*Short Communication*

Pak. j. sci. ind. res., vol. 35, no. 9, September 1992

# Triterpene Constituents from the Flowers of *Inula grantioides* Boiss.

D.K. Burdi, Mashooda Hasan, Viqar Uddin Ahmad*, M.A. Qureshi** and M.A. Mahesar**

*Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan*

(Received May 26, 1991; revised September 3, 1992)

*Inula* is one of the genera belonging to the cosmopolitan tribe inuleae of the Compositae. This tribe is well represented in the European flora (27 genera, 116 spp.) and is especially abundant in South Africa and Australia. There are some 200 genera and 2000 species in this tribe. The inuleae includes a selection of plants which have been prized by man for their useful properties [1].

Various plants belonging to the genus *Inula* are reported to possess bactericidal [2], toxic [3] and physiological properties [4,5]. It is reported that the oil obtained from *Inula grantioides* has antibiotic properties [6]. The plant has been used by local people in Lasbela for the patients suffering from asthma [7]. Lupeol palmitate and β-amyrin palmitate isolated from the plant are reported [8] to possess a protective effect against the $CCl_4$ induced hepatic damage. This supports the possibility of this plant for an antihepatotoxic action.

Though the presence of terpenoids sterols, flavonoids, alkaloids, lipids, polyacetylenes etc. have been reported in the literature [9,10] from various other species, no systematic work on chemical constituents of *Inula grantioides* has been done so far. Therefore, an attempt was made to isolate and identify the terpenoids present in the flowers of this species. Five triterpenes, lupeol palmitate [1], β-amyrin palmitate [2], lupeol acetate [3], taraxasterol acetate [4] and taraxasterol [5] were isolated from the flowers of *Inula grantioides* and identification was carried out mostly by spectroscopic methods.

The lupeol acetate is found [11] to possess bactericidal and fungicidal activities. It is effective against *Staphylococcus aureus* and *Candida albicans*. Taraxasterol acetate demonstrated [12] anti-inflammatory activity in albino rats against carrageenan, formaldehyde and adjuvant induced inflammations.

*Extraction.* The dried and coarsely milled flowers of *Inula grantioides* were extracted separately in two parts. One part (2.5 kg) was extracted by Soxhlet extraction with petroleum ether (40-60°). Evaporation of solvent from this extract yielded a thick syrupy brownish material (35 g). Another part (1.5 kg) was extracted three times with acetone at room temperature. Evaporation of solvent from the collective acetone extract under reduced pressure yielded 67 g of thick syrupy brownish material.

*Chromatographic separation.* The petroleum ether extract (35 g) was chromatographed on a column of silica gel. The elution was carried out with petroleum ether (40-60°) followed by a petroleum ether- benzene (1:1) mixture to obtain fraction A. Fraction A was evaporated (5 g) and taken in acetone. The acetone-soluble portion (4 g) was eluted on a column of silica gel with petroleum ether followed by petroleum ether-ethyl acetate (1:1) and then pure ethyl acetate. The petroleum ether fraction was concentrated and separated by preparative thin layer chromatography, using the mixture of petroleum ether-chloroform (1:3) as an eluent system to obtain substance VII as a viscous mass (0.29 g), this was rechromatographed by flash column chromato-graphy. The column was eluted with hexane and with a mixture of hexane-chloroform in various ratios. Lupeol palmitate (1) was obtained as a waxy mass (0.006 g) by eluting the column with hexane-chloro form (9:1).

β-Amyrin palmitate [2] was obtained from the acetone extract (67 g) which was chromatographed on a column of silica gel and eluted with hexane followed by hexane-ethyl acetate (various ratios). Fraction B (5.82 g) obtained by eluting the column with hexane-ethyl acetate (4:1) was rechromatographed on a small column. 2 alongwith some amount of 1 was separated as a semisolid mass by eluting the column with hexane. It was further purified (0.33 g) by preparative thin layer chromatography using hexane- ethyl acetate (9:1) as eluents.

