Journal of King Saud University – Science 30 (2018) 283–285

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Short communication

Alkaloids as chemotaxonomic markers from the Philippine endemic *Uncaria perrottetii* and *Uncaria lanosa* f. *philippinensis*

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ARTICLE INFO

Article history:
Received 14 November 2017
Accepted 17 December 2017
Available online 18 December 2017

Keywords:
Rubiaceae
Uncaria
Oxindole alkaloids
Chemotaxonomy
Uncarine

ABSTRACT

Phytochemical investigation on the aerial parts of *Uncaria perrottetii* led to the isolation of three pentacyclic oxindole alkaloids uncarine A or isoformosanine (**1**), uncarine E or isopteropodine (**2**), and rauniticine-*allo*-oxindole A (**3**). Five oxindole alkaloids, isomitraphylline (**4**), mitraphylline (**5**), uncarine B or formosanine (**6**), uncarine F (**7**), corynoxine (**8**), and uncarine D or speciophylline (**9**), were isolated from the leaves of *Uncaria lanosa* f. *philippinensis*. Their structures were determined by spectroscopic techniques and in comparison with the literature data. These compounds proved to be important chemotaxonomic markers in the genus *Uncaria*.

1. Introduction

The genus *Uncaria* Schreb. (Rubiaceae) has been described commonly as lianas with characteristic paired fang hooks at the nodes (Ridsdale, 1978). The species are widely distributed among tropical areas such as Southeast Asia, Africa, and South America (Ridsdale, 1978). *Uncaria* species possessed interesting chemical structures including alkaloids, terpenes, quinovic acid glycosides, flavonoids and coumarins (Heitzman et al., 2005). Biological activities associated with various *Uncaria* species include cytotoxicity, anti-inflammatory, antiviral, immune-stimulant, antioxidant, CNS-related response, vascular, hypotensive, mutagenicity and antibacterial properties (Heitzman et al., 2005). A total of 34 *Uncaria* species are recorded worldwide (Ndagijimana et al., 2013). In the Philippines, the genus *Uncaria* was represented by ten species in which two, *U. nervosa* Elmer and *U. perrottetii* (A. Rich.) Merr. are endemic (Ridsdale, 1978). *U. perrottetii* is an interesting Philippine endemic as it is the only species in the genus that bears a deeply bifid laciniate stipule and a distinct violet colored shoot system.

In our continuing studies on the endemic Philippine Rubiaceae species (Tan et al., 2012; Tan et al., 2014a,b), we herein report the isolation and identification of oxindole alkaloids from the leaves of *U. perrottetii* and *U. lanosa* f. *philippinensis*. The structures were elucidated based on 1D and 2D NMR, MS and by comparison with literature data. This is the first phytochemical study on *U. perrottetii* and *U. lanosa* f. *philippinensis*. The isolated alkaloids also served as chemotaxonomic markers for the different *Uncaria* species.

2. Materials and methods

2.1. General considerations

^{1}H and ^{13}C NMR spectra were recorded on 400 MHz or 500 MHz Agilent NMR spectrometer. HRESI-MS were recorded on a Thermo Fischer Scientific Exactive spectrometer. Silica gel (Merck 7734 for gravity CC or Merck 9385 for flash CC) was used for column chromatography. Solvents for chromatography were analytical grade.

2.2. Plant material

Fresh leaves of *U. perrottetii* were collected from Lanuza, Surigao del Sur, Philippines on May 2016 while fresh leaves of *U. lanosa*

