J. Indian Chem. Soc.,
Vol. 88, February 2011, pp. 303-305

NOTE

Lupeol, a pharmaceutically potent triterpenoid, from the ripe fruits of *Rauvolfia tetraphylla* L. (Apocynaceae)

Goutam Brahmachari*, Lalan C. Mandal, Rajiv Roy, Shyamal K. Jash, Avijit Mondal, Sasadhar Majhi and Dilip Gorai

Natural Products Laboratory, Department of Chemistry, Visva-Bharati University, Santiniketan-731 235, West Bengal, India

E-mail : brahmg2001@yahoo.co.in

Manuscript received 16 December 2009, accepted 07 July 2010

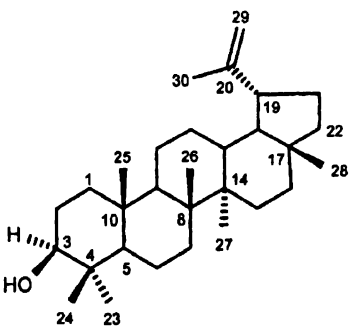
Abstract : *Rauvolfia tetraphylla* Linn. (Apocynaceae) has been established as new source of lupeol characterized on the basis of detailed spectral studies including 1D and 2D NMR; cold alcoholic extract of the ripe fruits of the plant yielded the pharmaceutically potent triterpenoid.

Keywords : *Rauvolfia tetraphylla*, Apocynaceae, triterpene, lupeol, spectral studies.

Introduction

Plant triterpenoids occupy a quite significant position among bioactive natural products as reported so far, and have contributed a lot in supplying useful and promising leads in drug discovery programmes[1]. Lupeol (**1**)[2], a natural pentacyclic triterpene, has evoked a lot of interests among the pharmacologists due to its multidirectional pharmaceutical potentials including antimicrobial[3], anti-inflammatory and anti-arthritic[4], anti-urolithiatic[5], anti-diabetic[6], anti-malarial[7], and anti-mutagenic[8] activities. The compound is also reported to possess potent enzyme-inhibitory activity against protein kinases and serine proteases as well as against DNA topoisomerase II, a target for anticancer chemotherapy[9]. Lupeol has been found to induce differentiation and to inhibit the growth of melanoma and leukemia cells[10] also. Recently, it has been demonstrated that lupeol bears significant potentiality to inhibit tumor promotion in two-stage mouse skin carcinogenesis by modulating various signaling pathways[11], and also to induce apoptotic cell death of human pancreatic adenocarcinoma cells via inhibition of *ras*-signaling pathway[12]. In continuation of our works on medicinal plants, we have found *Rauvolfia tetraphylla* Linn. (syn. *R. canescens* L.; family : Apocynaceae) as a new source of lupeol (**1**).

R. tetraphylla finds immense applications and uses in Indian traditional system of medicine[13]; roots of the plant are often used as substitute and adulterant of *R. serpentine*. Plant extract mixed with castor oil is applied to various skin ailments[13]. Occurrence of a number of alkaloids such as aricine, reserpiline, isoreserpiline, tetrahydroalstonine, ajmaline and α-yohimbine were reported earlier especially from the leaves and roots of the plant[14]. Still there is no report on the phytochemical composition of fruits of this plant, and we, herein, report the occurrence of a pentacyclic triterpenoid, lupeol (**1**), from the ripe fruits of *R. tetraphylla* for the first time.



