Isolation and Structure Elucidation of a New Prenylcoumarin from *Murraya paniculata* var. *omphalocarpa* (Rutaceae)

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A new C-8 prenylated 5,7-dimethoxycoumarin named omphamurrayin was isolated from the leaves of *Murraya paniculata* var. *omphalocarpa*, and its structure was established as 5,7-dimethoxy-8-(1-oxo-2-senecioyl-3-methyl-3-butenyl)-2*H*-1-benzopyran-2-one on the basis of the spectroscopic evidence. The taxonomic status of *M. paniculata* var. *omphalocarpa* is briefly discussed, along with its synonymity to *M. paniculata* from the chemosystematic viewpoint.

Key words *Murraya paniculata* var. *omphalocarpa*; Rutaceae; prenylcoumarin; chemotaxonomy

Murraya paniculata (L.) JACK var. *omphalocarpa* (HAYATA) TANAKA is a rutaceous shrub endemic to Lan Yu (Botel Tobago) of Taiwan. This variety was instated by Tanaka as distinct from the mother species based on the following morphologic characters: fruits are larger with attenuated tips; flowers are larger; petals are narrowed at the base; calyx lobes are elongate, ovate to linear oblong; and leaflets are broad.[1] However, these characters are often linked by gradations with those of the mother species, and thus it has been disputed whether it is distinguishable from the mother species.[2] To establish the relationship between this variety and the mother species from the chemotaxonomic viewpoint, we started chemical investigation of this plant and reported earlier the isolation of a number of prenylated coumarins[3,4] and one flavone[5] referring to the distinguishing chemical properties of this plant. After completion of the phytochemical investigation of this plant, we found that Huang had reduced var. *omphalocarpa* to a synonym of *M. paniculata*.[6] This prompted us to reinvestigate this plant to obtain more detailed chemical information in view of its chemosystematic relation to the mother species, which finally led to the isolation of a new prenylcoumarin derivative from its leaves. This paper refers not only to the isolation and structure elucidation of this compound but also to its chemotaxonomic significance in relation to the taxonomic status of var. *omphalocarpa*.

Further investigation of the chromatographic fractions reported in previous papers[3,4] led to the isolation of a new compound for which the name omphamurrayin is proposed (Fig. 1). Omphamurrayin (**1**) was obtained as colorless needles of mp 129—132 °C, and its molecular formula was calculated as $C_{21}H_{22}O_7$ based on high-resolution (HR) mass spectral analysis. The 5,7-dimethoxy-8-substituted coumarin skeleton of **1** was indicated by a set of two doublets [δ 7.90 (d, J=9.8 Hz), δ 6.14 (d, J=9.8 Hz)], a singlet proton at δ 6.27, and two methoxyls at δ 3.92 and 3.97 in its ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum. The absorption maximum at 319 nm in the ultraviolet (UV) spectrum is also supportive of the 5,7-dimethoxycoumarin skeleton. The remaining part of the molecule attached at C-8 was deduced from analytical data to be a $C_{10}H_{13}O_3$ chain. The ^{1}H-NMR spectrum showed the presence of a senecioyl group [δ 5.79 (1H, m), δ 2.18 (3H, d, J=1.4 Hz), δ 1.91 (3H, d, J=1.2 Hz)], *exo*-methylene [δ 5.01 (1H, m), δ 5.07 (1H, m)], allyl methyl [δ 1.79 (3H, m)] and one methine proton [δ 6.31 (1H, s)] in the C-8 side chain. The ^{13}C-NMR spectrum indicated the presence of one carbonyl carbon (δ 194.7). The occurrence of the senecioyl group in the side chain was confirmed by reconstitution of omphamurrayin from its hydrolysate and senecioyl chloride. The structure of the side chain was finally deduced to be 1-*oxo*-2-senecioyl-3-methyl-3-butene by ^{13}C–^{1}H long-range correlation spectroscopy (COSY) in which ^2J and ^3J cross peaks were observed in the following ^{13}C–^{1}H pairs: δ 194.7/δ 6.31; δ 117.8/δ 6.31; δ 165.0/δ 6.31; δ 82.2/δ 1.79; δ 82.2/δ 5.01; and δ 82.2/δ