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2017.12.008
1018-3647/

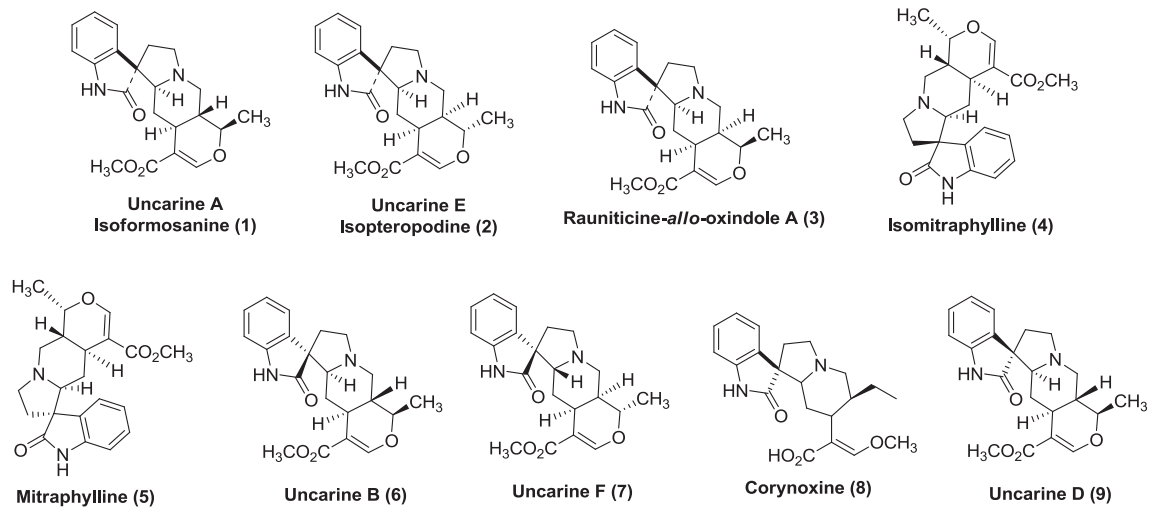

Fig. 1. Structures of isolated alkaloids.

f. *philippinensis* from Mt. Mingan, Aurora, Philippines on April 2016. Identity of the specimens was confirmed by the first author (JECO) using characters distinctive of the two species, *U. perrottetii* by having deeply bifid laciniate stipules and distinct violet colored shoot system and *U. lanosa* f. *philippinensis* by possessing deltoid calyx lobes. Voucher specimens of *U. perrottetii* (USTH 012900) and *U. lanosa* f. *philippinensis* (USTH 013915) were deposited at the UST Herbarium, Research Center for the Natural and Applied Sciences, University of Santo Tomas.

2.3. Extraction and isolation

The air-dried, ground leaves of *U. perrottetii* (1.2 kg) were percolated with distilled MeOH (14 L) for three days at room temperature and filtered. The combined filtrate was concentrated under reduced pressure to obtain the crude extract (86 g). The crude extract was dissolved in 1 M HCl and extracted thrice with EtOAc. The aqueous acid layer was basified to pH 8–9 and extracted exhaustively with $CHCl_3$. The $CHCl_3$ layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure affording the crude base. The crude base was subjected to silica gel gravity CC using a gradient of hexane-EtOAc (neat hexane, 9:1, 4:1, 7:3, 3:2, 1:1, neat ethyl acetate) resulting to five pooled fractions. Fraction 2, with distinct positive orange spots in Dragendorff, was re-purified by silica gel flash CC using isocratic hexane-EtOAc (3:2) to afford alkaloids **1** (uncarine A or isoformosanine, 3 mg) and **2** (uncarine E or isopteropodine, 4 mg). Alkaloid **3** (rauniticine-*allo*-oxindole A, 3 mg) was obtained from fraction 3 by silica gel flash CC using isocratic hexane-EtOAc (1:1).

The air-dried, ground leaves of *U. lanosa* f. *philippinensis* (1.9 kg) were percolated with distilled MeOH (16 L) for three days at room temperature and filtered. The combined filtrate was concentrated under reduced pressure obtaining the crude extract (100 g). The crude extract was dissolved in 1 M HCl and extracted thrice with EtOAc. The resulting aqueous acid layer was basified to pH 8–9 and extracted exhaustively with $CHCl_3$. The $CHCl_3$ layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain the crude base. The crude base was subjected to silica gel flash CC using gradient elution of hexane-EtOAc (neat hexane, 3:1, 1:1, 1:3, neat EtOAc) resulting to thirteen pooled fractions. Several fractions were isolated as pure compounds including fraction 1 (isomitraphylline, **4**, 45 mg), fraction 2 (mitraphylline, **5**, 38 mg), fraction 6 (formosanine, **6**, 21 mg), fraction 10 (uncarine F, **7**, 23 mg), fraction 12 (corynoxine, **8**, 4 mg), and fraction 13 (uncarine D, **9**, 7 mg).

3. Results and discussion

Chromatographic purification of the crude base of *U. perrottetii* yielded uncarine A or isoformosanine (**1**), uncarine E or isopteropodine (**2**), and rauniticine-*allo*-oxindole A (**3**) (Fig. 1). This is the first report of alkaloids **1** and **3** from *U. perrottetii* while uncarine E (**2**) was previously identified from this plant (Lleander et al., 1974). The presence of **2** was also identified from *U. bernaysii*, *U. donisii*, *U. guaianensis*, *U. homolalla*, *U. laevigata*, *U. lanosa*, *U. longiflora*, *U. orientalis*, *U. roxburghiana*, *U. scandens*, *U. sinensis*, *U. sterrophylla*, *U. tomentosa*, and *U. veluntina* (Arbain et al., 1993; Aquino et al., 1997; Kam et al., 1991; Lee et al., 1999a, 1999b; Phillipson et al., 1978; Tanahashi et al., 1997; Wagner et al., 1985). From the 34 species, alkaloid **3** was previously identified from *U. elliptica* (Phillipson and Supavita, 1983). Uncarine A (**1**) was previously identified from *U. attenuata*, *U. cordata*, *U. gambir*, *U. hirsuta*, *U. laevigata*, *U. orientalis*, and *U. sessilifructus* (Phillipson et al., 1978; Wu and Chan, 1994).

