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THREE NEW CARBAZOLE ALKALOIDS ISOLATED FROM *MURRAYA SIAMENSIS*[1]

NIJSIRI RUANGRUNGSI, JONGJIT ARIYAPRAYOON,

Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

GORDON L. LANGE,* and MICHAEL G. ORGAN

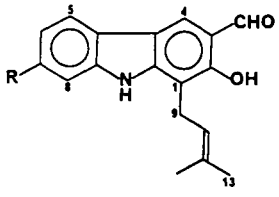
Guelph-Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT.—Eight components were isolated from the roots of *Murraya siamensis* and their structures determined by spectroscopic techniques. Seven of the components are carbazole alkaloids, three of which, compounds **4**, **6**, and **7**, are new natural products. The other component is the coumarin xanthoxyletin. A detailed ^{1}H-nmr study of the seven carbazoles in three different solvents ($CDCl_3$, Me_2CO-d_6, and C_6D_6) is reported, as well as the ^{13}C-nmr spectra of these compounds in Me_2CO-d_6.

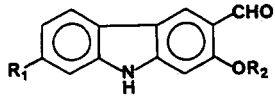
Murraya L., in the tribe Clauseneae, subfamily Aurantioideae, family Rutaceae, is a genus of shrubs or small trees distributed from Southeast Asia to Australia (2). *Murraya siamensis* Craib is one of the three species found throughout Thailand and is known locally as "Prong faa" (3). The powdered root of this species mixed with H_2O is claimed to be taken externally and internally for eye sores, for snakebite, and for tuberculosis (4). There have been no previous phytochemical reports on any part of *M. siamensis*, so in this study we describe the isolation and structural elucidation of eight constituents, including three new carbazole alkaloids **4**, **6**, and **7**. The eight components extracted from the roots were isolated by a combination of chromatographic techniques as described in the Experimental section. The structural elucidation of these compounds will be described in the order in which they were eluted from the chromatography column.

The first and least polar compound was shown to be the carbazole heptaphylline [**1**] by comparison with the mp and spectral data reported in the literature (5). In Table 2 we include the previously unreported ^{13}C-nmr spectrum of **1**. After we had determined independently the structures of the second and third components, these new carbazoles, **2** and **3**, were reported to be present in *Clausena harmandiana*, another Rutaceae species (6). We believe the assignments in the previous report (6) for C-2 and C-7 in **3** should be reversed (Table 2).

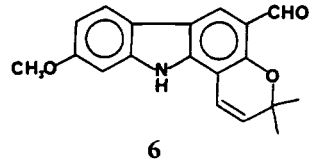
The fourth component was a pale yellow solid that exhibited a parent ion in its eims at *m/z* 255 and an accurate mass consistent with the molecular formula $C_{15}H_{13}NO_3$. The uv spectrum of the compound indicated a 3-formylcarbazole chromophore (7,8),



1 R=H
2 R=OMe



3 R_1=OMe, R_2=H
4 R_1=OMe, R_2=Me
5 R_1=H, R_2=H
7 R_1=H, R_2=Me



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[1]Part XI in the series of "Studies on Thai Medicinal Plants." For Part X, see Ruangrungsi *et al.* (1).

TABLE 1. 400 MHz ^{1}H-nmr Spectra of Components **1–7** in Three Solvents.[a]

