Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

Isolation and structural elucidation of chemical constituents of *Amanoa almerindae*

Kam Iu Leong, Paola F. Alviarez, Reinaldo S. Compagnone & and Alírica I. Suárez

To cite this article: Kam Iu Leong, Paola F. Alviarez, Reinaldo S. Compagnone & and Alírica I. Suárez (2009) Isolation and structural elucidation of chemical constituents of *Amanoa almerindae*, Pharmaceutical Biology, 47:6, 496-499, DOI: 10.1080/13880200902838717

To link to this article: https://doi.org/10.1080/13880200902838717

Published online: 01 Jun 2009.

Submit your article to this journal

Article views: 770

View related articles

Citing articles: 2 View citing articles

Full Terms & Conditions of access and use can be found at
https://informahealthcare.com/action/journalInformation?journalCode=iphb20

Pharmaceutical Biology, 2009; 47(6): 496–499

informa
healthcare

RESEARCH ARTICLE

Isolation and structural elucidation of chemical constituents of *Amanoa almerindae*

Kam Iu Leong[1], Paola F. Alviarez[2], Reinaldo S. Compagnone[2], and Alírica I. Suárez[1]

[1]*Facultad de Farmacia, Universidad Central de Venezuela, Apartado 40109 Caracas, Venezuela, and* [2]*Escuela de Química, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela*

Abstract

The present work describes the phytochemical investigation of the aerial parts of *Amanoa almerindae* Leal (Euphorbiaceae). After fractionation of the organic extracts, four pentacyclic triterpenes (friedelin, lupeol, betulin, betulinic acid), three steroids (β-sitosterol, sitostenone, daucosterol), a lignan (4′-demethyl-deoxypodophyllotoxin) and for first time in this genus the presence of the biflavones amentoflavone, sequoiaflavone, and putraflavone were identified. The structures of isolated compounds were established by spectroscopic methods and confirmed by comparison with reference samples and literature data. No previous phytochemical study of *Amanoa almerindae* has been reported, and to the best of our knowledge, this is the first report of biflavonoids in the *Amanoa* genus.

Keywords: *Euphorbiaceae;* Amanoa almerindae*; biflavones; triterpenes; lignan*

Copyright © 2009 Informa UK Ltd. Not for sale or commercial distribution. Unauthorised use prohibited. Authorised users can download, display, view and print a single copy for personal use

Introduction

The genus *Amanoa* belongs to subfamily Phyllanthoideae in the Euphorbiaceae, and comprises about 15 species, which are spread in tropical regions of America and Africa (Pittier et al., 1947). In Venezuela, six are present in the Guayana and Amazonas regions. *Amanoa almerindae* Leal, known by the vernacular name "reventillo", is a medium-sized tree that is widespread in the Venezuelan Amazonas, and in the Punos region in Perú (Steyermark et al., 1999). According to our search, this is the second report on the chemical composition of *Amanoa* species. The literature shows few studies of the genus *Amanoa*. The most known studies are from *Amanoa oblongifolia* Muell., which report the isolation of terpenes and lignans (Fang et al., 1985; Nanayakkara et al., 1986; MacRae et al., 1988) and its antiviral activity (Jassim & Naji, 2003). In the continuation of our investigation on phytochemical and pharmacological evaluation of the Euphorbiaceae of Venezuela (Suarez et al., 2003, 2004, 2005, 2006; Canelon et al. 2005) we chose the species *Amanoa almerindae,* which was collected on the borders of the Sipapo River, Amazonas state, Venezuela. The aim of the present paper is to report the first phytochemical investigation on the aerial parts of this plant, which led to the isolation and structural elucidation of four triterpenes, **1**, **2**, **3**, **7**, the steroids, **4**, **5**, **6**, the lignan **8** and, for first time in this genus, the presence of the biflavones amentoflavone (**9**), sequoiaflavone (**10**), and putraflavone (**11**), see Figure 1. The structures of the isolated compounds were established by spectroscopic analysis, mainly ^{1}H and ^{13}C using extensive 2D experiments and comparison with literature data. Pharmacological evaluation of *Amanoa almerindae,* which is underway in our lab, shows that the aqueous extract presented interesting antinociceptive activity.