Lupeol (**1**)

Results and discussion

Compound **1**, $C_{30}H_{50}O$ ($[M]^+$ at *m/z* 426), gave a characteristic red color with concentrated H_2SO_4 and acetic

anhydride when in $CHCl_3$ solution. It displayed infrared absorptions suggesting the presence of hydroxyl function (3325, 1032 cm^{-1}) and exomethylene (3068, 1637, 881 cm^{-1}) moiety in its molecule. The ^1H NMR and ^{13}C NMR spectral properties of the isolate clearly revealed that it is a pentacyclic triterterpene bearing lupeol-type of skeleton[15]. The ^1H NMR spectrum displayed signals for (i) six tertiary methyl groups at δ 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03 (each 3H, s), (ii) an isopropenyl moiety at δ 1.68 (3H, s), 4.57 (1H, br s), 4.68 (1H, br s), and (iii) a secondary carbinol-methine proton at δ 3.18 (1H, m); the remaining protons appeared as complex multiplets between δ 1.0 to 2.38. ^{13}C NMR spectrum of the compound showed 30 carbon signals for the triterpenoid of lupene skeleton having seven methyl, eleven methylene, six methine groups and six quaternary carbons. The complete assignments (see Experimental) were made with the help of DEPT as well as ^1H-^{13}C COSY (heteronuclear multiple-quantum coherence, HMQC) experiments. The secondary carbinol carbon (C-3) appeared at δ 79.4, while carbon atoms of the isopropenyl group displayed signals at δ 151.4 (C_{20}-alkenic), 109.7 (C_{29}-alkenic) and 19.7 (C_{30}-methyl); the HMQC spectrum of the compound also recorded the expected ^1H -^{13}C one-bond correlations for such functionalities – the secondary carbinol carbon ($\delta_{C\text{-}3}$ 79.4) correlated with the carbinol-methine proton ($\delta_{H\text{-}3}$ 3.18); the methyl carbon ($\delta_{C\text{-}30}$ 19.7) and the olefinic carbon ($\delta_{C\text{-}29}$ 109.7) of isopropenyl moiety correlated, respectively with the methyl protons at δ 1.68, and the methylene protons at δ 4.57 and 4.68. All other ^1H-^{13}C one-bond correlations as expected for compound **1** were observed in the HMQC spectrum. The afore-discussed 1D and 2D NMR spectral results are characteristics of lup-20(29)-en-3β-ol[15,16]. The structure of compound **1** was confirmed by comparison of physical (m.p., optical rotation) and spectral (IR, ^1H and ^{13}C NMR) data of our compound to those described for lupeol[17], and confirmed also by its mass (EI) spectrum, which displayed characteristic fragment ions[18] at *m/z* 426 [M^+], 411, 409, 219, 218, 207, 189, 188 and 41 (see Experimental).

Lupeol is found in various edible fruits as well as traditionally used medicinal plants[19], but its occurrence in *R. tetraphylla* has not been reported earlier. The present study establishes *R. tetraphylla* not only as a new source of such pharmaceutically potent triterpene, but also offers a basis for correlating some of the pharmacological properties of the plant to the presence of lupeol as one of its chemical constituents.

