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THE ISOLATION OF A PRENYLCOUMARIN OF CHEMOTAXONOMIC SIGNIFICANCE FROM *MURRAYA PANICULATA* VAR. *OMPHALOCARPA*

TAKESHI KINOSHITA,* JIN-BIN WU† and FENG-CHI HO‡

Faculty of Pharmaceutical Sciences, Teikyo University, 1091-1 Suarashi, Sagamiko-machi, Kanagawa199-01, Japan; †China Medical College, Taichung, Taiwan, Republic of China; ‡International Institute for Botanical Resources of Tropics, Hengchun 946, Taiwan, Republic of China

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Key Word Index—*Murraya paniculata* var. *omphalocarpa*; Rutaceae; coumarins; omphamurrayone; chemotaxonomy.

Abstract—A new C-8 prenylated 5,7-dimethoxycoumarin, omphamurrayone, was isolated from the leaves of *Murraya paniculata* var. *omphalocarpa*, and its structure was established as 5,7-dimethoxy-8-(3-methyl-1-*O*-isovaleryl-2-oxobutyl)-coumarin on the basis of spectroscopic evidence. Eight other coumarins were also obtained and identified as murralongin, isomurralonginol isovalerate, murrangatin, minumicrolin (murpanidin), coumurrayin, toddalenone, aurapten and toddasin. The chemosystematic status of *M. paniculata* var. *omphalocarpa* is briefly discussed with respect to the infraspecific differentiation of *M. paniculata*. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Murraya paniculata var. *omphalocarpa* (Hayata) Tanaka is indigenous to Taiwan (Orchid Island and Green Island), and recognized as one of three occurring varieties of *M. paniculata*, a rutaceous plant that has found wide medicinal value in tropical and subtropical Asia. This variety differs from the mother species in the following characters: fruits, flowers and leaflets are much larger than those of the mother species; petals are narrowed at the base; calyx lobes are elongate, ovate to linear-oblong [1]. However, these differences between the variety and the mother species are often viewed as gradational. Thus, it is worth investigating this plant chemically in view of the chemotaxonomic relationship between the variety and the species. The isolation of several alkaloids, coumarins and flavones has been reported from fruits [2], flowers [3], leaves [4, 5] and root barks [6, 7] of this plant, but the status of the variety has never been discussed from the chemosystematic viewpoint. Hence we have undertaken a more detailed chemical investigation of the leaves of this plant. In this report we describe the isolation and characterization of nine prenylated coumarins including one new compound.

RESULTS AND DISCUSSION

The acetone extract of the dried leaves of *M. paniculata* var. *omphalocarpa* on successive chromatographic separation afforded nine coumarins, murralongin (**1**), isomurralonginol isovalerate (**2**), murrangatin (**3**), minumicrolin (**4**), coumurrayin, toddalenone (**5**), aurapten, toddasin and a new coumarin for which a new name of omphamurrayone (**6**) was proposed. Murralongin (**1**) [8] and isomurralonginol isovalerate (**2**) [9] are 7-methoxy coumarin derivatives with the unusual rearranged prenyl units that have been reported from *M. paniculata*, and their occurrence in *M. paniculata* var. *omphalocarpa* was confirmed by this investigation. Murrangatin (**3**), its *erythro*-diastereoisomer minumicrolin (murpanidin) (**4**) and coumurrayin have already been obtained from the root bark of this plant [7]. Toddalenone (**5**) is a characteristic coumarin derivative possessing the (*E*)-3-oxo-1-butenyl unit at C-7 which is one carbon short of the ordinary prenyl units. It was first reported from *Toddalia asiatica* (Rutaceae) [10], and is also known to occur in *M. gleinei* [11]. Toddasin is an optically inactive dimeric coumarin derivative isolated from *T. asiatica* [12], and this is the first report of its isolation from a plant source other than the genus *Toddalia*. Aurapten, a 7-*O*-geranylcoumarin widely distributed in the genus *Citrus* [13], is now proved to occur in the genus *Murraya* for the first time.

