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New Carbazole Alkaloids from *Murraya euchrestifolia*

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Three new monomeric carbazole alkaloids, 3-formylcarbazole (**1**), *N*-methoxy-3-formylcarbazole (**2**), and pyrayaquinone-C (**3**), and two new binary carbazoles, murrafoline-E (**4**) and murrafoline-F (**5**), were isolated from root bark of *Murraya euchrestifolia* HAYATA (Rutaceae) collected in Taiwan, and their structures were elucidated.

Keywords——alkaloid; carbazole; *Murraya euchrestifolia*; Rutaceae; 3-formylcarbazole; *N*-methoxy-3-formylcarbazole; pyrayaquinone-C; murrafoline-E; murrafoline-F

Introduction

Previously, we have shown that the root and stem barks of *Murraya euchrestifolia* HAYATA (Rutaceae) contain many kind of monomeric carbazoles, carbazolequinones, and binary carbazole alkaloids.[1)] In a continuation of our investigation of the alkaloidal components of this plant, we have isolated another five new carbazole alkaloids. This paper describes the structural elucidation of these new carbazole alkaloids.

Results and Discussion

Structure of 3-Formylcarbazole (1)

The alkaloid was obtained as a colorless oil. The high-resolution mass spectrum (MS) gave the molecular formula as $C_{13}H_9NO$. The ultraviolet (UV) bands at λ_{max} 212, 233, 244 (sh), 273, 288, and 325 nm were typical of a carbazole nucleus.[2)] A lower field 1H singlet at δ 10.10 in the proton nuclear magnetic resonance (^{1}H-NMR) (acetone-d_6) spectrum, coupled with an infrared (IR) band at ν_{max} 1685 cm^{-1}, indicated the presence of a formyl group in this alkaloid. The appearance of a four-spin proton system in the aromatic region at δ 8.29 (d), 7.61 (d), 7.49 (t), and 7.31 (t) [each 1H, $J=7.7$ Hz] in the ^{1}H-NMR spectrum, showed the absence of substituents in the A-ring of the carbazole nucleus. Further, among the ABX-type signals at δ 8.73 (1H, d, $J=1.7$ Hz), 7.98 (1H, dd, $J=8.4$ and 1.7 Hz), and 7.68 (1H, d, $J=8.4$ Hz), a lower field signal at δ 8.73 assignable to deshielded H-4[3)] appeared as a *meta*-coupled doublet, suggesting the location of the formyl substituent at C-3. These spectral data showed the structure of this alkaloid to be 3-formylcarbazole (**1**).

Structure of *N*-Methoxy-3-formylcarbazole (2)

This compound was isolated as a yellow oil. The molecular formula $C_{14}H_{11}NO_2$ was established by high-resolution MS. The UV spectrum at λ_{max} 236, 272, 288, and 320 nm, IR bands at ν_{max} 1680, 1620, and 1600 cm^{-1}, ^{1}H-NMR (acetone-d_6) signal at δ 10.12 (1H, s), and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) signal at δ 191.7 (d) showed this alkaloid to have a carbazole nucleus with a formyl group. The ^{1}H-NMR spectrum of this compound showed close similarity with that of 3-formylcarbazole (**1**) described above, except for the 3H-singlet at δ 4.27 due to a methoxy group (see Experimental). This fact, together with the observation of a strong fragment ion peak at m/z 194, the loss of a methoxy group from the molecular ion in the MS, and seven sp^2 methine carbon signals in the ^{13}C-NMR spectrum (see Experimental) led us to propose the structure of *N*-methoxy-3-formylcarbazole (**2**) for this alkaloid. *N*-Methoxyindole alkaloid have been identified in a plant of the same genus, *Murraya* (*M. paniculata*).[4] However, the isolation of **2** represents the first example of an *N*-methoxycarbazole alkaloid from a natural source.

Structure of Pyrayaquinone-C (3)

Pyrayaquinone-C was isolated as dark violet prisms, mp 223 °C (dec.), $C_{23}H_{23}NO_3$. Together with the UV [λ_{max} 248, 275 (sh), 294, and 392 nm] and IR spectra [ν_{max} 1640 and 1600 cm^{-1}], the appearance of a long-range-coupled allyl methyl signal at δ 2.15 (3H, d, J= 1.7 Hz) and an olefinic proton at δ 6.45 (1H, d, J = 1.7 Hz) in the ^{1}H-NMR spectrum indicated the 3-methyl carbazolequinone system, as in the alkaloid isolated previously from the same plant.[1a,g] The AB-type proton signals at δ 6.86 (1H, d, J = 8.7 Hz) and 7.94 (1H, d, J = 8.7 Hz) could be assigned to H-6 and H-5 on the carbazolequinone nucleus, respectively. Other AB-type signals at δ 5.68 and 6.63 (each 1H, d, J = 9.8 Hz) accompanied with a 3H singlet at δ 1.44 indicated the presence of a pyran ring having a methyl group. The ^{1}H-NMR signals at δ 5.09 (1H, t, J = 1.3 Hz), 2.12 (2H, m), 1.76 (1H, m), 1.65 (3H, s), and 1.56 (3H, s) and a mass fragment base peak at m/z 278 suggested the presence of [$-CH_2CH_2-CH=C(CH_3)_2$] attached to the methylpyran ring.