The subsequent elution of fraction B with more hexane gave lupeol acetate [3] and taraxasterol acetate [4] as a white crystalline homogeneous mass (0.157 g). It was recrystallized from ether-methanol.

On further elution of acetone extract (see β-amyrin palmitate) on column with hexane-ethyl acetate (4:1) resulted in fraction C (3.38 g). This was rechromatographed on small scale column of silica, and eluted with hexane and mixtures of various ratios of hexane-ethyl acetate. Taraxasterol [5] was separated out as a crystalline material (0.01 g) with the hexane-ethyl acetate (9:1) as eluent system. It was recrystallized from ether- methanol.

*Identification.* The triterpenoids were identified mostly

* H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan.
**Institute of Chemistry, University of Sindh, Jamshoro, Pakistan.

on the basis of their spectral characteristics (IR, MS, $^1$H-NMR, $^{13}$C- NMR).

*Spectral data*. Lupeol palmitate (1) [8]. $\upsilon_{max}$CHCl$_3$ cm$^{-1}$: 2890, 1715, 1370, 1165, 882; MS: *m/z* 664 (M$^+$, $C_{46}H_{80}O_2$), 649, 445, 409, 408, 239, 218, 189, 175, 121; $^1$H-NMR ($CDCl_3$, 300 MHz): δ0.78 (3H, s, H-24), 0.83 (3H, s, H-28), 0.85 (3H, s, H-25), 0.87 (3H, s, H-23), 0.94 (3H, s, H- 26), 1.02 (3H, s, H-27), 1.68 (3H, s, $CH_2$=C-$CH_3$), 2.26 (2H, t, J=7.0 Hz, -$CH_2$-COO-), 4.46 (1H, m, H-3), 4.56 (1H, br.s, H-29B), 4.68 (1H, br.s, H-29A).

β-Amyrin palmitate (2) [13-15]. $\upsilon_{max}$CHCl$_3$ cm$^{-1}$: 2920, 1720, 1380, 1175, 990; MS: *m/z* 664 (M$^+$, $C_{46}H_{80}O_2$), 649, 445, 409, 408, 239, 218, 203, 189, 175, 121; $^1$H-NMR ($CDCl_3$, 400 MHz): δ0.78 (3H, s, H- 24), 0.82 (3H, s, H-28), 0.85 (3H, s, H-29), 0.86 (3H, s, H-30), 0.87 (3H, s, H-25), 0.96 (3H, s, H-23), 1.02 (3H, s, H-26), 1.13 (3H, s, H-27), 2.27 (2H, t, J=7.0 Hz, -$CH_2$-COO-), 4.50 (1H, m, H- 3), 5.17 (1H, t, J=3.6 Hz, H-12). $^{13}$C-NMR ($CDCl_3$, 100 MHz): δ38.25 (C-1), 26.92 (C-2), 80.58 (C-3), 37.47 (C-4), 55.26 (C-5), 18.24 (C-6), 32.58 (C-7), 39.81 (C-8), 47.55 (C-9), 36.84 (C-10), 23.52 (C-11), 121.68 (C-12), 145.24 (C-13), 41.71 (C-14), 29.15 (C-15), 26.12 (C-16), 32.47 (C-17), 47.24 (C-18), 46.79 (C-19), 31.04 (C- 20), 34.84 (C-21), 37.13 (C-22), 28.03 (C-23), 15.51 (C-24), 16.47 (C-25), 16.79 (C-26), 25.93 (C-27), 28.37 (C-28), 33.30 (C- 29), 23.66 (C-30), 173.72 (C-1'), 34.72 (C-2'), 25.15 (C-3'), 29.33, 29.44, 29.56, 29.67 (C-4'-12'), 29.23 (C-13'), 31.90 (C- 14'), 22.65 (C-15'), 14.07 (C-16').