Experimental

Ripe fruits (1.5 kg) of *Rauvolfia tetraphylla* Linn. were extracted with cold ethanol; the extract was concentrated under reduced pressure. The semi-solid mass (55 g) was stirred with dil. acetic acid; allowed to settle down; filtered, and the filtrate was neutralized with ammonia solution. The precipitate obtained was then subjected to column chromatography on basic alumia (300 g); the petroleum ether (60–80 C°) : benzene (2 : 1) eluent afforded lupeol as white microcrystalline powder (yield 121 mg; 0.0081%), molecular formula $C_{30}H_{50}O$ (Calcd. C, 84.51; H, 11.74; Found C, 84.53; H, 11.75), m.p. 211 °C (uncorrected), $[\alpha]^{26}_D$ + 26.2° ($CHCl_3$, *c* 0.75); IR (KBr) ν cm^{-1} : 3325, 3068, 2941, 2869, 1637, 1458, 1377, 1032, 881; ^1H NMR ($CDCl_3$, 400 MHz; TMS) : δ 0.76, 0.79, 0.83, 0.94, 0.97, 1.03 (each 3H, s, Me-23, Me-24, Me-25, Me-26, Me-27, Me-28), 1.68 (3H, br s, Me-30), 3.18 (1H, m, H_α-3), 4.57 (1H, br s, H_b-29), 4.68 (1H, br s, H_α-29); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 39.1 (C-1), 27.9 (C-2), 79.4 (C-3), 39.3 (C-4), 55.7 (C-5), 18.7 (C-6), 34.7 (C-7), 41.2 (C-8), 50.9 (C-9), 37.6 (C-10), 21.3 (C-11), 25.6 (C-12), 38.5 (C-13), 43.2 (C-14), 27.8 (C-15), 36.0 (C-16), 43.4 (C-17), 48.4 (C-18), 48.7 (C-19), 151.4 (C-20), 30.3 (C-21), 40.4 (C-22), 28.4 (C-23), 15.8 (C-24), 16.5 (C-25), 16.4 (C-26), 15.0 (C-27), 18.4 (C-28), 109.7 (C-29), 19.7 (C-30); EIMS (70 eV) : *m/z* 426 ([M]$^+$), 425 [M-H]$^+$, 411 [M-Me]$^+$, 409 [M-OH]$^+$, 408 [M-H_2O]$^+$, 385 [M-C(Me)=CH_2]$^+$, 384, 366, 365, 219, 218, 207, 206, 189, 188, 178, 163, 135, 107, 105, 95, 79, 41.

Acknowledgement

Financial support from UGC, New Delhi [Project No. F.34-357/2008(SR) dt. 02.01.2009] is deeply acknowledged. The authors are also grateful to Bose Institute, Kolkata and RSIC, IIT, Madras for spectral measurements.

References

1. Y.-W. Chin, M. J. Balunas, H. B. Chai and A. D. Kinghorn, *The AAPS Journal*, 2006, **8**, (http://www.aapsj.org); S. M. Jachak and A. Saklani, *Curr. Sci.*, 2007, **92**, 1251; G. M.

Cragg, P. G. Grothaus and D. J. Newman, *Chem. Rev.*, 2009, **109**, 3012; G. Brahmachari (ed.), "Natural Products : Chemistry, Biochemistry and Pharmacology", Narosa Publishing House Pvt. Ltd., New Delhi, 2009; G. Brahmachari, "Handbook of Pharmaceutical Natural Products", Wiley-VCH, Germany, 2010.