Materials and methods

General

NMR spectra were recorded on a Bruker AMX-500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. ^{1}H and ^{13}C chemical shifts (δ, ppm) are relative to residual solvent signals. MS were determined

Address for Correspondence: Prof. Alirica I. Suarez, Facultad de Farmacia, Universidad Central de Venezuela, Caracas 1040, Venezuela. Tel. 58-212-605-2755; Fax 58-212-605-2707; E-mail: asuarez@ciens.ucv.ve

(Received 12 March 2008; revised 15 May 2008; accepted 16 May 2008)

ISSN 1388-0209 print/ISSN 1744-5116 online © 2009 Informa UK Ltd
DOI: 10.1080/13880200902838717

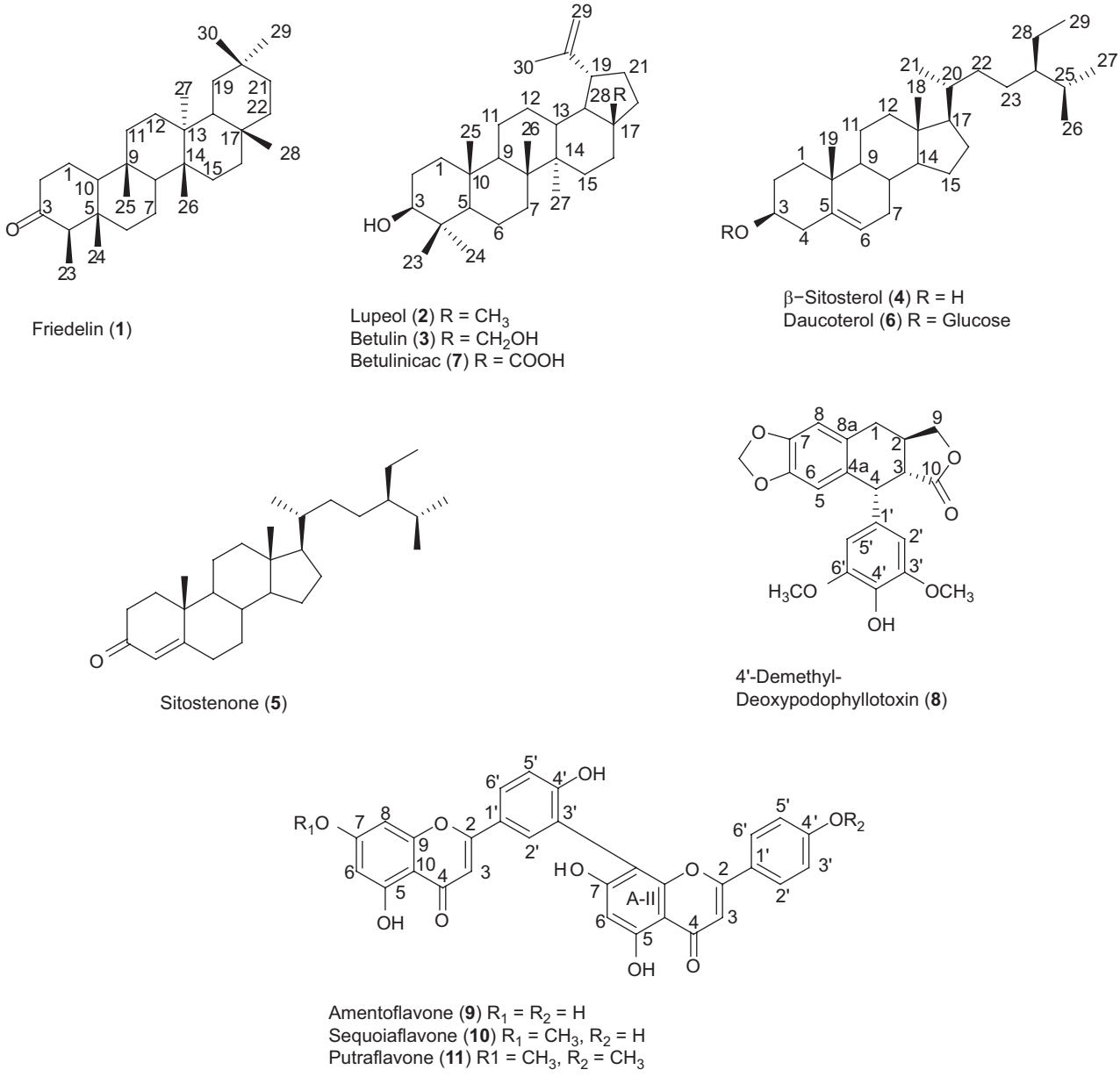

Figure 1. Structures of compounds from *Amanoa almerindae*.

on a Varian Saturn-2000 instrument. IR spectra were recorded on a Perkin Elmer 1600 Series FTIR spectrometer. UV spectra were measured on a Shimadzu UV-3101 spectrometer. Thin layer chromatograms were run on aluminum sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick) or with RP-18 F_{254} from Merck. The visualization of the TLC plates was carried out with a UV lamp (254 and 366 nm) before spraying the plates with *p*-anisaldehyde in H_2SO_4.

Plant material