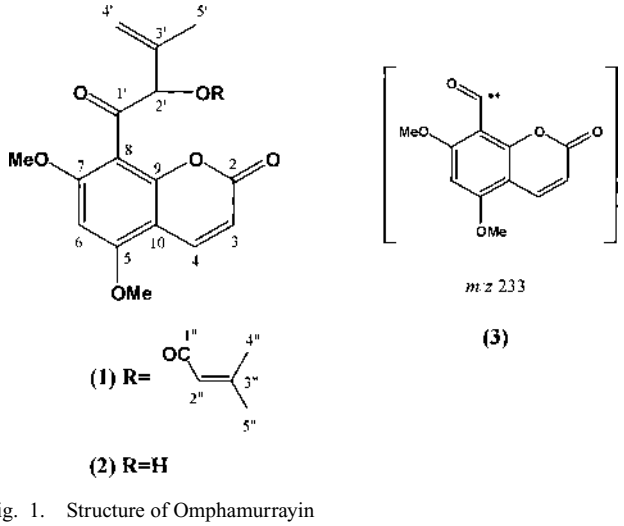

Fig. 1. Structure of Omphamurrayin

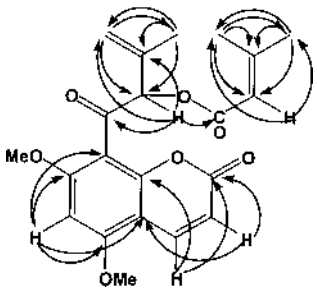

Fig. 2. ^2J and ^3J Interactions Observed in the Long-Range CH COSY of Omphamurrayin

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5.07) (Fig. 2). The location of a carbonyl group at C-2′ was further substantiated by the appearance of a strong base ion peak (**3**) at *m*/*z* 233 in the mass spectrum (Fig. 1).

Omphamurrayin possesses one asymmetric carbon at the 2′-position. Since its optical rotation was small ($[\alpha]_D^{25}$ −6.0°), it is probable that **1** was partially racemic. To determine its absolute stereochemistry, **1** was hydrolyzed under mild conditions. However, the hydrolysate lost optical activity completely. It is not surprising that hydrolysis of **1** led to the loss of optical activity, since the 2′-position of **1** is an active methine adjacent to the both carbonyl and vinyl group that is thus prone to racemization. Hydrolysis of **1** under acidic conditions yielded unsatisfactory results.

There is no doubt that chemical species characterizing *M. paniculata* and its allied species are a variety of coumarins. A feature common to those coumarins is the presence of an isoprenoid unit attached at the 8-position of either the 7-methoxy- or 5,7-dimethoxycoumarin skeleton. These isoprenyl units occur in a tremendous variety of either oxidized, esterified, or skeletally rearranged forms. We considered these isoprenylated coumarins to be useful chemical markers suitable for discussing the chemotaxonomy of *Murraya* species, and then tried to classify coumarin derivatives occurring in *Murraya* by the types of their presumed biogenetic precursors. We assumed that variegated forms of the isoprenyl group of coumarins occurring in the genus *Murraya* are biogenetically derived from the corresponding epoxide precursors such as 5,7-dimethoxy-8-(2,3-epoxy-3-methylbutyl)-2*H*-1-benzopyran-2-one (sibiricin), 5,7-dimethoxy-8-(1,2-epoxy-3-methyl-3-butenyl)-2*H*-1-benzopyran-2-one (gleinadiene epoxide), 8-(2,3-epoxy-3-methylbutyl)-7-methoxy-2*H*-1-benzopyran-2-one (meranzin), and 8-(1,2-epoxy-3-methyl-3-butenyl)-7-methoxy-2*H*-1-benzopyran-2-one (phebalosin), and expediently designated coumarins arising from each of the above epoxides as the A_1, A_2, B_1 and B_2 types, respectively.[7] Finally, *M. paniculata* from various localities and its related species were subjected to the analysis of constituents for their presumed biogenetic precursors in an attempt to reconstruct the taxonomy of *M. paniculata*, of which the morphologic diversity has long puzzled taxonomists. It has already been pointed out by the first author that there is distinct chemical difference between Formosan and Indonesian *M. paniculata*, indicating the possible occurrence of chemical races.[8,9] The former race is characterized as containing the B_1 and B_2 types and completely lacking the A_1 and A_2 types, whereas the latter contains the A_1 in addition to the B_2 type. Since A_1- and A_2-type coumarins possess a 5,7-dimethoxycoumarin skeleton showing strong bright blue fluorescence under long-wavelength UV light (365 nm), both races can be easily distinguished using thin-layer chromatography. The phytochemical investigations[3—5] carried out so far on *M. paniculata* var. *omphalocarpa* demonstrated the presence of the A_1, A_2, and B_2 types, suggesting that from the chemosystematic viewpoint var. *omphalocarpa* is not derived from the Formosan race of which the locality is geographically proximate to that of this variety. Although it is certain that var. *omphalocarpa* is akin to the Indonesian race rather than the Formosan race as indicated previously,[3] there are notable chemical differences between var. *omphalocarpa* and the Indonesian race in terms of the presence/absence of A_2 coumarins. Two A_2-type coumarins have been reported so far: omphamurrayone[3] and omphamurrayin (**1**), both of which are known so far to occur only in var. *omphalocarpa*. Therefore these coumarins that are presumed to arise biogenetically from gleinadiene epoxide as stated above may characterize the discriminative chemical property of var. *omphalocarpa*. These findings appear to suggest that this variety is botanically differentiated from *M. paniculata*, nullifying its synonymity with the mother species which Huang proposed in the taxonomic dichotomy of *M. paniculata*.[6] However, it should be noted that there might be other chemical races that remain to be chemically investigated. Elucidation of chemical information on the Philippine *M. paniculata* will probably play a key role in determining the chemosystematic status of var. *omphalocarpa*, since the Philippines are geographically located between Taiwan and Indonesia, and the flora of Lan Yu, where var. *omphalocarpa* occurs as an endemic variety, is considered to be closer to Philippine flora than that of Taiwan.[10] The chemical investigation of Phillipine *M. paniculata* is currently under way from this point of view.

