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A Pentacyclic Triterpenoid Possessing Analgesic Activity from the Fruits of *Dregea volubilis*

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ABSTRACT

Fruits of *Dregea volubilis* were extracted by petroleum ether and subjected to column chromatography for obtaining the pure compound. The structure was determined on the basis of IR, MASS, NMR (PMR, CMR and DEPT) spectroscopic analysis. The compound was screened for analgesic activity in swiss albino mice by inducing writhing reflex with acetic acid. The petroleum ether extract of the fruits of *Dregea volubilis* Benth led to isolation of a pentacyclic triterpenoid designated as taraxerol and characterized as D- friedoolean- 14- en, 3 ol (Fig.1). In vitro analgesic activity has been shown by the isolated taraxerol. The compound taraxerol obtained from the petroleum ether extract of the fruits of *Dregea volubilis* showed analgesic activity in swiss albino mice.

KEYWORDS: *Dregea volubilis* Benth, pentacyclic triterpenoid, taraxerol, analgesic activity.

INTRODUCTION

Dregea volubilis (Linn. f.) Benth ex. Hook f. Syn: *Wattakaka volubilis* (Linn. f.) Stapf; *Marsedenia volubilis* (Cooke) belongs to the family Asclepiadaceae and is commonly known as "Jukti" in Bengal. It is a tall woody climber of 11 m. of height and 95 cm. in girth with densely lenticulate branches, occurring throughout the hotter parts of India and Car Nicober Islands ascending to an altitude of 1500m. The parts of the plant are used traditionally as medicines. The juice of the plant is used as a sternutatory and leaves are employed in application for boils and abscesses. The roots and tender stalks are used as emetic and expectorant (1). It is reported that an alcohol (50%) extract of the plant showed activity on the central nervous system as well as anticancer activity against Sarcoma 180 in mice. The maximum tolerated dose was found to be 500 mg/kg body weights of albino mice (2). Reichstein and co- workers studied the components of the seeds of the plant and deduced the structure of drevogenins A, B, D and P. Previous investigation, reported the isolation and characterization of twelve polyhydroxy C/D cis-pregnane glycosides from the same plant collected from Thailand (3–4). Isolation of β- sitosterol, kaempherol-3-galactoside, a 2- deoxy sugar, drevogenin A, drevogenin P, D–cymarose and L- olendrose from the plant has also been reported (5) from the same plant collected from Shibpur, Howrah, West Bengal.

Present work is based on the chemical studies on naturally occurring bioactive triterpene. It is reported herein the isolation and characterization of a pentacyclic triterpenoid designated as taraxerol having analgesic

activity, from the petroleum ether extract of the fruits of this medicinal plant. Previously taraxerol (6) was isolated from the plant *Myrica Rubra* and had shown its inhibitory activity on reverse transcriptase on human immunodeficiency virus and of kinesin motor proteins (7).

MATERIALS AND METHODS

General procedure

All melting points were measured on Yanagimoto micromelting apparatus and are uncorrected. IR spectra were determined using JASCO 7300FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively using a Jeol ECP-500 spectrometer in C_5D_5N with TMS as internal standard. HRFABMS was performed on a JEOL MS-700 mass spectrometer. TLC was carried out on silica gel $60F_{254}$ and spots were visualized by spraying with Libermann- Buchard's reagent followed by heating. Silica gel (silica gel 60, Merck) was used for column chromatography.

Plant material

The plant material was collected from Indian Botanic Garden, Howrah, West Bengal, India. A voucher specimen has been preserved for any future reference.

Extraction and Isolation

The shade dried powdered fruits of *Dregea volubils* (2.4kgs) were extracted successively with Petroleum ether (3x8lt) at 40–45°C temperature. The combined Petroleum ether extract was concentrated and 18gms of extract was applied to a column of silica gel 60 (400gm) and washed with 100% Petroleum ether. Gradient elution was carried out with mixture of petroleum ether- chloroform (1:9, 1:4, 3:7, 2:3 and 1:1). A total of 72 fractions (50ml) were collected and 50 fractions giving similar spots on TLC were combined. Fractions eluted with chloroform-petroleum ether (1:4) were combined and subjected to re-chromatography over silica gel (20g), the fraction contained taraxerol along with β-sito sterol and a long chain lipid fraction. Fractions (collected 15ml lots) eluted with chloroform-petroleum ether mixture (1:1) furnished Taraxerol (1.5g).