Proton	Compound						
	1	**2**	**3**	**4**	**5**	**6**	**7**
H-1 . . .	—	—	6.85 (s) [6.84, 6.61]	7.11 (s) [6.85, 6.13]	6.89 (s) [6.88, 6.59]	—	7.12 (s) [6.88, 6.10]
H-4 . . .	8.22 (s) [8.04, 7.40]	8.12 (s) [7.94, 7.34]	8.29 (s) [8.05, 7.36]	8.37 (s) [8.45, 8.82]	8.43 (s) [8.18, 7.42]	8.23 (s) [8.30, 8.69]	8.50 (s) [8.56, 8.88]
H-5 . . .	8.01 (d, 7.6) [7.97, 7.80]	7.88 (d, 8.7) [7.84, 7.66]	7.92 (d, 8.8) [7.85, 7.61]	7.97 (d, 8.3) [7.87, 7.56]	8.07 (d, 7.6) 7.99, 7.75]	7.97 (d, 8.4) [7.84, 7.53]	8.10 (d, 8.0) [8.00, 7.70]
H-6 . . .	7.20 (t, 7.6) [7.27, 7.21]	6.82 (dd, 8.7, 2.2) [6.87, 6.93]	6.83 (dd, 8.8, 2.2) [6.88, 6.86]	6.83 (dd, 8.3, 2.2) [6.88, 6.74]	7.22 (t, 7.6) [7.28, 7.18]	6.83 (dd, 8.4, 2.2) [6.86, 6.73]	7.15 (t, 8.0) [7.25, 7.09]
H-7 . . .	7.35 (t, 7.6) [7.40, 7.27]	—	—	—	7.38 (t, 7.6) [7.40, 7.26]	—	7.35 (t, 8.0) [7.38, 7.25]
H-8 . . .	7.48 (d, 7.6) [7.40, 6.90]	7.01 (d, 2.2) [6.91, 6.44]	7.02 (d, 2.2) [6.89, 6.50]	7.02 (d, 2.2) [6.90, 6.62]	7.48 (d, 7.6) [7.40, 6.88]	6.97 (d, 2.2) [6.90, 6.55]	7.47 (d, 8.0) [7.40, 6.96]
H-9 . . .	3.61 (d, 6.8) [3.64, 3.53]	3.59 (d, 6.9) [3.63, 3.57]	—	—	—	6.91 (d, 9.8) [6.61, 6.03]	—
H-10 . . .	5.34 (br t, 6.8) [5.32, 5.22]	5.32 (br t, 6.9) [5.32, 5.26]	—	—	—	5.90 (d, 9.8) [5.80, 5.32]	—
H-12 . . .	1.64 (d, 1.0) [1.77, 1.57]	1.64 (d, 1.0) [1.78, 1.58]	—	—	—	1.54 (s) [1.55, 1.26]	—
H-13 . . .	1.81 (s) [1.90, 1.69]	1.81 (s) [1.90, 1.71]	—	—	—	1.54 (s) [1.55, 1.26]	—
CHO . . .	9.91 (s) [9.91, 9.47]	9.90 (s) [9.90, 9.52]	9.95 (s) [9.93, 9.49]	10.43 (s) [10.50, 10.93]	9.98 (s) [9.95, 9.45]	10.45 (s) [10.49, 10.92]	10.45 (s) [10.49, 10.89]
NH . . .	10.62 (br s) [8.20, 7.48]	10.51 (br s) [8.10, 7.39]	10.72 (br s) [8.12, 6.31]	10.64 (br s) [8.11, 6.58]	10.72 (br s) [8.22, 6.31]	10.68 (br s) [8.14, 6.55]	10.61 (s) [8.88, 6.65]
OH . . .	11.75 (s) [11.70, 12.35]	11.72 (s) [11.60, 12.38]	11.42 (s) [11.43, 12.18]	—	11.46 (s) [11.46, 12.16]	—	—
OMe . . .	—	3.84 (s) [3.90, 3.46]	3.85 (s) [3.90, 3.47]	3.85, 3.99 (s)[b] [3.90, 3.99; 3.46, 3.32]	—	3.85 (s) [3.89, 3.46]	4.00 (s) [3.99, 3.30]

[a]For each proton, the first number is the chemical shift in the solvent Me_2CO-d_6 while the two numbers in square brackets are values obtained in $CDCl_3$ and C_6D_6, respectively. In parentheses are given the multiplicity for the proton and the coupling constant(s) in Hertz.

[b]In each pair, the first value is for the 7-OMe and the second for the 2-OMe.

TABLE 2. ^{13}C-nmr Spectra of Compounds **1–7**.[a]

