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TWO CARBAZOLE ALKALOIDS FROM LEAVES OF *MURRAYA EUCHRESTIFOLIA*

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Key Word Index—*Murraya euchrestifolia*; Rutaceae; carbazole alkaloids; murrayamines-D and -E.

Abstract—Two new carbazole alkaloids, murrayamines-D and -E, were isolated from the leaves of *Murraya euchrestifolia* in February 1995. Their structures were elucidated by spectral analyses.

INTRODUCTION

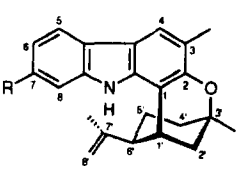
In continuing our examination of the acetone extract of the leaves of *Murraya euchrestifolia* collected in Taiwan during the winter, two new isomeric carbazole alkaloids, murrayamines-D (**1**) and -E (**2**), were obtained with two binary carbazole alkaloids, *bis*-7-hydroxygirinimbines-A (**3**) and -B (**4**) [1], as well as six known compounds. Herein, we report the structural elucidation of compounds **1** and **2**.

RESULTS AND DISCUSSION

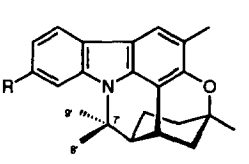
The acetone extract from the leaves of *M. euchrestifolia* was repeatedly chromatographed to afford two new carbazole alkaloids, murrayamines-D (**1**) and -E (**2**), as well as the seven carbazoles, *bis*-7-hydroxygirinimbines-A (**3**) [1] and -B (**4**) [1], girinimbine (**5**) [1], murrayamine-A (**6**) [2], bicyclomahanimbine (**7**) [3,4], mahanimbine (**8**) [2], (+)-mahanine (**9**) [2] and one steroid, β-sitosterol (**10**). The latter structures were characterized by spectroscopic analyses or by direct comparison with authentic samples.

Murrayamine-D (**1**) was isolated as a colourless oil. High-resolution mass measurement determined the molecular formula as $C_{25}H_{25}NO_2$. UV bands at 215, 239, 266, 315 and 324 nm suggested that this compound could be a 2,7-dioxygenated carbazole derivative [2]. In the aromatic region of the ^{1}H NMR spectrum of **1**, one set of a ABX mutually-coupled proton system at δ 6.65 (*dd*, J = 8.4, 2.2 Hz), 6.78 (*d*, J = 2.2 Hz) and 7.69 (*d*, J = 8.4 Hz) was assigned to H-6, H-8 and H-5, respectively, in ring A. A downfield shift singlet, together with a three-proton singlet at δ 7.54 and 2.31 were deduced for H-4 and 3-Me. The location of this methyl substituent could be further confirmed by a NOE difference experiment. Only the signal of H-4 (δ 7.52) was enhanced when irradiation of the methyl signal (δ 2.31) was made. On the other hand, in the decoupling experiment, a broad singlet signal at δ 7.52 (H-4) was changed into a sharp singlet on irradiation of the methyl signal at δ 2.31. The remaining characteristic signals at δ 4.74 and 4.81 (each 1H, *d*, J = 0.7 Hz) for vinylidene protons, (H-8'), a methyl singlet at δ 1.49 for vinyl methyl (7'-Me), another methyl singlet at δ 1.43 for tertiary methyl (3'-Me), a methine double doublet at δ 3.37 for a benzylic proton (H-1') and a complicated multiplet between δ 1.5–2.2 for three methylene and one methine protons were attributed to a 10-carbon bicyclic skeleton similar to that of cyclomahanimbine (**11**) [5]. An extra hydroxyl group on C-7 showed a broad IR band between 3600 and 3200 cm^{-1}. Based on the above analyses, murrayamine-D (**1**) thus has the following structure: 7-hydroxymurrayazolidine.

Murrayamine-E (**2**), an isomer of **1**, was obtained as optically active colourless prisms. By the comparison of the ^{1}H NMR spectrum of **2** with that of **1**, the similarity in the 1-substituted-3-methyl-2,7-dioxygenated carbazole moiety at δ 6.78 (*dd*, J = 9.0, 2.4 Hz, H-6), 6.99 (*d*, J = 2.4 Hz, H-8), 7.75 (*d*, J = 9.0 Hz, H-5) and 2.31 (3H, *s*, 3-Me) was apparent. The major difference was that the *iso*propenyl side-chain in **1** was replaced by two isolated dimethyls that were geminal substituents on a quaternary carbon atom. Thus, the three methyl singlets at



R
1: OH
11: H



R
2: OH
2a: OMe
12: H

*Author to whom correspondence should be addressed.

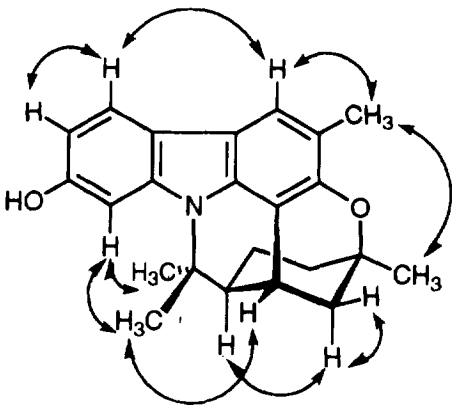


Fig. 1. NOESY correlations of compound 2.