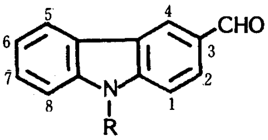
On the basis of these data, the structure of pyrayaquinone-C was proposed as formula **3**.

Structure of Murrafoline-E (4)

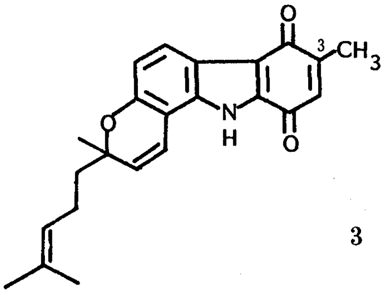
Murrafoline-E, a colorless oil, was found to have the molecular formula $C_{32}H_{28}N_2O_2$ by high-resolution MS. The presence of a carbazole chromophore in this molecule was easily deduced from the UV spectrum (see Experimental). The binary carbazole structure of murrafoline-E was suggested by the observation of two fragment peaks at m/z 262 (base peak) and 211 in the MS due to the fragments corresponding to the lower and upper halves of the molecule, respectively. The ^{1}H-NMR (400 MHz, acetone-d_6) spectrum indicated the presence of an aryl methyl (δ 2.51), a methoxy (δ 3.94), a dimethylpyran [δ 1.41 (6H, s), 5.79 and 6.92 (each 1H, d, J = 10 Hz)], and benzylic methylene attached to a nitrogen atom [δ 6.01 (2H, s)]. Further, proton–proton correlation spectroscopy (H–H COSY) of murrafoline-E showed the presence of two four-spin proton systems in the aromatic proton region (see Experimental), indicating the lack of substituents in the A-ring of both carbazole skeletons. The H–H COSY also showed a correlation between the 1H broad singlets at δ 7.58 (H-4) and 6.90 (H-2), and the 1H broad singlet at δ 7.34 (H-4′) and 2H singlet at δ 6.01 (benzylic methylene). In nuclear Overhauser effect (NOE) experiments, a 20% enhancement of the singlet at δ 6.90 (H-2) appeared on irradiation of the methoxy signal at δ 3.94, and 7% enhancements of both the aromatic protons at δ 7.58 (H-4) and 6.90 (H-2) on irradiation of the methyl proton at δ 2.51. Based on these results, we assigned the structure **4** to murrafoline-E.[1b,f] This is the third example of a binary carbazole alkaloid having an *N*-benzyl linkage in the molecule from a *Murraya* plant.[1c,i]

Structure of Murrafoline-F (5)

Murrafoline-F, $C_{28}H_{24}N_2O_2$, was obtained as a colorless oil and gave UV absorption typical of a carbazole nucleus (see Experimental). The ^{1}H-NMR (400 MHz, acetone-d_6) spectrum showed signals attributable to two methoxy groups (δ 4.14 and 3.93) and an aryl methyl group [δ 2.38 (3H, s)]. The H–H COSY of this compound showed the presence of an isolated proton signal (δ 7.74) having a long-range coupling with the aryl methyl (δ 2.38), as well as one three-spin, and two four-spin proton systems in the aromatic proton region (see



1 : R=H
2 : R=OCH3



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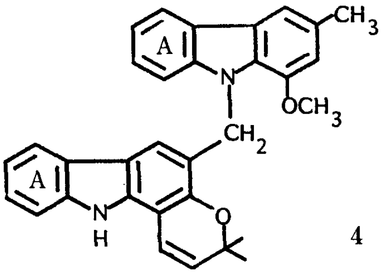
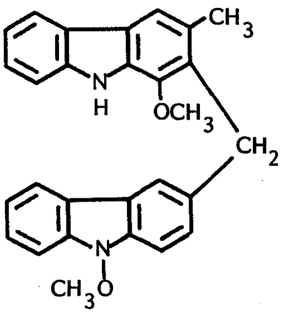