Carbon	Compound						
	1	**2**	**3**	**4**	**5**	**6**	**7**
C-1a	145.3(+)	145.5(+)	147.0(+)	146.6(+)	146.8(+)	141.6(+)	146.2(+)
C-1	109.7(+)	109.6(+)	97.0(−)	93.9(−)	97.1(−)	105.2(+)	93.5(−)
C-2	158.0(+)	157.5(+)	160.9(+)	161.6(+)	161.7(+)	154.4(+)	162.2(+)
C-3	115.7(+)	115.6(+)	115.8(+)	116.0(+)	116.1(+)	117.2(+)	117.7(+)
C-4	126.0(−)	124.5(−)	126.7(−)	121.5(−)	128.3(−)	121.5(−)	121.2(−)
C-4a	117.7(+)	117.9(+)[b]	118.4(+)	119.4(+)	118.6(+)	119.1(+)	119.3(+)
C-5a	124.1(+)	117.5(+)[b]	117.3(+)	118.2(+)	124.0(+)	118.0(+)	124.2(+)
C-5	120.7(−)[b]	120.7(−)	121.0(−)	120.2(−)	121.0(−)	118.4(−)	120.7(−)[b]
C-6	120.0(−)[b]	109.0(−)	109.2(−)	109.5(−)	120.4(−)	109.3(−)	120.5(−)[b]
C-7	125.9(−)	159.5(+)	159.8(+)	159.7(+)	126.5(−)	160.1(+)	126.2(−)
C-8	111.6(−)	95.9(−)	96.1(−)	96.2(−)	111.8(−)	96.2(−)	111.7(−)
C-8a	141.5(+)	142.9(+)	143.0(+)	143.0(+)	141.7(+)	143.2(+)	141.4(+)
2-OMe	—	—	—	56.3(−)	—	—	56.1(−)
3-CHO	196.3(−)	196.3(−)	196.2(−)	188.6(−)	196.1(−)	188.3(−)	188.4(−)
7-OMe	—	55.3(−)	55.5(−)	55.8(−)	—	55.7(−)	—
C-9	23.0(+)	22.9(+)	—	—	—	130.8(−)	—
C-10	121.7(−)	121.8(−)	—	—	—	117.4(−)	—
C-11	132.7(+)	132.6(+)	—	—	—	77.7(+)	—
C-12	17.6(−)	17.6(−)	—	—	—	27.6(−)	—
C-13	25.3(−)	25.3(−)	—	—	—	27.6(−)	—

[a]Chemical shifts are in ppm with the solvent Me_2CO-d_6 as internal reference.
[b]Assignments may be interchanged.

and, as addition of base did not alter the spectrum, it was concluded that no phenolic hydroxyl groups were present. The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) indicated the presence of two methoxyl groups and a formyl substituent. The ^{1}H resonances for H-5, H-6, and H-8 were very similar to those in **2** and **3** and indicated that one of the methoxyl groups was attached to C-7. The other two aromatic resonances as singlets, were assigned to H-1 and H-4 and showed that the second methoxyl group must be at C-2. Thus, this component was assigned the structure 3-formyl-2,7-dimethoxycarbazole [**4**], a new alkaloid and the methylated derivative of **3**. The related 3-formyl-2,6-dimethoxycarbazole (glycozolide) has been isolated from *Glycosmis pentaphylla* (9) and prepared by oxidation of the corresponding 3-methyl derivative (10).

The fifth component was shown to be the known coumarin xanthoxyletin (11) by comparison of its mp and ^{1}H-nmr spectrum with an authentic sample. This compound was also isolated from *C. harmandiana* (12) along with **2** and **3**, which are mentioned above (6).

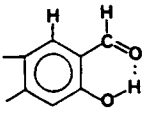
The next component was found to be mukonal [**5**], a carbazole previously isolated from *Murraya koenigii* (13). The ^{13}C-nmr spectrum of **5** is given in Table 2, as a number of our assignments, particularly in the unsubstituted benzene ring, differ from those reported previously for mukonal (13). Our assignments for this ring are similar to those reported for carbazole itself (14,15).

The seventh component was a yellow crystalline solid that showed a weak parent ion in its eims at *m/z* 307 and an accurate mass consistent with the molecular formula $C_{19}H_{17}NO_3$. The uv spectrum suggested the presence of a 3-formylcarbazole partial structure (7,8), and, as addition of base did not shift the uv maxima, it was assumed that the other oxygens are not present as phenolic hydroxyl groups. The ^{1}H-nmr spectra in the three solvents used in this study (Table 1) indicated the presence of a 7-methoxy substituent as the familiar pattern seen in the spectra of **2**, **3**, and **4** for H-5, H-6, and H-8 was observed here also. The only other aromatic proton, a significantly deshielded singlet, was assigned to H-4. Two vinyl protons, which were coupled to each other, and a 6-proton singlet were readily accounted for by a 2,2-dimethyl-Δ^3-pyran system fused to C-1 and C-2 of the carbazole nucleus, and, thus, this component is a new alkaloid possessing structure **6**. A compound without the methoxyl substituent, murrayacine, has been isolated previously from *M. koenigii* (16) and from *Clausena heptaphylla* (17), and we therefore suggest that **6** be called 7-methoxymurrayacine. A comparison of the ^{1}H-nmr spectrum of murrayacine (16) with that of **6** (Table 1) fully supports the structure proposed for the latter, as does the ^{13}C-nmr spectrum reported in Table 2. The weaker parent ion in the eims of **6** is explained by the facile loss of one of the methyl groups at C-11 to give a carbazolopyrilium ion (15), the base peak in the spectrum.

