried mixture was then dissolved and stirred in a methanolic K_2CO_3 solution (5%) at r. t. overnight to give romucosine C (**2**) (Fig. 2). The expected product was prepared by refluxing in HCl-MeOH (25%) for 2 h. The starting material, romucosine C (**2**), was opened at ring B to give the expected product, which was identified as compound **1** on the basis of the comparison of physical data. The process of converting aporphine to phenanthrene was monitored by ^{1}H-NMR and is illustrated in Fig. 3. The structure of **1** was identified as a ring opened aporphine and named romucosine I (**1**).

The phenolic oxidative coupling is an important process in the biosynthesis of aporphine alkaloids [15]. This oxidative coupling leads to the production of the unsubstituted and mono-substituted aporphines from proaporphines. Therefore, promucosine (**4**) may be converted into romucosine C (**2**), possessing substituted D-ring (Fig. 4). In the next step, romucosine C (**2**) could be opened at ring B by Hofmann-like degradation to romucisine I (**1**) (Fig. 4). Based on the previous literature [16], we propose the hypothesis that the opened aporphine alkaloid, romucisine I (**1**), is a metabolic product of the proaporphine promucosine (**4**). This hypothesis could be proven by simple chemical experiments that are practicable *in vivo*.

In conclusion, a new phenanthrene alkaloid, romucosine I (**1**), was isolated and its structural assignment was proven by spectral and chemical evidence. The relationship between compound **1** and other isolated alkaloids is supported by the literature. Our biogenetic considerations maybe important for the integrity of the chemotaxonomy study of Formosan Annonaceous plants.

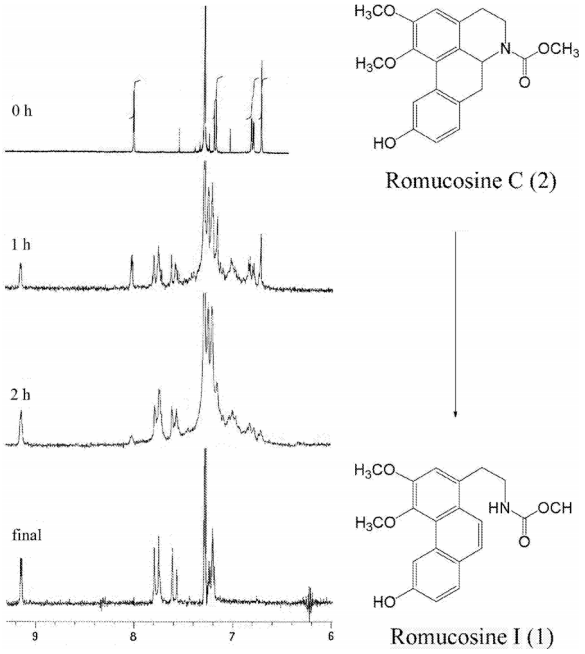


Fig. 3 ^{1}H-NMR investigation of the ring opening process from romucosine C (**2**) to romucosine I (**1**).

Experimental Section

General. The UV spectra were obtained on a Jasco UV-530 spectrophotometer, IR spectrum was measured on a Mattson Genesis II spectrophotometer. NMR spectra were obtained on Varian NMR spectrometers (Unity Plus 400 MHz and Gemini 200 MHz) using $CDCl_3$ as solvent for measurement. Low-resolution EIMS were collected on a Jeol JMS-SX/SX 102A mass spectrometer or Quattro GC/MS spectrometer having a direct inlet system. Silica gel 60 (Merck, 230-400 mesh) was used for column chromatography. Semi-prepared columns (LichroCART 250-10, Merck) used for HPLC system (Shimadzu LC-10AT), and the signals were recorded by UV detector (Shimadzu SPD-10A). The spots on TLC were detected by spraying with 50% H_2SO_4 and then heating on a hot plate.

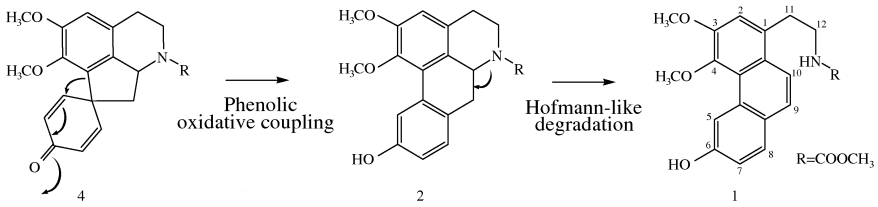


Fig. 4. Proposed biogenetic pathway of compound **1**, **2** and **4**.

Plant material. Fresh stems of *Rollinia mucosa* (11.5 kg) were collected from Chia-Yi County, Taiwan in August, 1997. A voucher specimen was characterized by Dr. Hsin-Fu Yen and deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and isolation. Fresh stems of *R. mucosa* (11.5 kg) were extracted repeatedly with MeOH at r. t. The combined MeOH extracts were evaporated under reduced pressure to yield a dark-brown syrup (305.5 g). The syrup was partitioned between $CHCl_3$ and H_2O to give two layers. The $CHCl_3$ layer was then extracted with 3% HCl to give a $CHCl_3$ solution (Part A) (108.7 g) and an acidic aqueous layer. The aqueous layer was basified with NH_4OH and extracted with $CHCl_3$ to give Part B (3.1 g). Part B gave a positive test for alkaloids employing Dragendorff's reagent. The crude alkaloid portion (Part B) was chromatographed over silica gel and eluted with increasing polarities of $CHCl_3$/MeOH mixtures to obtain 21 fractions. Romucosine I (**1**) was isolated as acetone-insoluble precipitate from fraction 5 and promucosine (**3**) was derived by preparative TLC with the solvent system ($CHCl_3$/MeOH 20:1, R_f = 0.33). Separation of fraction 14-1 by reversed phase HPLC with the solvent system (MeOH / H_2O 80:20, flow rate = 2.0 ml/min) gave romucosine C (**2**) (1.5 mg, $t_R = 23.5$ min). Further separation of fraction 11 by column chromatography with the solvent system ($CHCl_3$/MeOH 9:1) gave promucosine (**4**).

Romucosine I (**1**) [2-(6-Hydroxy-3,4-dimethoxy-phenanthren-1-yl)-ethyl]-carbamic acid methyl ester] brown amorphous powder. – $[\alpha]_D^{25}$: +0.5° (*c* 0.1, $CHCl_3$). – UV (MeOH): λ_{max} = 251, 259, 304, and 316 nm. – IR (neat): ν_{max} = 1696 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.11 (*d*, *J* = 2.4 Hz, 1H, 5-H), 7.76 (*d*, *J* = 8.4 Hz, 1H, 8-H), 7.74 (*d*, *J* = 9.2 Hz, 1H, 9-H), 7.56 (*d*, *J* = 9.2 Hz, 1H, 10-H), 7.19 (*dd*, *J* = 2.4, 8.4 Hz, 1H, 7-H), 7.18 (*s*, 1H, 2-H), 4.01, 3.93, and 3.70 (3×s, each 3H, C-4, C-3, and *N*CO-OCH_3), 3.55 (*q*, *J* = 6.8 Hz, 2H, 12H), 3.30 (*t*, *J* = 6.8 Hz, 2H, 11H). – EIMS (70 eV): m/z = 355 $[M]^+$ (49), 267 (100), 253 (12), 88 (20), 59(18).

Acknowledgement

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