4



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Experimental). Among these aromatic protons, two four-spin proton systems were assignable to non-substituted A-ring protons in two carbazole nuclei, and the three-spin system at δ 7.89 (1H, br s), 7.45 (1H, d, $J=8.4$ Hz), and 7.34 (1H, d, $J=8.4$ Hz) to 3-substituted carbazole ring protons. The methylene linkage between two carbazole moieties in this molecule was suggested by the signals at δ_H 4.43 (2H, s) and δ_C 32.2 (triplet) in the ^{1}H- and ^{13}C-NMR spectra, respectively, and of a mass fragment at m/z 223 corresponding to a half of the molecule bearing an additional methylene and the loss of a hydrogen atom, as well as fragments at m/z 210 (a half of the molecule) and 180 (a half of the molecule $-\cdot OCH_3$). In NOE experiments, irradiation of the aryl methyl protons at δ 2.38 revealed a 7% enhancement of the signal at δ 7.74 (H-4), and irradiation of the methylene protons at δ 4.43 caused 4% and 7% enhancements of the aromatic proton signals at δ 7.89 (H-4′) and 7.34 (H-2′), respectively. There was no NOE enhancement at any proton signal on irradiation of the two methoxy protons at δ 4.14 and 3.93. The occurrence of a strong fragment ion peak at m/z 390, associated with the loss of a methoxy substituent in the MS, and the appearance of 12 sp^2 carbons as doublets in the ^{13}C-NMR spectrum (see Experimental) suggested that one methoxy group is located on the nitrogen of a carbazole nucleus as in **2**. These results led us to propose the structure **5** for murrafoline-F.[1b,f)]

Experimental

A melting point was measured on a micromelting point hot-stage apparatus (Yanagimoto). ^{1}H- and ^{13}C-NMR spectra were recorded on GX-270 (JEOL) and GX-400 (JEOL) spectrometers, respectively, in $CDCl_3$, unless otherwise stated. Chemical shifts are shown in δ-values (ppm) with tetramethylsilane (TMS) as an internal reference. Electron impact-mass spectra (EI-MS) were taken with a Hitachi M-52 spectrometer having a direct inlet system, and high-resolution mass spectra with a Hitachi M-80 spectrometer. UV spectra were determined in methanol and IR spectra were recorded in $CHCl_3$.

Extraction and Separation——The dried, powdered root bark (900 g) of *M. euchrestifolia* collected at Kuantaochi, Nantou Hsien, Taiwan, in December 1982, was extracted with acetone. The acetone extract was treated in the manner described in the previous paper.[1g)] The benzene eluate from silica gel chromatography was further subjected to column and preparative chromatographies on silica gel (solvents: appropriate combinations of acetone, benzene, hexane, isopropyl ether and dichloromethane) to afford four new alkaloids: 3-formylcarbazole (**1**) (2 mg), *N*-methoxy-3-formylcarbazole (**2**) (4 mg), pyrayaquinone-C (**3**) (2 mg), murrafoline-E (**4**) (2 mg), and murrafoline-F (**5**) (4 mg).

3-Formylcarbazole (1)——Colorless oil. High-resolution MS: Calcd for $C_{13}H_9NO$: 195.0683. Found: 195.0652. UV λ_{max} nm: 212, 233, 244 (sh), 273, 288, 325. IR ν_{max} cm^{-1}: 3460, 1685, 1600. ^{1}H-NMR (acetone-d_6) δ: 10.10 (1H, s, CHO), 8.73 (1H, d, J=1.7 Hz), 8.29 (1H, d, J=7.7 Hz), 7.98 (1H, dd, J=8.4, 1.7 Hz), 7.68 (1H, d, J=8.4 Hz), 7.61 (1H, d, J=7.7 Hz), 7.49 (1H, t, J=7.7 Hz), 7.31 (1H, t, J=7.7 Hz). ^{1}H-NMR δ: 10.11 (1H, s, CHO), 8.62 (1H, br s), 8.42 (1H, br s, NH), 8.15 (1H, d, J=7.7 Hz), 7.98 (1H, d, J=8.4 Hz), 7.48—7.55 (3H, m), 7.33 (1H, m). MS m/z (%): 195 (M$^+$, 100%), 194 (88), 166 (94), 139 (94).

***N*-Methoxy-3-formylcarbazole (2)**——Yellow oil. High-resolution MS: Calcd for $C_{14}H_{11}NO_2$: 225.0790. Found: 225.0810. UV λ_{max} nm: 236, 272, 288, 320. IR ν_{max} cm^{-1}: 1680, 1620, 1600, 1570. ^{1}H-NMR (acetone-d_6) δ: 10.12 (1H, s, CHO), 8.73 (1H, d, J=1.4 Hz), 8.31 (1H, d, J=7.7 Hz), 8.08 (1H, dd, J=8.4, 1.4 Hz), 7.75 (1H, d, J=8.4 Hz), 7.68 (1H, d, J=7.7 Hz), 7.60 (1H, t, J=7.7 Hz), 7.37 (1H, t, J=7.7 Hz), 4.27 (3H, s, OCH_3). ^{1}H-NMR δ: 10.10 (1H, s, CHO), 8.57 (1H, d, J=1.7 Hz), 8.10 (1H, d, J=7.7 Hz), 8.02 (1H, dd, J=1.7, 8.4 Hz), 7.54—7.60 (3H, m), 7.33 (1H, m), 4.18 (3H, s, OCH_3). ^{13}C-NMR δ: 64.2 (q), 108.0 (d), 108.5 (d), 119.7 (s), 120.9 (d), 121.2 (d), 123.9 (d), 127.2 (d), 127.8 (d), 129.5 (s), 137.5 (s), 140.2 (s), 191.7 (d); One singlet signal may overlap with some other signal. MS m/z (%): 225 (M$^+$, 100%), 210 (67), 194 (69), 166 (97), 139 (23).