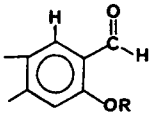
The final component isolated was a cream-colored solid that gave a parent peak in its eims (*m/z* 225) and an accurate mass corresponding to the molecular formula $C_{14}H_{11}NO_2$. The uv spectrum again suggested a 3-formylcarbazole skeleton, and, as it was unchanged upon addition of base, the remaining oxygen is not present as a phenolic hydroxyl group. The ^{1}H nmr was quite simple (Table 1, compound **7**); other than the aromatic, amine, and formyl protons, it showed only one methoxyl group, and the aromatic protons H-5 to H-8 displayed a pattern similar to that found in **1** and **5**. In addition, two aromatic singlets, with chemical shifts similar to the corresponding protons in **5**, were assigned to H-1 and H-4. Thus, component **7** is 3-formyl-2-methoxycarbazole or *O*-methylmukonal. This compound has been synthesized (10), but to our knowledge this is the first time it has been reported to occur in nature. The 3-formyl-1-methoxy isomer has been reported in *M. koenigii* (18) and *C. heptaphylla* (19). ^{1}H- or ^{13}C-nmr spectra of **7** were not previously reported; these are included in Tables 1 and 2, respectively.

The isolation of seven carbazole components from the species under investigation provides an unusual opportunity for a detailed comparison, so the 400 MHz ^{1}H- and 100 MHz ^{13}C-nmr spectra of all these compounds are recorded in Tables 1 and 2, respectively. A compilation of the ^{1}H-nmr spectra of known carbazole alkaloids was reported in 1977 (8). In our study we recorded the ^{1}H-nmr spectra of the seven carbazoles in three different solvents: the polar solvent Me_2CO-d_6, the common nmr solvent $CDCl_3$, and the aromatic medium C_6D_6. The solvent Me_2CO-d_6 was found to be particularly effective in resolving the aromatic protons H-5 to H-8. For all compounds, the NH proton in C_6D_6 is shielded, while in Me_2CO-d_6 it is deshielded relative to its position in $CDCl_3$. The aromatic solvent-induced shift (ASIS) is explained by assuming that C_6D_6 is aligned perpendicular to the polar N-H bond at the positive end of the dipole, resulting in shielding of the amine proton by the π cloud (20). It should be noted that with an alkyl group at the 1 position (e.g., in **1** and **2**), this shielding effect is not as pronounced. However, in Me_2CO-d_6 this proton is deshielded because of the interaction between the amine as proton-donor and the Me_2CO-d_6 oxygen as electron pair-donor (21). Interestingly, in compounds **1–3** and **5**, the 2-OH, which is H-bonded to the 3-formyl oxygen, is deshielded in C_6D_6 compared with the other two solvents.

A comparison of the chemical shift of H-4 in compounds containing a 2-OH substituent (**1–3**, **5**) with those containing a 2-OR substituent (**4**, **6**, **7**) is instructive. In the latter compounds in C_6D_6, H-4 resonates considerably further downfield than in the former compounds. We suggest that in compounds **1–3** and **5**, the 3-formyl group exists in conformation **A** because of intramolecular H-bonding with the 2-OH substituent, while with compounds **4**, **6**, and **7** conformation **B** predominates. Application of the so-called carbonyl plane rule (21,22) would predict that in C_6D_6, H-4 in conformation *B* would be deshielded because it is in front of the carbonyl plane, whereas in conformation **A** it would be shielded relative to its position in $CDCl_3$. It should also be noted that in the **A**-type compounds (**1–3**, **5**) the formyl proton is more shielded in all solvents compared with the **B**-type compounds (**4**, **6**, **7**). On the other hand, the formyl carbon (Table 2) is deshielded in the **A**-type group (~196 ppm) because of intramolecular H-bonding, as compared with the other group (~188.5 ppm). A comparison of the chemical shifts of the two methoxyl groups in **4** with the single methoxyl groups in **2**, **3**, **6**, and **7** establishes unambiguously that in **4** the downfield resonance in Me_2CO-d_6 and $CDCl_3$ (3.99 ppm), but the upfield resonance in C_6D_6 (3.32) is the 2-methoxyl group. It is hoped that knowledge of these solvent effects may find application in the structural elucidation of new carbazole natural products.



