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Waltherione C and cleomiscosin from *Melochia umbellata* var. Degrabrata K. (Malvaceae), biosynthetic and chemotaxonomic significance

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1. Subject and source

Melochia, which comprises fifty-four species spread from India easterly across to the central region of the Americas, is a genus of the family Malvaceae[1] (Chase and Reveal, 2009) and grows well in tropical forests (Goldberg, 1967). Plants from this genus are well known in eastern Indonesia for their medicinal properties.

The *Melochia* are rich in alkaloids, notably cyclopeptides (Tschesche and Reutel, 1968; Kapadia et al., 1977a; Bhakuni et al., 1987a; Dias et al., 2007a; Emile et al., 2007), isatins (Kapadia et al., 1977b, 1980; Bhakuni et al., 1991; Kapadia and Shukla, 1993), and 4-pyridinone and 4-quinolinone derivatives (Kapadia et al., 1975, 1978; Medina and Spiteller, 1979; Medina and Spiteller, 1981; Arriaga Giner et al., 1983; Dias et al., 2007a, 2007b; Jadulco et al., 2014), in addition to compounds in the expected secondary metabolite classes, flavonoids, triterpenes, steroids etc. (Shukla et al., 1976; Nair et al., 1977; Gunasegaran et al., 1980; Borges del Castillo et al., 1983; Bhakuni et al., 1986, 1987b; Dias et al., 2007a; Tripathi et al., 2010; Ridhay et al.,

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[1] The genus *Melochia* was formerly classified in the family Sterculiaceae. The genera of the Sterculiaceae have recently been placed into the family Malvaceae and the use of the family name Sterculiaceae discontinued.

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2012). One of the species in this genus is *Melochia umbellata*, known in South Sulawesi, Indonesia, as paliasa, and is used to treat many kinds of disease such as hepatitis, hypercholesterolemia, diabetes, and hypertension (Hadi and Bremner, 2001).

Herein we report the isolation and a quinolinone alkaloid, waltherione C (Fig. 1), as well as a coumarinolignan, cleomiscosin A (Fig. 2), both known compounds. A possible biosynthetic scheme explaining the relationship between waltheriones A–D and other 4-quinolinones isolated from plants from the Malvaceae family is proposed.

Melochia umbellata Stapf. was collected from Makassar city, South Sulawesi, Indonesia (5° 08′ 30″ S, 119° 27′ 11″ E) in February, 2007. The plant was identified Dr Eko Baroto Walujo of the Herbarium Bogoriense, LIPI Bogor, Indonesia and a voucher specimen was lodged in the Herbarium Bogoriense collection (voucher specimen number BO-1912171).

2. Present study

The dried heartwood of *M. umbellata* (12.7 kg) was extracted with MeOH and the dried MeOH extract (167 g) was re-suspended in water and sequentially partitioned with 4 × 200 mL n-hexane (yield 10 g), 4 × 250 mL $CHCl_3$ (20 g), and 4 × 200 mL EtOAc (10 g). After removal of solvent under reduced pressure, the $CHCl_3$ fraction was further fractionated using vacuum column chromatography (silica gel, n-hexane-EtOAc eluents of increasing polarity and finally acetone) to give ten major fractions. The fifth fraction (590 mg) was fractionated by flash chromatography using hexane-acetone with ratios 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 0:10 to give eight fractions, of which the second fraction was subjected again to flash chromatography column with n-hexane-EtOAc eluent ratios of 8:2, 7.5:2.5; 7:3; 6:4; 0:10 to give 4 major fractions. The third, and largest, fraction was recrystallized from chloroform-hexane (8:2) to obtain waltherione C (13 mg, 1.0×10^{-4} % yield from dried heartwood).

The fourth fraction from the vacuum chromatography of the $CHCl_3$ partition (397 mg) was subjected to flash chromatography, eluting with hexane/acetone (9:1; 8:2; 7:3; 6:4; 0:10), to obtain ten fractions, of which the ninth (64 mg) was further separated by flash chromatography column (hexane-acetone; 7:3; 6:4; 5:5; 0:10) to give three subfractions.