2. L. Ruzicka and M. Brenner, *Helv. Chim. Acta*, 1939, **22**, 1523; 1940, **23**, 1325; L. J. Swift and E. D. Walter, *J. Am. Chem. Soc.*, 1942, **64**, 2539.
3. B. Mohamed Khadeer Ahamed, V. Krishna, H. B. Gowdru, H. Rajanaika, H. M. Kumaraswamy, S. Rajshekarappa, C. J. Dandin and K. M. Mahadevan, *Res. J. Med. Plant*, 2007, **1**, 72; V. Kuete, J. D. Wansi, A. T. Mbaveng, M. M. K. Sop, A. T. Tadjong, V. P. Beng, F.-X. Etoa, J. Wandji, J. J. M. Meyer and N. Lall, *South African J. Bot.*, 2008, **74**, 572.
4. R. B. Agarwal and V. D. Rangari, *Ind. J. Pharm.*, 2003, **35**, 384.
5. R. Anand, G. K. Patnaik D. K. Kulshreshtha and B. N. Dhawan, *Phytother. Res.*, 2006, **7**, 417.
6. M. Na, B. Y. Kim, H. Osada and J. S. Ahn, *J. Enzyme Inhibit. Med. Chem.*, 2009, **24**, 1056.
7. J. Fotie, D. S. Bohle, M. L. Leimanis, E. Georges, G. Rukunja and A. E. Nkengfack, *J. Nat. Prod.*, 2006, **69**, 62.
8. M. Saleem, A. Alam, S. Arifin, M. S. Shah, B. Ahmed and S. Sultana, *Pharmacal. Res.*, 2001, **43**, 127.
9. L. D. Hodges, G. Kweifio-Okai and T. A. Macrides, *Mol. Cell. Biochem.*, 2003, **252**, 97; S. Wada, A. Iida and R. Tanaka, *J. Nat. Prod.*, 2001, **64**, 1545.
10. Y. Aratanechemuge, H. Hibasami, K. Sanpin, H. Katsuzaki, K. Imai and T. Komiya, *Oncol. Rep.*, 2004, **11**, 289; K. Hata, K. Hori and S. Takahashi, *J. Biochem.*, 2003, **134**, 441; K. Hata, K. Hori, H. Ogasawara and S. Takahashi, *Toxicol. Lett.*, 2003, **143**, 1.
11. M. Saleem, F. Afaq, V. M. Adhami and H. Mukhtar, *Oncogene*, 2004, **23**, 5203.
12. M. Saleem, S. Kaury, M.-H. Kweony, V. M. Adhami, F. Afaq and H. Mukhtar, *Carcinogenesis*, 2005, **26**, 1956.
13. R. N. Chopra, S. L. Nayar and I. C. Chopra, "Glossary of Indian Medicinal Plants", PID, CSIR, New Delhi, 1956, p. 210; S. P. Ambasta (Editor-in-Chief), "The Useful Plants of India", PID, CSIR, New Delhi, 1986, p. 517.
14. J. A. M. Peraz, J. M. Tonamou and Z. A Campa, *Rev. Cubana Farm*, 1982, **16**, 28; J. A. M. Peraz, Z. A. Campa and J. M. Tonamou, *Rev. Cubana Farm*, 1982, **16**, 251; H. A. Lastra, M. Palacios, R. Menendez, M. Larionova, F. Rodriguez and V. Fuste, *Rev. Cubana Farm*, 1982, **16**, 316.
15. J. D. Connolly and R. A. Hill, *Nat. Prod. Rep.*, 1999, **16**, 221; M. Sholichin, K. Yamasaki, R. Kasai and O. Tanaka, *Chem. Pharm. Bull.*, 1980, **28**, 1006; W. F. Reynolds, S. Mclean, J. Poplawski, R. G. Enriquez, L. I. Escobar and I. Leon, *Tetrahedron*, 1986, **42**, 3419.
16. H. Tomosaka, H. Koshino, T. Tajika and S. Omata, *Biosci. Biotechnol. Biochem.*, 2001, **65**, 1198.
17. S. Imam, I. Azhar, M. M. Hasan, M. S. Ali and S. W. Ahmed, *Pak. J. Pharm. Sci.*, 2007, **20**, 125; R. D. Rofi and A. B. Pomilio, *J. Nat. Prod.*, 1987, **50**, 767; D. Shang-Jiang, P. Gariboldi and G. Jomni, *Planta Med.*, 1986, 317; S. C. Pakrashi, J. Bhattacharyya, S. Mookerjee, T. B. Samatan and H. Vorbruggen, *Phytochemistry*, 1968, **7**, 461.
18. G. H. Aynilian, N. R. Farnsworth and G. J. Persinos, *Phytochemistry*, 1972, **11**, 2885; P. Monaco and L. Privitera, *J. Nat. Prod.*, 1984, **47**, 673.
19. A. K. Jamal, W. A. Yaacob and L. B. Din, *J. Phys. Sci.*, 2008, **19**, 45; T. H. Beveridge, T. S. Li and J. C. Drover, *J. Agric. Food Chem.*, 2002, **50**, 744; R. Kakuda, T. Iijima, Y. Yaoita, K. Machida and M. Kikuchi, *Phytochemistry*, 2002, **59**, 791; M. Saleem, A. Alam, S. Arifin, M. S. Shah, B. Ahmed and S. Sultana, *Pharmacol. Res.*, 2001, **43**, 127; J. A. Blair, P. A. Ongley, J. Chiswell and M. H. G. Griffiths, *Phytochemistry*, 1970, **9**, 671.