Experimental

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: ^{1}H- and ^{13}C-NMR spectra with a JEOL JNM GSX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer with tetramethylsilane (TMS) as internal standard; mass spectra (MS) with a JEOL SX-102A mass spectrometer; IR spectra with a JASCO FT/IR-8000 IR spectrometer; optical rotations with a JASCO DIP-370 polarimeter; and UV spectra with a Shimadzu UV-240 spectrometer. Column chromatography was carried out with Wakogel C-200 or Merck Kieselgel 60 (eluted with hexane–ethyl acetate or benzene–acetone), Sephadex LH-20 (Pharmacia, eluted with MeOH–$CHCl_3$), and RP-8 reversed-phase silica gel (eluted with MeOH–H_2O).

Plant Material The leaves of *M. paniculata* var. *omphalocarpa* were collected in Lan Yu (Botel Tobago), Taiwan, in 1988. A voucher specimen and its duplicate were deposited in the Herbarium of the Taiwan Forestry Research Institute, Hengchun, Taiwan, and Medicinal Plant Research Station, Teikyo University, Kanagawa, Japan, respectively.

Extraction and Isolation The dried leaves (2 kg) of *M. paniculata* var. *omphalocarpa* were extracted two times with distilled acetone at room temperature, and the combined extracts were evaporated to dryness under reduced pressure to yield a greenish viscous syrup (127.4 g). The entire extract was dissolved in acetone and adsorbed on silica gel (120 g). The adsorbed material was transferred to a silica gel column (1 kg) packed in hexane. The column was eluted with the hexane–ethyl acetate mixed solvent system, and 22 fractions (fr. I—fr. XXII) were collected as described in the previous papers.[3,4] Fraction XIII was subjected to a series of chromatographic separation using silica gel, Sephadex LH-20, or RP-8 reversed-phase silica gel to afford an omphamurrayin-rich fraction. This fraction was recrystallized from MeOH–H_2O to give pure omphamurrayin (114 mg) as colorless needles of mp 129—132 °C.