U. lanosa f *philippinensis* has elaborated isomitraphylline (**4**), mitraphylline (**5**), formosanine or uncarine B (**6**), uncarine F (**7**), corynoxine (**8**), and uncarine D or speciophylline (**9**) (Fig. 1). Isomitraphylline (**4**) was isolated from 18 species namely *U. africana*, *U. bernaysii*, *U. callophylla*, *U. elliptica*, *U. guianensis*, *U. hirsuta*, *U. homomalla*, *U. laevigata*, *U. lancifolia*, *U. lanosa*, *U. longiflora*, *U. orientalis*, *U. perrottetii*, *U. scandens*, *U. sessilifructus*, *U. sterrophylla*, *U. tomentosa*, and *U. veluntina* (Diyabalanage et al., 1997a, 1997b; Phillipson et al., 1978; Tantivatana et al., 1979; Wagner et al., 1985). The presence of mitraphylline (**5**) was perceived in *U. africana*, *U. attenuata*, *U. bernaysii*, *U. callophylla*, *U. elliptica*, *U. gambir*, *U. guianensis*, *U. hirsuta*, *U. homomalla*, *U. laevigata*, *U. lancifolia*, *U. lanosa*, *U. longiflora*, *U. orientalis*, *U. perrottetii*, *U. scandens*, *U. sessilifructus*, *U. sterrophylla*, *U. tomentosa*, and *U. veluntina* (Phillipson et al., 1978; Wagner et al., 1985; Tantivatana et al., 1979; Tantivatana et al., 1980; Herath et al., 1978). The species *U. attenuata*, *U. elliptica*, *U. gambir*, *U. hirsuta*, *U. laevigata*, *U. orientalis*, and *U. sessilifructus* were known to contain uncarine B (**6**) (Phillipson et al., 1978; Wu and Chan, 1994; Tantivatana et al., 1980; Herath et al., 1978). Uncarine F (**7**) was previously identified from *U. bernaysii*, *U. donisii*, *U. homomalla*, *U. lanosa*, *U. longiflora*, *U. orientalis*, *U. perrottetii*, *U. roxburghiana*, *U. scandens*, *U. sessilifructus*, *U. sinensis*, *U. sterrophylla*, and *U. veluntina* (Phillipson et al., 1978; Tanahashi et al., 1997). Six species contained corynoxine B (**8**) including *U. attenuata*, *U. cordata*, *U. kunstleri*, *U. macrophylla*, *U. sessilifructus*, and *U. sterrophylla* (Phillipson et al., 1978; Lee et al.,

2000). Uncarine D (**9**) was isolated from *U. attenuata*, *U. bernaysii*, *U. donisii*, *U. guianensis*, *U. homomalla*, *U. laevigata*, *U. lanosa*, *U. longiflora*, *U. orientalis*, *U. perrottetii*, *U. roxburghiana*, *U. scandens*, *U. sinensis*, *U. sterrophylla*, *U. tomentosa*, and *U. veluntina* (Arbain et al., 1993; Phillipson et al., 1978; Tanahashi et al., 1997).

Except for rauniticine-*allo*-oxindole A (**3**), all the alkaloids from this study were identified from at least six different *Uncaria* species. Thus, the isolated alkaloids may provide as chemotoxanomic markers for the genus *Uncaria*.

Löfstrand et. al. (2014), supported the monophyly (PP = 1.00; BS = 100%) of *Uncaria* indicating the distinctness of members of the genus from other members of the family Rubiaceae. It must be noted that only members of *Uncaria* exhibit the following character combinations: (1) lianescent growth habit and (2) presence of paired fang hooks at the nodes. This distinctness can be directly correlated to the presence of unique oxindole alkaloids in *Uncaria*, which are of chemotaxonomic significance. Phylogenetically, Löfstrand et al. suggested that members of the genus can be subdivided into an Asian and Afro-Neotropical clades. It is no surprise that *U. perrottetii* and *U. lanosa* f. *philippinensis* share oxindole alkaloids with other members, as they are representatives from the strictly Asian clade. These alkaloids, therefore, maybe be used to delimit members of the clade.

Acknowledgement

The Philippine Tropical Forest Conservation Foundation (PTFCF) of the Philippines is acknowledged for the funding of the field collection.

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