The aerial parts of *Amanoa almerindae*, were collected on the borders of the Sipapo River, Amazonas state, Venezuela, in May 2000. Taxonomic identification was made by Dr. Anibal Castillo. A voucher specimen (AC 8989) has been deposited in the Herbarium Victor Manuel Ovalles (MYF) of the Faculty of Pharmacy, Universidad Central de Venezuela.

Extraction and isolation

The air-dried powdered leaves (100 g) were extracted by Soxhlet with methanol. After evaporation of the methanol (25.5 g), water was added and partitioned with hexane and with dichloromethane. Evaporation of the solvents at reduced pressure furnished 4.5 g of hexane extract, 2.4 g of dichloromethane extract and the

Table 1. ^{13}C NMR data of *Amanoa almerindae* biflavones.

Compound	Unity	C2	C3	C4	C5	C6	C7	C8	C9	C10	C1′	C2′	C3′	C4′	C5′	C6′	OCH3
9	**I**	164.4	103.5	182.3	161.9	99.3	164.2	94.6	157.9	104.2	120.6	128.3	121.9	160.3	116.8	131.9	
	II	164.6	103.1	182.6	161.5	99.4	162.7	104.6	155.0	104.1	121.4	128.7	116.3	116.3	116.3	128.7	
10	**I**	164.2	103.6	182.5	161.6	98.6	165.7	93.2	157.8	104.7	120.7	128.4	121.9	116.8	116.8	132.1	56.6
	II	164.7	103.1	182.6	161.5	99.3	162.8	105.3	155.1	104.1	121.3	128.7	116.3	116.3	116.3	128.7	
11	**I**	163.7	103.8	182.5	162.7	98.6	165.6	93.2	157.8	104.7	120.7	128.5	121.3	116.8	116.8	131.9	56.6
	II	164.6	103.7	182.7	161.5	99.3	162.9	105.2	155.0	104.2	123.5	128.5	115.0	115.0	115.0	128.5	56.0