Omphamurrayone (**6**) was obtained as optically active needles of mp 147–149° which analysed for $C_{21}H_{26}O_7$ (M^+ m/z 390). The 5,7-dimethoxy-8-substituted coumarin skeleton for this compound was indicated by a set of two doublets [δ 6.16 (1H, *d*, $J = 9.8$ Hz) and 7.97 (1H, *d*, $J = 9.8$ Hz)] and a singlet proton at δ 6.32 in the aromatic region of the ^{1}H NMR spectrum and absorption maxima at 249, 258 and 322 nm in the UV spectrum. The remaining part of the

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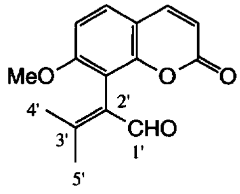
*Author to whom correspondence should be addressed.



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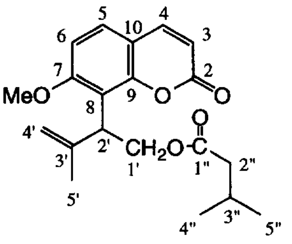

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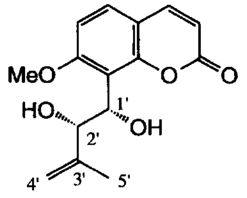

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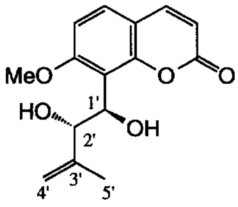

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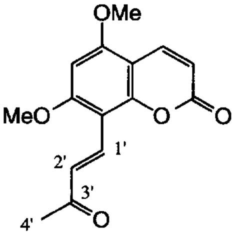

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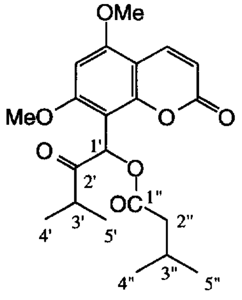

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molecule attached at C-8 was deduced from analytical data to be a $C_{10}H_{17}O_3$ side chain. The ^1H NMR spectrum showed the presence of an isovaleryl group [δ 0.95, 0.96 (3H each, *d*, $J = 6.7$ Hz), 2.14 (1H, *m*), 2.25 (1H, *dd*, $J = 6.7$ and 15.0 Hz) and 2.31 (1H, *dd*, $J = 7.6$ and 15.0 Hz)], an isopropyl group [δ 1.03, 1.17 (3H each, *d*, $J = 6.7$ Hz) and 2.85 (1H, *hept*, $J = 6.7$ Hz)] and a methine proton at δ 6.85. The ^{13}C NMR spectrum showed the presence of four methyl carbons [δ 18.1, 19.1, 22.4 ($2 \times C$)], one methylene carbon (δ 43.2), three methine carbons (δ 25.7, 36.2 and 69.4) and two carbonyl carbons [δ 171.9 (COO) and 208.1 (CO)] for the side chain (Table 1). From these spectral data the side chain at C-8 was elucidated as $-CH(OCOR)COCH(CH_3)_2$ (R = isovaleryl). Hence, the structure for omphamurrayone (**6**) was determined as 5,7-dimethoxy-8-(3-methyl-1-*O*-isovaleryl-2-oxo-butyl)-coumarin.

There has been little information available on the ^{13}C NMR spectra of prenylcoumarins isolated from the rutaceous plants. Therefore the carbon signals of coumarins isolated from *M. paniculata* var. *omphalocarpa* leaves were assigned by use of ^{13}C–^1H COSY and long range ^{13}C–^1H COSY, and the results are as shown in Table 1.