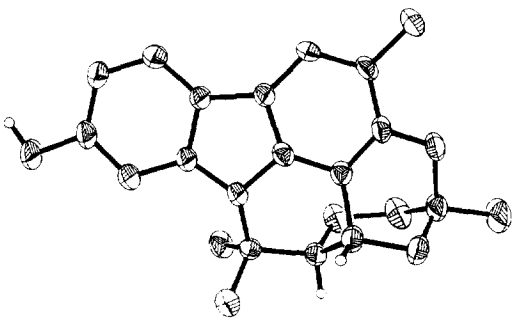


Fig. 2. Structure and solid-state conformation of compound 2.

δ1.38, 1.45 and 1.88, as well as a benzylic broad doublet at δ 3.28, along with three methylene and one methine overlapping multiplicities between δ 0.2–2.6 constructed a 10-carbon tricyclic structural unit attached to carbazole, as found murrayazoline (**12**) [5]. The complete structure and relative stereochemistry of **2** were determined by a NOESY experiment (Fig. 1), along with single crystal X-ray analysis (Fig. 2). Consequently, the structure of murrayamine-E (**2**) was suggested as 7-hydroxymurrayazoline.

Furthermore, treatment of **2** with excess CH_2N_2 produced a methyl ether **2a**. An extra methoxyl singlet at δ 3.89 was observed in the ^{1}H NMR spectrum of **2a**. In a NOE difference experiment, irradiation of the aryl methyl (δ 2.31) caused a 13.86% increase of the signal at δ 7.34 (H-4). Enhancements of 11.93% and 9.42% for the signals at δ 6.78 (H-8) and 6.99 (H-6), respectively, were observed when the methoxyl signal at δ 3.89 (7-OMe) was irradiated. These results strongly supported the structure **2** proposed for murrayamine-E.

EXPERIMENTAL

Mps: uncorr. UV: in MeOH. IR: in KBr, unless otherwise stated. ^{1}H NMR and ^{13}C NMR: in $CDCl_3$, TMS as int. standard except where noted. MS: direct inlet system.

Plant material. Leaves of *M. euchrestifolia* Hayata were collected in Kuantaochi, Nantou Hsien, Taiwan, in February 1987 and identified by Prof. C. S. Kuoh. A specimen of the plant has been deposited at the Herbarium of the National Cheng Kung University, Tainan, Taiwan.

Extraction and separation. Air-dried leaves (1.64 kg) were extracted with Me_2CO at room temp. The comb. Me_2CO extracts were concd under red. pres. to yield a dark-green syrup (1.03 kg) which was subjected to chromatography over silica gel and eluted with benzene–Me_2CO (9:1) to give 7 frs. Frs 1 and 2 were comb. and rechromatographed over a silica gel column using hexane–EtOAc (6:1) to furnish **5** (3.0 g), **6** (22 mg) and **10** (0.3 g), successively. Fr 3 was also rechromatographed on silica gel using hexane–Me_2CO (9:1) to give **2** (25 mg) and **7** (12 mg). In a similar way, **8** (0.2 g) was obtained from frs 4 and 5 using hexane–Me_2CO (9:1), whereas **1** (4 mg), **9** (10 mg), **3** (5 mg) and **4** (6 mg) and an unknown dimer C (4 mg) were afforded from fr. 6 by TLC separation using $CHCl_3$–Me_2CO (25:1).

Murrayamine-D (**1**). Oil. HRMS: calcd for $C_{23}H_{25}NO_2$, *m/z* 347.1887 $[M]^+$, found 347.1874. UV λ_{max}^{MeOH} nm 215, 239, 266, 315, 324. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3429, 1620, 1495, 1427. EIMS *m/z* (rel. int.): 347 ($[M]^+$, 100), 264 (44). ^{1}H NMR ($CDCl_3$) δ1.43 (3H, *s*, 3′-Me), 1.49 (3H, *s*, 7′-Me), 1.5–2.2 (6H, *m*, H-2′, -4′ and -5′), 2.31 (3H, *d*, *J* = 0.7 Hz, 3-Me), 2.56 (1H, *m*, H-6′), 3.37 (1H, *dd*, *J* = 6.9, 3.3 Hz, H-1′) 4.68 (1H, *s*, 7-OH), 4.74 and 4.81 (each 1H, *br s*, H-8′), 6.65 (1H, *dd*, *J* = 8.4, 2.2 Hz, H-6), 6.78 (1H, *d*, *J* = 2.2 Hz, H-8), 7.52 (1H, *br s*, H-4), 7.60 (1H, *br s*, NH), 7.69 (1H, *d*, *J* = 8.4 Hz, H-5).