Lupeol acetate (3) [14,16,17,18]. $\upsilon_{max}$KBr cm$^{-1}$: 2950, 1722, 1450, 1360, 1242, 880; MS:*m/z* 468 (M$^+$, $C_{32}H_{52}O_2$), 453, 408, 218, 189, 175, 121; $^1$H-NMR ($CDCl_3$, 400 MHz): δ0.78 (3H, s, H-24), 0.84 (6H, s, H-25, H-28) 0.87 (3H, s, H-26), 0.92 (3H, s, H-23), 1.00 (3H, s, H-27), 1.68 (3H, s, H-30), 2.03 (3H, s, $CH_3$-CO-), 4.47 (1H, m, H-3), 4.56 (1H, br. s, H-29B), 4.68 (1H, br. s, H-29A), $^{13}$C-NMR ($CDCl_3$, 100 MHz): δ38.91 (C-1), 26.68 (C-2), 81.03 (C-3), 37.10 (C-4), 55.44 (C-5), 18.23 (C-6), 34.26 (C-7), 40.90 (C-8), 50.40 (C-9), 37.83 (C-10), 20.98 (C-11), 25.15 (C-12), 38.09 (C-13), 43.03 (C-14), 27.47 (C-15), 35.61 (C-16), 34.56 (C-17), 48.35 (C- 18), 48.04 (C-19), 151.04 (C-20), 29.88 (C-21), 40.03 (C-22), 27.97 (C-23), 14.47 (C-24), 16.19 (C-25), 15.91 (C-26), 14.53 (C- 27), 18.02 (C-28), 109.40 (C-29), 19.31 (C-30), 171.07 (*CO*-$CH_3$), 21.32 (CO-*CH*$_3$).

Taraxasterol acetate (4) [14,16,17,18]. $\upsilon_{max}$KBr cm$^{-1}$: 2950, 1722, 1450, 1360, 1242, 880; MS:*m/z* 468 (M$^+$, $C_{32}H_{52}O_2$), 453, 408, 357, 218, 189, 135, 109, 95, 69; $^1$H-NMR ($CDCl_3$, 400 MHz): δ0.78 (3H, s, H-24), 0.84 (6H, s, H-25, H-28), 0.87 (3H, s, H-23), 0.92 (3H, s, H-26), 1.00 (3H, s, H-27), 1.02 (3H, d, J=6.5 Hz, H-29), 2.03 (3H, s, $CH_3$-CO), 4.47 (1H, m, H-3), 4.60 (2H, m, H-30). $^{13}$C-NMR ($CDCl_3$, 100 MHz): δ38.49 (C-1), 26.18 (C-2), 81.03 (C-3), 37.10 (C-4), 55.51 (C-5), 18.23 (C-6), 34.26 (C-7), 40.96 (C-8), 50.46 (C-9), 37.83 (C-10), 21.50 (C-11), 25.51 (C-12), 39.42 (C-13), 42.08 (C-14), 25.65 (C-15), 39.21 (C-16), 34.56 (C-17), 48.72 (C- 18), 38.33 (C-19), 154.74 (C-20), 23.74 (C-21), 38.91 (C-22), 27.97 (C-23), 14.74 (C-24), 16.51 (C-25), 16.0 (C-26), 14.53 (C- 27), 26.18 (C-28), 19.49 (C-29), 107.18 (C-30), 171.07 (CO-$CH_3$), 21.32 (CO*CH*$_3$).