The presence of two distinct chemical races of *M. paniculata*, Formosan and Indonesian, has been indicated [14]. The Formosan race differs chemically from the Indonesian race in that the former race is devoid of 5,7-dimethoxy-8-prenylcoumarins that are principal constituents of the latter race [14, 15]. Since characteristic 5,7-dimethoxy-8-prenylcoumarin derivatives are shown to be present in var. *omphalocarpa* as mentioned above, it is apparent that this variety is closely related to the Indonesian race rather than to the Formosan race that occurs geographically in proximity. In view of the chemosystematic relation of var. *omphalocarpa* with the mother species (var. *paniculata*), this variety is perhaps derived not from the Formosan race but from the one related to the Indonesian race. The distribution of *M. paniculata* extends in the west to India, in the north to southern China and the Okinawa Islands, and in the south and east to New Guinea, New Caledonia and northern Australia [1]. Thus there may be other chemical types different from those referred to so far.

Table 1. ^{13}C NMR (100 MHz, δ ppm, TMS as internal standard) spectral data of the coumarins isolated from *Murraya paniculata* var. *omphalocarpa* leaves

C	1	2	3	4	5	6
2	160.9	160.7	160.2	160.2	160.3	160.0
3	112.7	113.0	113.4	113.5	111.3	111.6
4	143.6	143.9	143.8	143.7	138.5	138.3
5	128.5	127.8	128.6	128.5	158.2	158.0
6	107.5	108.0	107.9	108.0	90.2	90.5
7	159.8	161.1	160.2	160.6	163.1	162.0
8	112.7	116.1	116.1	116.5	104.6	104.6
9	152.2	153.6	152.9	153.3	154.9	154.6
10	112.7	113.1	113.1	113.3	103.8	103.9
1′	188.7	64.1	69.6	68.6	131.7	69.4
2′	159.7	40.7	78.4	78.5	129.8	208.1
3′	128.9	142.5	143.9	145.1	199.8	36.2
4′	24.6	111.7	113.7	113.8	27.5	18.1*
5′	19.5	22.1	17.4	18.0	—	19.1*
1″	—	172.8	—	—	—	171.9
2″	—	43.4	—	—	—	43.2
3″	—	25.6	—	—	—	25.7
4″	—	22.2	—	—	—	22.4
5″	—	22.2	—	—	—	22.4
5-OMe	—	—	—	—	56.1*	56.1
7-OMe	56.0	56.1	56.3	56.4	56.2*	56.3

*Assignments in the same column may be interchangeable. The numbering of carbons is shown in the structures.

The chemical investigation of *M. paniculata* from various localities is currently under way in order to clarify the infraspecific differentiation of *Murraya paniculata*.

EXPERIMENTAL

General procedures. Mps: uncorr; IR: JASCO FT/IR-8000, KBr; UV: Shimadzu UV-240, MeOH; ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR: JEOL JNM GSX-400, $CDCl_3$ with TMS as int. standard; MS: JEOL SX-102A; optical rotations: JASCO DIP-370, $CHCl_3$; Si gel CC: (Wakogel C-200), C_6H_6–Me_2CO (B–A) or *n*-hexane–AcOEt; Sephadex LH-20 CC: (Pharmacia), $CHCl_3$–MeOH (C–M); reversed-phase silica gel CC: (Merck RP-8), MeOH–H_2O(M–W); TLC: 0.25 mm precoated silica gel ($60F_{254}$, Merck), spots were detected by inspection under UV light (254 and 365 nm) or by the colours developed with 10% H_2SO_4 spraying followed by heating.

Plant materials. The leaves of *M. paniculata* var. *omphalocarpa* were collected in 1988 in Lan Yu (Orchid Island), Taiwan, and identified by one of the authors (F.-C. H.). A voucher specimen is on deposit at the Herbarium of Taiwan Forestry Research Institute, Hengchun, Taiwan.