Murrayamine-E (**2**). Prisms (MeOH), mp 275–276° (dec). $[\alpha]_D$ + 39.68° (*c*, 0.133, $CHCl_3$). HRMS: calcd for $C_{23}H_{25}NO_2$, *m/z* 347.1887 $[M]^+$, found 347.1889. UV λ_{max}^{MeOH} nm (log ε) 219 (3.60), 245 (3.87), 273 (3.51), 316 (3.37), 335 (sh, 3.10). IR ν_{max}^{KBr} cm^{-1}: 3358, 1623, 1608, 1571. EIMS *m/z* (rel. int.): 347 ($[M]^+$, 100), 332 (53), 265 (43). ^{1}H NMR ($CDCl_3$) δ0.20 (1H, *m*, H-5′$_{ax}$), 1.28 (3H, *s*, H-8′), 1.30 (1H, *dd*, *J* = 13.8, 6.6 Hz, H-5′$_{eq}$), 1.45 (3H, *s*, 3′-Me), 1.50 (1H, *m*, H-4′$_{ax}$), 1.62 (1H, *m*, H-4′$_{eq}$). 1.88 (3H, *s*, H-9′), 1.90 (1H, *br d*, *J* = 13.0 Hz, H-2′$_{ax}$), 1.96 (1H, *m*, H-6′$_{ax}$), 2.31 (3H, *s*, 3-Me), 2.37 (1H, *m*, H-2′$_{eq}$), 3.28 (1H, *br d*, *J* = 1.8 Hz, H-1′$_{eq}$), 4.70 (1H, *s*, 7-OH), 6.78 (1H, *dd*, *J* = 9.0, 2.4 Hz, H-6), 6.99 (1H, *d*, *J* = 2.4 Hz, H-8), 7.38 (1H, *s*, H-4), 7.75 (1H, *d*, *J* = 9.0 Hz, H-5). ^{13}C NMR ($CDCl_3$) δ14.0 (*q*, 3-Me), 20.3 (*t*, C-5′), 21.1 (*q*, C-8′), 26.7 (*d*, C-6′), 27.8 (*q*, 3′-Me), 28.4 (*q*, C-9′), 34.5 (*t*, C-2′), 35.1 (*t*, C-4′), 46.9 (*d*, C-1′), 58.9 (*s*, C-7′), 74.5 (*s*, C-3′), 99.3 (*d*, C-8), 106.0 (*s*, C-1), 106.9 (*d*, C-6), 112.8 (*s*, C-4a), 116.0 (*s*, C-4b), 116.8 (*d*, C-4), 118.4 (*s*, C-3 and *d*, C-5), 140.7 (*s*, C-8a and C-9a), 152.3 (*s*, C-2), 152.9 (*s*, C-7). Crystal data: M = 347.4, monoclinic, space group $P2_1/c$ *a* = 10.899 (6), *b* = 11.285 (3), *c* = 15.525 (7) Å, β = 107.74 (4)°, *U* = 1818.8 (14) Å^3, *Z* = 4, D_c = 1.269 mg m^{-3}, μ($Mo K_\alpha$ radiation, λ = 0.71073 Å), crystal dimensions: 0.3 × 0.4 × 0.45 mm. Intensity data ($+h$, $+k$, $+l$, $2\theta_{max}$ = 50.0°) were recorded on a Siemens R 3m/V diffractometer. The crystal structure was solved by a direct method. Full-matrix least-squares refinement of atomic parameters (anisotropic C, O; isotropic H) converged at *R* = 0.0631 (R_w = 0.0744) over 1829 reflections with $I > 4.00\,(I)$.

O-*Methylmurrayamine-E* (**2a**). Treatment of **2** (10 mg) with excess CH_2N_2 in the usual way afforded colourless needless of **2a**, mp 224–225° (Me_2CO). UV λ_{max}^{MeOH} nm 218, 241, 267, 312, 323. IR ν_{max}^{KBr} cm^{-1}: 1615, 1460. EIMS *m/z* (rel. int.): 293 ($[M]^+$, 21), 278 (100), 263 (13). ^1H NMR ($CDCl_3$) δ0.21 (1H, *m*, H-5′$_{ax}$), 1.28 (3H, *s*, H-8′), 1.30 (1H, *dd*, J = 9.1, 5.5 Hz, H-5′$_{eq}$), 1.45 (3H, *s*, 3′-Me), 1.53 (1H, *m*, H-4′$_{ax}$), 1.62 (1H, *m*, H-4′$_{eq}$), 1.88 (3H, *s*, H-9′), 1.87 (1H, *br d*, J = 13.2 Hz, H-2′$_{ax}$), 1.97 (1H, *ddd*, J = 11.2, 5.5, 2.3 Hz, H-6′$_{ax}$), 2.31 (3H, *s*, 3-Me), 2.39 (1H, *ddd*, J = 13.2, 5.5, 3.2 Hz, H-2′$_{eq}$), 3.28 (1H, *br d*, J = 2.2 Hz, H-1′$_{eq}$), 3.89 (3H, *s*, 7-OMe), 6.78 (1H, *dd*, J = 8.4, 2.4 Hz, H-6), 6.99 (1H, *d*, J = 2.4 Hz, H-8), 7.37 (1H, *s*, H-4), 7.75 (1H, *d*, J = 8.4 Hz, H-5).

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