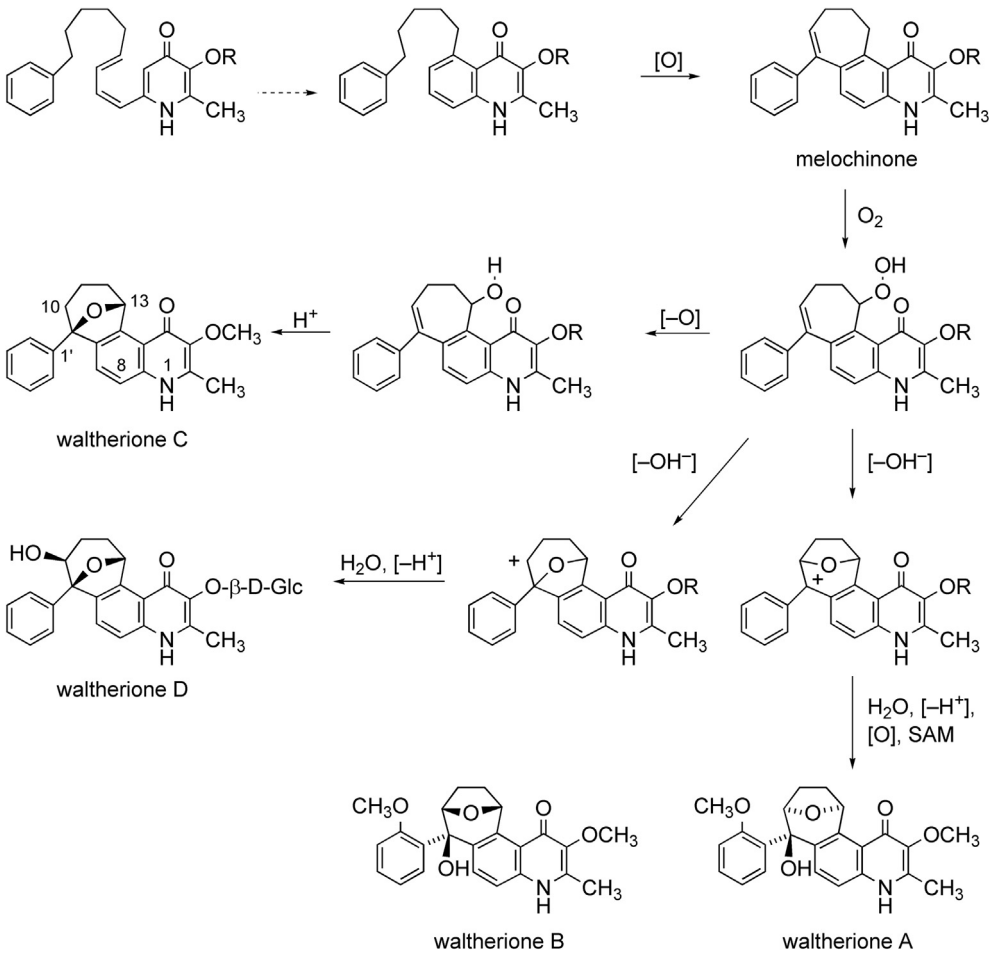

Fig. 1. Suggested mode of cyclic ether formation in the biosynthesis of waltheriones A–D via melochinone.

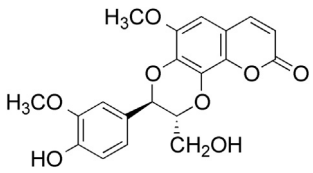

Fig. 2. Structure of cleomiscosin A.

Cleomiscosin A (5.8 mg, 4.6×10^{-5} % yield from dried heartwood) crystallized from the second subfraction from acetone-chloroform (1:1).

