N-methyl-5-carboxamide-2-pyridone from *Mallotus barbatus*: A chemosystematic marker of the Euphorbiaceae genus *Mallotus*

Céline Rivière a,e,1,*, Van Nguyen Thi Hong b,1, Nam Nguyen Hoai c, Bieke Dejaegher d, Christophe Tistaert d, Kiem Phan Van c, Yvan Vander Heyden d, Minh Chau Van c, Joëlle Quetin-Leclercq a

a Pharmacognosy Research Unit, Louvain Drug Research Institute, Avenue E. Mounier, 72, Université catholique de Louvain, Brussels B-1200, Belgium
b Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Viet Nam
c Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Viet Nam
d Department of Analytical Chemistry and Pharmaceutical Technology, Laarbeeklaan, 103, Vrije Universiteit Brussel – VUB, Brussels B-1090, Belgium
e Laboratoire de Pharmacognosie, EA4481 (GRIIOT), Faculté des Sciences Pharmaceutiques et Biologiques, Université Lille Nord de France (Lille 2), F-59006 Lille Cedex, France

1. Subject and source

*Mallotus* Lour., in the Malpighiales order and in the Euphorbiaceae family, consists of a large paleo(s)ub)tropical genus of shrubs, trees and rarely climbers with a wide distribution in different habitats of Southeast Asian forests, whether in the primary forest understories or in the more disturbed secondary forests (Kulju et al., 2007a, 2007b; Kulju and van Welzen, 2008). Thus some species are used as indicators of forest disturbance (Slik et al., 2003). *Mallotus* is placed in the tribe Acalyphae pro-parte of the uniovulate sub-family Acalypoideae and has been classified in subtribe Rottlerinae with seven small genera (*Coccoceras*, *Cordemoya*, *Deuteromallotus*, *Neotrewia*, *Octospermum*, *Rockinghamia* and *Trewia*) according to the Euphorbiaceae classifications of Webster and Radcliffe-Smith (Webster, 1994; Radcliffe-Smith, 2001; Nowicke and Takahashi, 2002). *Mallotus* is morphologically close to the large monophyletic genus *Macaranga*, classified in the same tribe Acalyphaeae but in a separate monogenic subtribe Macaranginae (Kulju et al., 2007a). A recent molecular phylogenetic study on *Mallotus* and its eight related genera underlined the paraphyly of *Mallotus* (Kulju et al., 2007a). This genus, commonly known as *Ba bet*...
in Vietnam, is one of the most diverse and richest genera of the Euphorbiaceae family in this country, where about 40 Mallotus species may be found among which six species and one variety are endemic (Thin, 2003). 

Mallotus barbatus (Wall.) Müll. Arg., is referred as Rung buec in Vietnam, is a small tree growing particularly in the evergreen forests all over the mountainous areas from the North to the South of Vietnam at an altitude inferior to 1100 m. Mallotus barbatus is also distributed in other countries (China, Laos, Cambodia, Thailand, Malaysia, Philippines, Indonesia, Myanmar and India). Different parts of this plant (roots, stem bark, leaves) are used in traditional medicine for treating gastrointestinal disorders, oedema and headache (Thin, 2003). The leaves of M. barbatus were collected in May 2006 at the SonLa mountains (Northern Province) in Vietnam and identified by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, VAST, Vietnam. A voucher specimen has been deposited at the Institute of Natural Products Chemistry, VAST, Vietnam (N° M. b. 03).

2. Previous works

Some Mallotus species are known to contain different natural compounds, mainly diterpenoids, triterpenoids, steroids, flavonoids, coumarinolignoids, phloroglucinol derivatives or benzopyrans, and to exhibit interesting biological activities such as antimicrobial, antioxidant, antiviral, or cytotoxic ones (Rivière et al., 2010). In the course of our ongoing project to investigate the biologically active chemical constituents from Mallotus growing in Vietnam (Chau et al., 2004, 2005, 2009; Rivière et al., 2009, 2010; Hoai et al., 2009), we reported herein the identification of some compounds from M. barbatus leaves. An earlier phytochemical study revealed that the leaves contained polyphenols (Sasak and Chonjnacki, 1973). However, to our knowledge, no other reports on the chemical composition of M. barbatus have been made in the literature so far.

3. Present study

The dried and powdered leaves of M. barbatus (840 g) were extracted three times with MeOH (3 x 2 L) to give 80 g residue. This extract was subjected to column chromatography on silica gel 0.04–0.063 mm with CHCl3–MeOH and CHCl3 conditions to give 25 fractions (A–Y). Fraction X (100 g) was subjected to preparative thin-layer chromatography (PTLC) on silica gel (60 F 254, Merck) 0.25 mm with CHCl3–MeOH (99:1) as developing systems to give 10 fractions (A–I). Fraction II (6 g) was subjected to preparative TLC on silica gel 60 F 254 (Merck) 0.25 mm with CHCl3–MeOH (9:1) as developing systems to give 5 fractions (A–E).