residual methanol extract was 18.7 g. The stems (170 g) were extracted successively in a Soxhlet first with hexane, then with dichloromethane, and finally with ethyl acetate to give, respectively, 0.53, 0.58, and 0.77 g. The bulk of each extract was chromatographed separately over Si-gel using hexane and increasing amounts of dichloromethane, EtOAc and then methanol. From the hexane extract obtained from leaves, 60 fractions (ca. 25 mL each) were collected, which were combined on the basis of their profiles on TLC to give: friedelin (**1**) (27.2 mg) (Gottlieb et al., 1985), lupeol (**2**) (11.2 mg) (Schulichin et al., 1980), betulin (**3**) (12.8 mg) (Tinto et al., 1992), and β-sitosterol (**4**) (32.5 mg) (De Pascual et al., 1987; Satyanarayana et al., 1992). From the hexane extract of the stems, the compounds isolated were: **1**, **3**, and β-sitostenone (**5**) (17.8 mg) (Della-Greca et al., 1990). The dichloromethane extract obtained from the leaves provided, after column chromatography with Si-gel using dichloromethane-EtOAc and MeOH in mixtures of increasing polarity, the compounds daucosterol (**6**) (23.6 mg) (Kojima et al., 1990) and betulinic acid (**7**) (34.2 mg) (Schulichin et al., 1980). The methanol extract obtained from the leaves was subjected to column chromatography using C18 reverse-phase column chromatography, eluted with water-acetone (80:20 -70-30) to give 4′-demethyl-deoxypodophyllotoxin (**8**) (56.3 mg) (Hudson et al., 1989) and the biflavones: amentoflavone (**9**) (25.7 mg) (Chaabi et al., 2007), sequoiaflavone (**10**) (18.2 mg) (Li et al., 2003), and putraflavone (**11**) (15.9 mg) (Suàrez et al., 2003). The lignan **8** and the flavonoid **11** were also obtained from the EtOAc extract of the stems.

Results and discussion

The ^{1}H NMR and ^{13}C NMR spectra were used to assign the signals corresponding to methyl, methylene, methine, and quaternary carbons in the structural elucidation of the steroids and triterpene compounds (**1**–**7**). Comparison with authentic substances and literature data confirmed the well established structure of each of these metabolites, isolated from leaves and stems of *Amanoa almerindae*.

The ^{1}H, ^{13}C NMR, HMQC, and HMBC experiments were used to confirm the occurrence of 4′-demethyl-deoxopodophyllotoxin (**8**). All the data obtained were consistent with the NMR data reported for this lignan, which was previously isolated from *Amanoa oblongifolius* (Fang et al., 1985). The presence of this lignan among the steroids and triterpenes previously described could be considered as a valuable chemotaxonomic marker for the genus.

To identify completely each one of the biflavones, amentoflavone (**9**), sequoiaflavone (**10**), and putraflavone (**11**), a series of UV and NMR experiments provided strong supporting evidence for each of the proposed structures (Agrawal, 1989), which were corroborated with mass spectrometry and ^{13}C NMR analysis (Table 1). The first report of biflavonoids in species of *Amanoa*, presented by us in this communication, may be useful for future chemical profiles of the genus. The occurrence of the biflavonoids could be considered as valuable chemotaxonomic markers for the genus, and this whole work strongly supports the placement of *Amanoa* within the *spurge* family, as triterpenes, steroids and biflavonoids are common metabolites in many of its species.

Acknowledgement

This work was supported in part by a Grant for FONACIT (G200500389) and by CDCH of the Universidad Central de Venezuela Providence No. 06-017-2007 to A.I. Suàrez.

References

Agrawal PK (1989). *Carbon-13 NMR of Flavonoids*. Amsterdam: Ed. Elsevier, pp. 283–354.

Canelón D, Compagnone RS, Castillo A, Suárez AI (2005): Chemical constituents of *Senefelderopsis chiribiquetensis*. *Biochem Sys Eco* 33:1303–306.

Chaabi M, Antheaume C, Weniger B, Justininiano H, Lugnier C, Lobstein A (2007): Biflavones of *Decussocarpus rospigliossi* as phosphodiesterase inhibitors. *Planta Med* 73:1284–1286.