Waltherione C (Fig. 1), isolated as pale yellow powder, has a molecular formula of $C_{22}H_{21}NO_3$ determined by HRESI-MS. The UV spectrum shows λ_{max} (MeOH) at 217, 245, 334, and 347 nm, indicating the presence of an extended aromatic conjugated system. The IR spectrum exhibits absorptions for secondary amine (3273 cm^{-1} N–H), aromatic (3065 and 1554 cm^{-1}), and aliphatic (C–H) (2933, 2906, 2827, 2782, and 2785 cm^{-1}) groups. The ^1H NMR spectrum of waltherione C shows aromatic signals consistent with a mono-substituted phenyl group at δ_H 7.55 (d, $J = 7.2$ Hz, 2H), 7.39 (t, $J = 7.2$ Hz, 2H) and 7.31 (tt, $J = 7.2, 1.3$ Hz, 1H). A pair of signals at δ_H 7.28 (d, $J = 8.5$ Hz, 1H) and 7.04 (d, $J = 8.5$ Hz; 1H) are consistent with a pair of isolated vicinal aromatic protons. Other easily distinguishable proton signals appearing in the ^1H NMR spectrum are an oxymethine proton at δ_H 6.04 (bd, $J = 3.3$ Hz), apparently coupled to a complex spin system of a further six aliphatic protons, an aromatic methyl group singlet at δ_H 2.50, an aromatic methoxy group at δ_H 3.88 and a broadened proton singlet at δ_H 9.50 attached to a heteroatom, supporting the presence of a secondary nitrogen group suggested by the infrared spectrum. The ^{13}C NMR spectrum shows a carbonyl carbon (δ_C 173.7), an additional fourteen sp^2 hybridized carbon signals, two sp^3 hybridized carbons carrying oxygen (δ_C 85.4, C and 80.4, CH), three aliphatic methylene groups, and methyl and methoxyl groups (δ_C 15.5 and 60.1, respectively). These data, and taking into account the highly unsaturated nature of the compound and comparison of spectroscopic data obtained from secondary metabolites previously reported from *Melochia* spp., indicated that the compound has the same structure as that recently reported by Jadulco et al. (2014) for waltherione C, isolated from *Melochia odorata* L.f. Despite the NMR spectroscopic data being obtained in a different solvent (CD_3OD) compared to the current report ($CDCl_3$), the data is a close match: ^1H average difference 0.04 ppm (largest difference 0.1 ppm), ^{13}C 0.8 (2.8). The structure was fully supported by 2D NMR spectroscopy. Ultra-violet and IR spectroscopic data in both reports are also consistent with each other.

The second compound obtained has a molecular formula of $C_{20}H_{18}O_8$ by ESI-MS and NMR data and, as indicated by its UV spectrum, is highly unsaturated. The IR spectrum of this compound exhibited absorbances consistent with hydroxyl (3453 cm^{-1}), aromatic (3078, 1577, and 1446 cm^{-1}) and conjugated carbonyl groups (1701 cm^{-1}). Its ^1H NMR spectrum showed, with the assistance of COSY correlations, the presence of aromatic signals typical of a phenyl group ABX system (δ_H 6.95 (1H, d, $J = 1.8$ Hz), 6.84 (1H, dd, $J = 8.1, 1.8$ Hz), 6.80 (1H, d, $J = 8.1$ Hz)) in which protons are shielded due to the presence of electron donating substituents on the phenyl ring. HMBC correlations originating from the protonated α,β-unsaturated carbonyl methine groups (δ_H 6.23 (1H, d, $J = 9.5$ Hz), 7.83 (1H, d, $J = 9.5$ Hz)) led to the incorporation of a further six aromatic carbons and a methoxyl group (δ_H 3.792 (3H, s)) into an isolated spin system which could be accommodated by a coumaryl moiety, similar to the 6-methoxy-7,8-dioxymethylenecoumarin previously isolated from *M. tomentosa* (Shukla et al., 1976). These considerations led to the assignment of this compound as one of the cleomiscosins A or B. The ^{13}C NMR chemical shifts of the compound are within 2 ppm of published values (Kan et al., 2011), despite being recorded in a different solvent, viz. pyridine-d_5. It is notoriously difficult to distinguish between cleomiscosins A and B using spectroscopic data from just one regioisomer (Arnoldi et al., 1984; Begum et al., 2010). However, a weak correlation observed between H-7′ and C-7 in the isolated compound's HMBC spectrum indicated that the compound is most likely cleomiscosin A (Fig. 2). So far, these cleomiscosins have been found to be optically inactive and thus are racemates (Begum et al., 2010).