Pyrayaquinone-C (3)——mp 223 °C (dec.), dark-violet prisms from acetone. High-resolution MS: Calcd for $C_{23}H_{23}NO_3$: 361.1676. Found: 361.1666. UV λ_{max} nm: 248, 275 (sh), 294, 392. IR ν_{max} cm^{-1}: 3440, 1640, 1600. ^{1}H-NMR δ: 9.37 (1H, br s), 7.94 (1H, d, J=8.7 Hz), 6.86 (1H, d, J=8.7 Hz), 6.63 (1H, d, J=9.8 Hz), 6.45 (1H, d, J=1.7 Hz), 5.68 (1H, d, J=9.8 Hz), 5.09 (1H, t, J=1.3 Hz), 2.15 (3H, d, J=1.7 Hz), 2.12 (2H, m), 1.76 (1H, m), 1.65 (3H, s), 1.56 (3H, s), 1.44 (3H, s). MS m/z (%): 361 (M$^+$, 17%), 346 (3), 279 (20), 278 (100).

Murrafoline-E (4)——Colorless oil. High-resolution MS: Calcd for $C_{32}H_{28}N_2O_2$: 472.2149. Found: 472.2155. UV λ_{max} nm: 228 (sh), 238, 255 (sh), 263 (sh), 287, 328, 340, 352. IR ν_{max} cm^{-1}: 3460, 1650, 1610, 1580. ^{1}H-NMR (400 MHz, acetone-d_6) δ: two four-spin protons [δ 8.07 (1H, d, J=7.8 Hz), 7.47 (1H, d, J=7.8 Hz), 7.30 (1H, t, J=7.8 Hz), 7.13 (1H, t, J=7.8 Hz); 7.58 (1H, d, J=7.8 Hz), 7.35 (1H, d, J=7.8 Hz), 7.20 (1H, t, J=7.8 Hz), 6.99 (1H, t, J=7.8 Hz)], 7.58 (1H, s), 7.34 (1H, s), 6.92 (1H, d, J=10 Hz), 6.90 (1H, s), 6.01 (2H, s), 5.79 (1H, d, J=10 Hz), 3.94 (3H, s), 2.51 (3H, s), 1.41 (6H, s). MS m/z (%): 472 (M$^+$, 23%), 420 (20), 263 (21), 262 (100), 247 (23), 211 (18), 196 (10), 180 (8), 168 (8), 167 (11).

Murrafoline-F (5)——Colorless oil. High-resolution MS: Calcd for $C_{28}H_{24}N_2O_2$: 420.1835. Found: 420.1834. UV λ_{max} nm: 218 (sh), 239, 252 (sh), 261 (sh), 285 (sh), 296, 326, 340. IR ν_{max} cm^{-1}: 3460, 1610. ^{1}H-NMR (400 MHz, acetone-d_6) δ: two four-spin protons [δ 8.06 (1H, d, J=7.8 Hz), 7.54 (1H, d, J=7.8 Hz), 7.37 (1H, t, J=7.8 Hz), 7.16 (1H, t, J=7.8 Hz); 7.99 (1H, d, J=7.8 Hz), 7.52 (1H, d, J=7.8 Hz), 7.44 (1H, t, J=7.8 Hz), 7.17 (1H, t, J=7.8 Hz)], three-spin protons [δ 7.89 (1H, s), 7.45 (1H, d, J=8.4 Hz), 7.34 (1H, d, J=8.4 Hz)], 7.74 (1H, s), 4.43 (2H, s), 4.14 (3H, s), 3.93 (3H, s), 2.38 (3H, s). ^{13}C-NMR δ: 20.3 (q), 32.2 (t), 61.2 (q), 63.4 (q), 108.3 (d), 108.4 (d), 110.9 (d), 117.3 (d), 119.5 (d), 119.6 (d), 119.9 (d), 120.3 (d), 120.5 (d), 125.6 (d), 126.0 (d), 126.6 (d). Singlet signals could not be detected clearly, because the yield of the compound from the natural source was so small. MS m/z (%): 420 (M$^+$, 33%), 390 (100), 375 (29), 359 (19), 223 (33), 210 (19), 208 (24), 280 (52).

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