Taraxasterol (5) [16,17]. $\upsilon_{max}$KBr cm$^{-1}$: 3425, 2925, 1450, 1380, 1040, 878; MS: *m/z* 426 (M$^+$, $C_{30}H_{50}O$), 411, 408, 357, 344, 315, 218, 189, 136, 109, 107, 95, 81, 69; $^1$H-NMR ($CDCl_3$, 400 MHz): δ0.76 (3H, s, H-24), 0.85 (6H, s, H-25, H-28), 0.92 (3H, s, H-27, 0.97 (3H, s, H-23), 1.00 (3H, s, H-26), 1.02 (3H, d, J=6.5 Hz, H- 29), 3.20 (1H, dd, J=10.5, 6.5 Hz, H-3α), 4.60 (2H, m, H-30); $^{13}$C-NMR ($CDCl_3$, 100 MHz): δ 38.82 (C-1), 27.46 (C-2), 79.06 (C-3), 38.90 (C-4), 55.42 (C-5), 18.36 (C-6), 34.13 (C-7), 40.96 (C-8), 50.55 (C-9), 37.19 (C-10), 21.51 (C-11), 25.67 (C-12), 39.24 (C- 13), 42.09 (C-14), 26.72 (C-15), 38.94 (C-16), 34.58 (C-17), 48.76 (C-18), 39.42 (C-19), 154.63 (C-20), 26.24 (C-21), 38.36 (C-22), 28.06 (C-23), 15.41 (C-24), 15.95 (C-25), 16.31 (C-26), 14.80 (C-27), 25.53 (C-28), 19.51 (C-29), 107.14 (C-30).

***Key words***: *Inula grantioides*, Compositeae, Lupeol palnitate, β-Amyrin palmitate, Lupeol acetate, Taraxasterol acetate.

## References

1. J.B. Harborne, *Inuleae - Chemical Review* (Academic Press, London, 1977), Vol. I, pp. 604.
2. O. Ya Rashba, S.I. Zelepukha and M.B. Kaghani Ka: Microb. Zhur., Akad. Nauk. Ukr. R.S.R. No. 2, 16, 67 (1954).
3. T. Go, Jap. J. Med. Sci., IV, Pharmacol II, No. 2/3, Proc. Japan Pharm. Soc., **12**, 123 (1938).
4. I.C. Chopra, J.D. Kohli and K.L. Handa, Indian J. Med. Res. **33**, 139 (1945).
5. I.M. Eadie, J. Aust. Inst. Agr. Sci., **19**, 45 (1953).
6. M. Ahmed and M.I.D. Chughtai, Pak. j. sci. ind. res., **4**, 273 (1961).
7. K.R. Kirtikar and B.D. Basu, *Indian Medicinal Plants* (1933), Vol. II, 2nd ed., pp. 1353.
8. C.N. Lin and W.P. Tome, Planta Med., **54**, 223 (1988).
9. J.B. Harborne, *Inuleae - Chemical Review* (1977), Vol. I, pp. 603-19.
10. (a). V.K. Sethi, R.K. Gupta and M.P. Jain, Indian J. Pharm. Sci., **41**(5), 208 (1979).

    (b) B. Kaur and P.S. Kalsi, Phytochemistry, **24** (9), 2007 (1985).

    (c) A. Ulubelen, S. Oksuz and N. Goren, Phytochemistry, **26** (4), 1223 (1987).

11. S.B. Mathur, T.P. Garcia, F.C. Marcano and V. Mora-Arellano, Rev. Latinoam. Quim., **6** (4), 201 (1975).
12. Bupinder Singh, S.N. Ram, V.B. Pandey, V.K. Joshi and S.S. Gambhir, Phytother. Res., **5** (3), 103 (1991).
13. S. Seo, Y. Tomita and K. Tori, Tetrahedron Lett. **1**, 7 (1975).
14. J. Bhattacharyya and C.B. Barros, Phytochemistry, **25** (1), 274 (1986).
15. S. Oksuz and G. Topcu, Phytochemistry, **26** (11), 3084 (1987).
16. V. Anjaneyulu, K. Ravi, K.H. Prasad and J.D. Connolly, Phytochemistry, **28**, 1471 (1989).
17. R. Tanaka and S. Matsunaga, Phytochemistry, **27**, 3579 (1988).
18. H.W. Kircher, Phytochemistry, **19**, 2708 (1980).