3. Biosynthesis and chemotaxonomy

The biosynthesis of compounds with this unusual skeleton may be more interesting than first appears. Firstly, the presence of 4-pyridinone in addition to quinolone metabolites in *Melochia* spp. suggests that the precursor of the heterocyclic portion of the molecule is not anthranilic acid, as might be anticipated (Kapadia et al., 1975, 1978; Medina and Spiteller, 1979; Medina and Spiteller, 1981). Secondly, the observation that the configuration of the oxygen substituents at C-9 and C-10 of waltheriones B and D are not *trans*, as might be expected if the are formed by oxygen nucleophile attack on an epoxide derived from an alkene such as found in melochinone, suggests the existence of a stable carbonium ion intermediate which can be attacked by a nucleophile from either side. It may be that the formation of the epoxide across the seven membered ring is initiated by oxidation at the benzylic position C-13, as shown in Fig. 1. In fact, the formation of a hydroperoxy intermediate followed by concerted addition of the peroxide to a C-9,10 double bond is a neat explanation of the formation of the *syn* stereochemistry of the oxygen substituents at C-9 and C-10 in waltheriones B and D. However, invocation of a concerted mechanism may not be necessary since molecular modeling shows that formation of the *syn* configuration is sterically favorable.

The absolute configurations of waltheriones A and B have been determined (Gressler et al., 2008) and are as shown in Fig. 1 whereas only relative configurations in waltheriones C and D have been proposed (Jadulco et al., 2014). Despite the difference in the magnitude of the specific rotation of waltherione C isolated from *M. odorata* (Jadulco et al., 2014) and *M. umbellata* ($[\alpha]_D$ -17° and -65°, respectively), both being levorotatory suggests that they are the same enantiomer.

Consideration of the 4-pyridones and 4-quinolines isolated from *Melochia* spp. suggests a biosynthesis of the pentacyclic systems that originates from a polyunsaturated sphingolipid-like compound with a benzoic acid starter unit. This kind of chemistry is thus far unknown in the angiosperms, making these compounds a chemotaxonomic marker for the *Melochia* and *Waltheria* (Hoelzel et al., 2005), both of the Malvaceae family.

Cleomiscosins A and B appear to be widely distributed amongst the angiosperms but reports of their isolation appear to be concentrated in the superorders Rosanae (particularly among the Sapindales and Malpighiales) and Asteranae. *Melochia umbellata* belongs to the order Malvales, superorder Rosanae.

4. Biological activity

The crude extract from *M. umbellata* was active in the brine shrimp (*Artemia salina* Leach) assay (LC_{50} 1.80 μg/mL) and waltherione C and cleomiscosin A showed LC_{50} activities in this assay of 0.29 μg/mL and >1000 μg/mL, respectively (Meyer et al., 1982). Waltherione C has been reported as having anti-HIV activity (EC_{50} 0.3 μg/mL) and toxicity against CEM-TART cells (LC_{50} 3.8 μg/mL) (Jadulco et al., 2014). In our hands, waltherione C showed significant cytotoxicity against P-388 murine leukemia cells while cleomiscosin A appeared to be inactive, with IC_{50} values of 0.26 and >100 μg/mL, respectively (Alley et al., 1988).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2014.03.020.

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