Omphamurrayin (**1**): $[\alpha]_D^{25}$ −6.0° (*c*=0.269, MeOH). IR (KBr) cm^{-1}: 3444, 2938, 1715, 1595, 1456, 1224, 1146. UV λ_{max} (MeOH) nm (log ε): 283 sh (3.99), 319 (4.21). ^{1}H-NMR ($CDCl_3$) δ: 1.79 (3H, m, 5′-CH_3), 1.91 (3H, d, *J*=1.2 Hz, 5″-CH_3), 2.18 (3H, d, *J*=1.4 Hz, 4″-CH_3), 3.92 (3H, s, 7-OCH_3), 3.97 (3H, s, 5-OCH_3), 5.01 (1H, m, 4′-H), 5.07 (1H, br m, 4′-H), 5.79 (1H, m, 2″-H), 6.14 (1H, d, *J*=9.8 Hz, 3-H), 6.27 (1H, s, 6-H), 6.31 (1H, s, 2′-H), 7.90 (1H, d, *J*=9.8 Hz, 4-H). ^{13}C-NMR ($CDCl_3$) δ: 18.4 (C-5′), 20.4 (C-5″), 27.5 (C-4″), 56.2 (7-OCH_3), 56.3 (5-OCH_3), 82.2 (C-2′), 90.0 (C-6), 103.5 (C-10), 108.9 (C-8), 111.8 (C-3), 115.6 (C-2″), 117.8 (C-4′), 138.0 (C-4), 139.1 (C-3′), 153.6 (C-9), 157.9 (C-3″), 158.5 (C-5), 159.7 (C-2), 160.9 (C-7), 165.0 (C-1″), 194.7 (C-1′). Electron impact (EI-MS) *m*/*z* (int. %): 386 (M^+, 2), 233 (100), 83 (12). HR-EI-MS *m*/*z*: 386.1362 (Calcd for $C_{21}H_{22}O_7$: 386.1366).

Hydrolysis of 1 Omphamurrayin (30 mg) was dissolved in 10 ml of methanol, and 20 mg of powdered sodium methoxide was added to this solution. The reaction mixture was left overnight at room temperature, poured into icy water (100 ml) and extracted three times with ether. The ether layer was washed with 1 N hydrochloric acid, dried, and concentrated to dryness to afford an oily residue (19 mg). $[\alpha]_D^{25}$ 0° (*c*=0.38, MeOH). IR ($CHCl_3$) cm^{-1}:

3490, 2960, 1715, 1598, 1436. UV λ_{max} (MeOH) nm: 283 sh, 319. ^{1}H-NMR ($CDCl_3$) δ: 1.83 (3H, m, 5′-CH_3), 3.93 (3H, s, 7-OCH_3), 3.96 (3H, s, 5-OCH_3), 4.23 (1H, br, 2′-OH), 4.90 (1H, m, 4′-H), 4.98 (1H, br m, 4′-H), 5.11 (1H, br s, 2′-H), 6.13 (1H, d, J=9.7 Hz, 3-H), 6.25 (1H, s, 6-H), 7.92 (1H, d, J=9.7 Hz, 4-H). ^{13}C-NMR ($CDCl_3$) δ: 18.2 (C-5′), 56.2 (7-OCH_3), 56.3 (5-OCH_3), 81.6 (C-2′), 90.0 (C-6), 103.4 (C-10), 109.4 (C-8), 111.7 (C-3), 117.8 (C-4′), 138.2 (C-4), 142.4 (C-3′), 153.8 (C-9), 158.9 (C-5), 159.8 (C-2), 161.0 (C-7), 195.6 (C-1′). EI-MS *m/z* (int. %): 304 (M^+, 0.3), 287 (5), 233 (100).

Reconstitution of Omphamurrayin from 2 The hydrolysate (**1**; 15 mg) was dissolved in 5 ml of dry pyridine and a small amount of senecioyl chloride was added to this mixture. After standing overnight, the reaction mixture was evacuated to dryness and the residue was crystallized from MeOH–H_2O to give the semisynthetic omphamurrayin as pure colorless needles (12 mg), which was identified as natural omphamurrayin by the IR spectrum. This procedure unequivocally confirmed the presence of a senecioyl group in omphamurrayin.

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References and Notes

1) Tanaka T., *J. Soc. Trop. Agric*. (Taiwan), **1**, 23—44 (1929).
2) In this paper the scientific name of *M. paniculata* var. *omphalocarpa* is retained for convenience, since all papers published so far concerning the chemistry of this plant have used this name.
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7) Details of the classification of coumarins occurring in *Murraya* species will appear elsewhere, and are available from T. Kinoshita upon request.
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