The chloroformic partition was subjected to a vacuum liquid chromatography (VLC) on silica gel normal phase with CH2Cl2–MeOH and CH2Cl2–AcOEt to give 10 fractions (A–I). Friedelin 4 was obtained in fraction A by crystallization in CH2Cl2–MeOH and identified by GC–MS and comparison with reference sample. The gas chromatograph was a TRACE GC 2000 series, equipped with an autosampler AS2000 Thermo-Quest. The GC system was interfaced to a Trace MS mass spectrometer in the electron-impact mode. Chromatographic separations were performed on a capillary nonpolar column (DX-XLB; column length 15 m x 0.25 mm with 0.25 μm film thickness) from Agilent Technologies (Rivière et al., 2009) (Fig. 1).

4. Chemotaxonomic significance

N-methyl-2-pyridone-5-carboxamide 3 is a metabolite of nicotinic acid in mammals. Nicotinic acid, also known as niacin, is a member of the B-vitamin family and has been identified in Mallotus apelta (Kang and Lu, 2007). N-methyl-2-pyridone-5-carboxamide has been already isolated from Euphorbiaceae, from Trewia nudiflora L. (Sastry and Waller, 1972) and more recently from Mallotus anisopodus Airy Shaw (Chau et al., 2009). In the Euphorbiaceae, Mallotus and Trewia are placed in the tribe Acalypheae pro parte in the subtribe Rottlerinae (Webster, 1994; Radcliffe-Smith, 2001). Trewia is an Asiatic ditypic genus (T. nudiflora L. present from India to the Philippines and T. polyacarpa Benth. & Hook. f., an Indian endemic species) morphologically very close to Mallotus. They share the pollen type, the extrafloral nectaries on the upper leaf surface and a similar type of glandular hairs, a character which is also typical for most species of Macaranga but rare for species in other sub-families within Euphorbiaceae (Kulju et al., 2007a, 2007b). Trewia differs from Mallotus only in the fruit type. The fruits in T. nudiflora L. are indehiscent and drupaceous instead of generally dehiscent in Mallotus (Kulju and van Welzen, 2008).
M. hookerianus N-methyl-2-pyridone-5-carboxamide could be a chemotaxonomical indicator of the genus Mallotus from other genera have been reported. Thus, the isolation of this compound from (Kulju and van Welzen, 2008). To our knowledge, no other reports on the isolation of N-methyl-2-pyridone-5-carboxamide thus merged recently with a separate generic status. Although the fruit type is a noticeable morphological difference, this character is not a suf
to be part of the main Mallotus s.s. clade; whereas, four Rottlerinae genera, including Coccoceras, Neotrewia, Octospermum and Trewia, were found to be part of the main Mallotus clade named Mallotus s.s. clade, sister group with the Macaranga clade (Kulju et al., 2007a). Although the fruit type is a noticeable morphological difference, this character is not a sufficient justification to maintain a separate generic status. Trewia, along with the other three Rottlerinae genera Coccoceras, Neotrewia and Octospermum, were thus merged recently with Mallotus Kulju et al., 2007b). T. nudiflora L. is now called Mallotus nudiflorus (L.) Kulju & Welzen (Kulju and van Welzen, 2008). To our knowledge, no other reports on the isolation of N-methyl-2-pyridone-5-carboxamide from other genera have been reported. Thus, the isolation of this compound from T. nudiflora L., as from some species of Mallotus, could justify the molecular phylogeny data leading to the merger of the genus Trewia with the genus Mallotus. N-methyl-2-pyridone-5-carboxamide could be a chemotaxonomical indicator of the genus Mallotus.

Friedelin 4, a pentacyclic triterpene, has been isolated from other species of Mallotus: M. apelta (Chau et al., 2005), M. hookerianus (Hui and Li, 1976), M. paniculatus (Hui et al., 1969), M. philippensis (Nair and Rao, 1993) and M. repentus (Hui and Li, 1976). The flavonoid, quercitrin 1, has been isolated from M. apelta (Chau et al., 2004) and from M. metcalfianus (Rivière et al., 2009), and more recently identified by LC-ESI-MS in M. nanus, M. cuneatus, M. paniculatus growing in Vietnam (Nguyen et al., 2011). 3-O-α-L-rhamnosyl kaempferol 2 has been also isolated from M. metcalfianus and identified in M. nanus (Rivière et al., 2009; Nguyen et al., 2011).

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