A



B

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were obtained on a Nicolet Model 20 SX/C Ft-ir spectrometer, uv spectra on a Perkin-Elmer Lambda 3 uv/vis spectrophotometer in 95% EtOH with and without 0.1 M NaOH solution, and ms on a VG Micromass 7070F or a ZAB-E spectrometer. ^{1}H- and ^{13}C-nmr spectra were obtained on a Bruker WH-400 spectrometer with TMS and Me_2CO-d_6 as internal standards, respectively. The multiplicities for ^{13}C-nmr spectra were determined by the attached proton test which produced positive (+) quaternary C and CH_2 signals and negative (−) CH and Me signals.

PLANT MATERIAL.—The roots of *M. siamensis* used in this study were obtained from Sukhothai Province, Thailand, during October of 1988. The plant material was authenticated by comparison with a

voucher specimen at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. The voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

EXTRACTION AND ISOLATION OF COMPONENTS.—The dried, chipped roots (1 kg) were macerated twice for 2-day periods with 95% EtOH (6 and 5 liters). The combined extracts were evaporated in vacuo, and the residue was suspended in H_2O (2 liters). The aqueous phase was extracted with $CHCl_3$ (4.5 liters). This organic phase was dried (anhydrous Na_2SO_4) and filtered, and the solvent was removed to yield 9.3 g of a gummy residue. This residue was divided into four equal portions, and each was purified by Si gel flash chromatography using $CHCl_3$ as eluent. Fractions of 25 ml each were collected and examined by tlc, and those containing the same material were combined to give the following components: fractions 2–3 gave 230 mg of **1**, fractions 5–7 gave 120 mg of **2**, fraction 9 gave 24 mg of **3**, fractions 11–23 gave residue A and fractions 26–32 gave 16 mg of **4**. Residue A was further purified by Si gel preparative tlc using EtOAc-petroleum ether (30–60°) (8:2) to yield 143 mg of xanthoxyletin, 28 mg of **5**, and residue B. Purification of residue B by Si gel flash chromatography using EtOAc-petroleum ether (30–60°) (1:1) gave 22 mg of **6** and 21 mg of **7**.

3-FORMYL-2,7-DIMETHOXYCARBAZOLE [**4**].—Mp 219–220°; uv (EtOH) λ max (log ε) 299 (4.69), 344 (4.14); ir (CCl_4) 3340, 2964, 1687, 1662, 1632, 1148, 1063 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims (rel. int.) *m/z* $[M]^+$ 255 (81), 240 (33); exact mass calcd for $C_{15}H_{13}NO_3$, 255.0895, found 255.0875.

7-METHOXYMURRAYACINE [**6**].—Mp 211–213°; uv (EtOH) λ max (log ε) 306 (4.58), 354 (4.09); ir ($CHCl_3$) 3460, 1666, 1628, 1603, 1156 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims (rel. int.) *m/z* $[M]^+$ 307 (44), 292 (100), 255 (58), 210 (65); exact mass calcd for $C_{19}H_{17}NO_3$, 307.1208, found 307.1208.

3-FORMYL-2-METHOXYCARBAZOLE (*O*-METHYLMUKONAL) [**7**].—Mp 189.0–189.5°; uv (EtOH) λ max (log ε) 296 (4.64), 350 (4.19); ir ($CHCl_3$) 1668, 1628, 1606, 1153 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims (rel. int.) *m/z* $[M]^+$ 225 (100), 208 (12), 179 (18), 154 (14); exact mass calcd for $C_{14}H_{11}NO_2$, 225.0790, found 225.0796.

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