Extraction and isolation of compounds. The dried leaves (2 kg) of *M. paniculata* var. *omphalocarpa* were extracted 2×with Me_2CO at room temp., and the combined extract was evapd to dryness under red. pres. to yield a greenish viscous syrup (127.4 g). The whole extract was dissolved in Me_2CO and adsorbed on silica gel (120 g). The adsorbed material was transferred to a silica gel column (1 kg) packed in *n*-hexane (column size: 10 × 28 cm). The column was eluted with a solvent system of *n*-hexane–EtOAc increasing the amount of EtOAc gradually. Frs of 500 ml each were collected and combined into 22 frs (Fr. I–XXII) on the basis of their TLC patterns. Fr. VIII (10.78 g) was subjected to silica gel CC on elution with B–A. Fractions eluted with 1% B–A were purified by RP-8 CC to afford aurapten (95 mg), mp 62–63° (lit. [13] 62–63°). Fr. IX (6.12 g) was separated by silica gel followed by LH-20 CC to give coumurrayin (203 mg), 156–157° (lit. [16] 158–158.5°). Fr. X (5.97 g) was chromatographed on a silica gel column (3.6 × 12 cm) on elution with the stepwise gradient solvent system (B–A). Frs of 200 ml each were collected. Frs 3–5 eluted with 3%, 4% and 6% B–A were combined and purified by repeated CC over silica gel, LH-20 and reversed-phase silica gel (RP-8) to give isomurralonginol isovalerate (128 mg), $[\alpha]_D + 25.1°$ (lit. [9] + 14.5°). Fr. XIII (18.53 g) was partitioned between MeOH (200 ml) and *n*-hexane (400 ml), and the MeOH layer was evapd to dryness. The residue was subjected to Sephadex LH-20 CC on elution with C–M (1:2) in order to remove chlorophylls. The coumarin-rich fractions were collected and chromatographed over silica gel (150 g) on elution with the B–A stepwise-gradient system. Frs eluted with 2% B–A were combined and further sepd by RP-8 CC to give omphamurrayone (222 mg). Fractions eluted with 6–8% B–A were repeatedly chromatographed over silica gel and RP-8, respectively, to give murralongin (163 mg), mp 135–137° (lit. [8] 137–138°). Fr. XIV (4.17 g) was separated by silica gel CC on elution with B–A followed by RP-8 CC to give murrangatin (390 mg), mp 133–134° (lit. [17] 133°) and minimicrolin (murpanidin) (1.58 g), mp 140–141° (lit. [18] 132–133°; [19] 163–164°). Fr. XVII (2.55 g) was recrystallized from Me_2CO to give toddasin (879 mg), mp 242–243° (lit. [12] 241°). Fr. XVIII (12.39 g) yielded yellow precipitates, which were recrystallized from MeOH to give toddalenone (**5**) (70 mg), mp 241–242° (lit. [10] 243–245°).

Omphamurrayone (**6**). Needles from MeOH–H_2O, mp 147–149°; $[\alpha]_D^{25} + 113.2°$ ($CHCl_3$; *c*, 0.718). IR ν_{max}^{KBr} cm^{-1}: 2965, 1732, 1603, 1472, 1341, 1248, 1115, 992. UV λ_{max}^{MeOH} nm (log ε): 249 (4.03), 258 (4.07), 322 (4.24). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95, 0.96 (3H each, *d*, $J = 6.7$ Hz, 4″-CH_3 and 5″-CH_3), 1.03, 1.17 (3H each, *d*, $J = 6.7$ Hz, 4′-CH_3 and 5′-CH_3), 2.14 (1H, *m*, 3″-H), 2.25(1H, *dd*, $J = 6.7$, 15.0 Hz, 2″-H), 2.31 (1H, *dd*, $J = 7.6$, 15.0 Hz, 2″-H), 2.85 (1H, *hept*, $J = 6.7$ Hz, 3′-H), 3.90 (3H, *s*, 7-OCH_3), 3.96 (3H, *s*, 5-OCH_3), 6.16 (1H, *d*, $J = 9.8$ Hz, 3-H), 6.32 (1H, *s*, 6-H), 6.85 (1H, *s*, 1′-H), 7.97 (1H, *d*, $J = 9.8$ Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1; EI-MS *m/z* (rel. int.): 390 $[M]^+$ (1), 319 (28), 235 (100), 219 (9); HR-MS: $[M]^+$ 390.1658 ($C_{21}H_{26}O_7$ requires 390.1678); Analyt. found: C, 64.83; H, 6.77 (calcd for $C_{21}H_{26}O_7$; C, 64.60; H, 6.71).

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