Della-Greca M, Monaco P, Previtera L (1990): Stigmasterols from *Typha latifolia*. *J Nat Prod* 5:1430–1435.

De Pascual TJ, Urones JG, Marcos IS, Basame P, Sexmero-Cuadrado MJ, Fernández-Moro R (1987): Triterpenes from *Euphorbia broteri*. *Phytochemistry* 26:1767–1776.

Fang X, Nanayakkara NPD, Phoebe CHH, Pezzuto JM, Kinghorn AD, Farnsworth NR (1985): Plant anticancer agents XXXVII. Constituents of *Amanoa oblongifolia*. *Planta Med* 51:346–347.

Gottlieb H, Ramaiah P, Lavie D (1985): 13C NMR Signal assigment of friedelin and 3α-hydroxyfriedelan-2-one. *Magn Reson Chem* 23:616–620.

Hudson JB, MacRa WD, Tower GH (1989): The antiviral action of lignans. *Planta Med* 55: 531–535.

Jassim SAA, Naji MA (2003): Novel antiviral agents: A medicinal plant perspective. *J Appl Microb* 95:412–427.

Kojima H, Sato N, Hatano A, Ogura H (1990): Sterol glucosides from *Prunella vulgaris*. *Phytochemistry* 29:2351–2355.

Li S-H, Zhang H-J, Niu X-M, Yao P, Sun H-D, Fong HHS (2003): Chemical constituents from *Amentotaxus yunnanensis* and *Torreya yunnanensis*. *J Nat Prod* 66:1002–1005.

MacRae WD, Hudson JD, Towers GH (1988): Alpha-(-)-peltatin, an antiviral constituent of *Amanoa aff. oblongifolia*. *J Ethnopharmacol* 22:223–236.

Nanayakkara DNP, Kinghorn AD, Farnsworth NR (1986): New hydroxylated eudesmane sesquiterpenoids from *Amanoa oblongifolia*. *J Chem Res* 12:454–455

Pittier H, Lasser T, Schee L, Luces Z, Badillo V (1947): Catalogo de la Flora Venezolana. Litografia y Tipografia Vargas, Caracas. Vol. II, p. 65.

Satynarayana V, Krupadanam GLD, Srimannarayana G (1992): Tetracyclic triterpenes from the latex of *Euphorbia nivula*. *Fitoterapia* 63:82–83.

Shulichin M, Yamasaki K, Kasai R, Tanaka O (1980): 13C Nuclear magnetic resonance of lupane-type triterpenes lupeol, betulin and betulinic acid. *Chem Pharm Bull* 28:1006–1008.

Steyermark, JA, Berry PE, Yatskievych K, Host BK (1999): *Flora of the Venezuelan Guayana*, Vol 5. Missouri Botanical Garden Press, St Louis, MO, USA, pp. 95–99.

Suarez AI, Diaz B, Delle Monache F, Compagnone RS (2003): Biflavonoids from *Podocalyx loranthoides*. *Fitoterapia* 74:473–475.

Suarez AI, Blanco Z, Delle Monache F, Compagnone RS (2004): Three new glutarimide alkaloids from *Croton cuneatus*. *Nat Prod Res* 18:421–426.

Suarez AI, Tapias E, Compagnone RS, Tillett S, Diaz B, Canelòn D, Blanco Z (2005): Chemical constituents from *Croton huberi*. *Rev Fac Farm* 68:14–19.

Suarez AI, Blanco Z, Compagnone RS, Salazar-Bookaman MM, Zapata V, Alvarado C (2006): Anti-inflammatory activity of *Croton cuneatus* aqueous extract. *J Ethnopharmacol* 105: 99–101.

Tinto WF, Blair LC, Alli A, Reynolds WF, McLean S (1992): Lupane triterpenoids of *Salacia cordata*. *J Nat Prod* 55:395–398.