Chemistry of Natural Compounds, Vol. 43, No. 3, 2007

PENTACYCLIC TRITERPENES FROM *Plumeria inodora*

M. Grignon-Dubois and B. Rezzonico

UDC 547.918.543.4

Plumeria, commonly known as frangipani, is a genus of shrubs and trees of the family Apocynaceae [1]. It originates from the New World Tropics, from southern Mexico to northen South America, but has been introduced into all tropical areas of the world and is now common in South-East Asia. This genus has medicinal value in folk medicine [2 and references cited]. Extracts of various *Plumeria* species have been shown to exhibit significant antibacterial, antifungal, and antiviral activity [2]. No phytochemical investigation of *Plumeria inodora* has been reported up to now, except our recent paper related to plumieride [3 and references therein]. Tetra- and pentacyclic triterpenes have been isolated previously from other species belonging to the *Plumeria* genus, in particular lupanes, ursanes, oleanes, and dammaranes [4–9]. From this point of view, *P. rubra, P. alba,* and *P. obtusa* were the most studied. In addition, antimutagenic activity has been reported for alcoholic extracts of the green leaves of *Plumeria acuminata* [10]. All these results prompted us to investigate the triterpene content of the aerial part of *Plumeria inodora*.

The crude methanolic extract of leaves (110 g) was partitioned between organic solvent of increasing polarity (i.e., pentane, dichloromethane, and ethyl acetate) and 10% aq. MeOH. From the four resulting fractions, only F2 (10 g, 9% yield) gave a positive Liebermann-Buchard test for triterpene. F2 was submitted to flash chromatography (silica gel, petroleum ether, petroleum ether–EtOAc, and EtOAc, in order of increasing polarity) to obtain 14 fractions (25 mL). Fractions 2–4 (730 mg), which eluted with petroleum ether–EtOAc (9:1), on subjecting to column chromatography (silica gel, petroleum ether, petroleum ether–dichloromethane in order of increasing polarity) afforded lupeol acetate (610 mg), lupenone (40 mg), and triglycerides (70 mg). Fractions 5–7 (840 mg), which eluted with petroleum ether–EtOAc (8:2 to 6:4), on subjecting to column chromatography (silica gel, petroleum ether–dichloromethane 1:1) afforded a further quantity of lupenone (120 mg) and lupeol (540 mg). Fractions 9–12 (3.15 g), which eluted with petroleum ether–EtOAc (4:6 to 1:9), was washed with cold dichloromethane. The resulting pale yellow solid (2.50 g) consisted almost exclusively of ursolic acid. On subjecting to flash column chromatography (silica gel, petroleum ether–dichloromethane–MeOH 45:52:3), it afforded pure ursolic acid (2.21 g). Ursolic acid has been reported to show significant cytotoxicity against some tumor cell lines [11–12]. For each compounds, MS, ^{1}H, and ^{13}C NMR spectra were recorded. Structures were assigned by comparison with authentic samples and literature data [13–15]. Although pentacyclic terpenes of the lupane and ursane- type skeleton were isolated earlier from other members of the *Plumeria* genus, this is the first report of their presence in *Plumeria inodora*.

General Experimental Procedures. TLC was carried out on precoated Si gel 60 F254 plates (Merck). Phenol-sulfuric acid and Liebermann reagents were used for visualization. Column chromatography was carried out on Si gel 60 (230–400 mesh, Merck). NMR spectra were recorded with a Bruker DPX 300 spectrometer using $CDCl_3$, CD_3OD, or pyridine-d_5 as solvent and internal standard. Mass spectra were recorded on an Autospec-Q apparatus at 70 eV and at a source temperature of 200°C. Authentic samples of lupeol, lupenone, acetyl lupeol, and ursolic acid were purchased from Extrasynthese.

Plant Material. Leaves of *Plumeria inodora* were collected on March 2001 in San Rafael de Lagunillas, Merida State, Venezuela, at an elevation of 1000 m above sea level. The plant material was identified by Dr. Gilberto Morillo, from the Faculty of Forestry, University of Los Andes, where a voucher specimen is kept [16].

Extraction and Isolation. The pulverized air-dried leave material (600 g) was extracted by maceration in methanol at room temperature. It was concentrated to dryness, yielding 110 g of a viscous material, which was suspended in 10% aq. MeOH and partitioned with petroleum ether, chloroform, and ethyl acetate to give a petroleum ether fraction (yield: 31.2%), a dichloromethane fraction (9%), an ethyl acetate fraction (2.8%), and a water-soluble fraction (57%).

Phytochimie et Valorisation de la Biomasse (PhyValBio), Universite Bordeaux I, 351, Cours de la Liberation, F 33405 Talence cedex, France, e-mail: m.grignon-dubois@phyvalbio.u-bordeaux1.fr. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 290-291, May-June, 2007. Original article submitted March 2, 2006.

0009-3130/07/4303-0351

REFERENCES

1. L. Allorge, *Succulentes (France),* **19** (2), 23 (1996).
2. M. O. Hamburger, G. A. Cordell, and N. Ruangrungsi, *J. Ethnopharmacol*., **33** (3), 289 (1991).
3. M. Grignon-Dubois, B. Rezzonico, A. Usubillaga, and L. Vojas, *Chem. Nat. Comp.*, **41** (6), 730 (2005).
4. J. Schmidt, N. T. Lien, N. H. Khoi, and G. Adam, *Phytochemistry,* **22** (4), 1032 (1983).
5. B. S. Siddiqui, F. Ilyas, M. Rasheed, and S. Begum, *Phytochemistry*, **65** (14), 2077 (2004).
6. N. Akhtar and A. Malik, *Phytochemistry*, **32** (6), 1523 (1993).
7. S. Siddiqui, B. Shaheen Siddiqui, A. Naeed, and S. Begum, *Phytochemistry*, **31** (12), 4279 (1992).
8. B. S. Siddiqui, F. Ilyas, and S. Begum, *Phytochemistry*, **52** (6), 1115 (1999).
9. S. Siddiqui, B. S. Siddiqui, S. Begum, and A. Naeed, *Phytochemistry*, **29** (11), 3615 (1990).
10. A. P. Guevara, E. Amor, and G. Russell, *Mutat. Res.*, **361**, 67 (1996).
11. K. H. Lee, Y. M. Lin, T. S. Wu, D. C. Zhang, T. Yamagishi, T. Hayashi, I. H. Hall, J. J. Chang, R. Y. Wu, and T. H. Yang, *Planta Med*., **54** 308 (1988).
12. C. N. Lin, C. M. Lu, M. K. Cheng, K. H. Gan, and S. J.Won, *J. Nat. Prod*., **53**, 513 (1990).
13. W. F. Reynolds, S. McLean, and J. Poplawski, *Tetrahedron*, **42** (13), 3419 (1986).
14. T. K. Razdan, S. Harkar, B. Qadri, M. A. Qurishi, and M. A. Khuroo, *Phytochemistry*, **27** (6), 1890 (1988).
15. W. Seebacher, N. Simic, R. Weis, R. Saf, and O. Kunert, *Magn. Reson. Chem.*, **41**, 636 (2003).
16. G. Morillo, *Analisis preliminar de la diversidad y distribución de las Asclepiadaceae de los bosques Andinos Venezolanos*. In: S. P. Churchill, H. Balslev, E. Forero, J. L. Luteyn, Biodiversity and Conservation of Neotropical Montane Forests, Proceedings of the Neotropical Montane Forest Biodiversity and Conservation Symposium, The New York Botanical Garden, New York, 433 (1995).