Analgesic activity

The pharmacological screening the compound obtained from the petroleum ether extract of the fruits of *Dregea volubilis* was carried out using standard protocols (8–9). The compound was suspended in 1% DMSO for administration to swiss albino mice.

Acetic acid induced writhing reflex (10–11). Eighteen mice were divided into three groups of 6 mice each for various treatments as shown in Table 1. The dose of 10ml/kg was injected intraperitonially to induce the writhing (12–13).

RESULTS

The petroleum ether extract of Dregea volubilis fruits showed moderate in vitro analgesic activity. The active fraction eluted with petroleum ether-chloroform mixture from the silica gel chromatography led to isolation of the pentacyclic triterpenoid.

Taraxerol(1) was crystallized from methanol as colorless shiny needles mp.276–278°C, $[\alpha]_D$ +0.55°. The compound gave positive Libermann-Burchard test indicating the triterpene nature of the compound. The IR spectrum exhibited an absorption band at 3482 cm^{-1} attributable to hydroxyl group and an olefinic double bond at 1638 cm^{-1}. It displayed a quasi-molecular ion peak at m/z 449[M+Na]+ in the MALDI-TOF mass spectrum indicating the molecular weight to be 426 gm. This information coupled with the ^{13}C NMR and DEPT spectral analysis suggested the molecular formula $C_{30}H_{50}O$.

The 1H NMR spectrum of the compound displayed eight methyl signals resonated at δ_C 0.80, 0.82, 0.90(2xCH_3), 0.92, 0.95, 0.97, and 1.09.Additional signals observed include those described to an olefinic proton at δ 5.25 and one oxymethine proton at δ 3.2.

The compound displayed 30 signals in its 13C NMR spectrum accounted for seven singlets, five doublets, ten triplets and eight quartrates. The signal appeared at δ 79.5(doublet) indicated the presence of a hydroxy group at C-3 of the triterpene skeleton and is β- oriented (equatorial). While the olefinic carbons resonated at δ 158.5(singlet) and 117.0(doublet) indicated the position of the double bond between C-14 and C-15 in ring D. Finally from the fore going evidences it was concluded that the triterpene core of compound was D-friedoolean-14- en- 3 ol or Taraxerol.

Analgesic Activity

To determine the analgesic activity of the compound, acetic acid solution was injected subcutaneously (0.5mg/ml) and writhing was produced due to algesia. The compound had shown the activity in the following manner as described in Table 1.

Table 1: Analgesic effect of test compounds on acetic-acid induced writhing in mice

Treatment	Dose	Mean no of writhing ±SEM	% Inhibition
Control	10ml/kg	52.83 ±1.400	——
Standard (Aspirin)	300mg/kg	17.66 ±1.606***	66.57
Taraxerol	5mg/kg	28.83 ±1.195***	45.42

n : six animals in each group; Values are mean ± SEM. One way ANOVA with Tukey-Kramer multiple comparison post test.
****P<0.001 when compared to control*

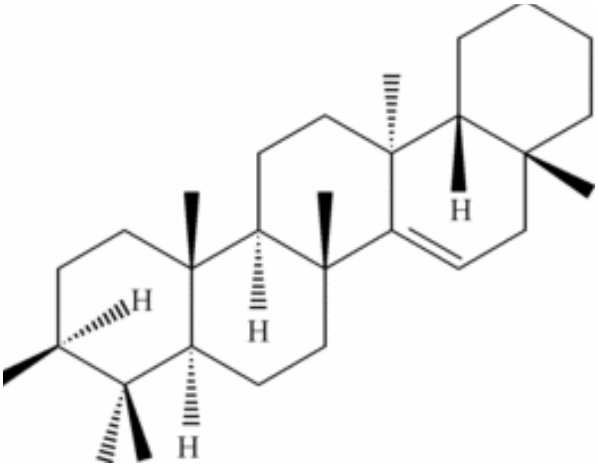

Figure 1. Taraxerol

DISCUSSION

A pentacyclic triterpene compound identified as Taraxerol obtained from the petroleum ether extract of the fruits of *Dregea volubilis*. The compound had showed analgesic activity, which was found to be statistically significant at higher concentration in acute acetic acid induced writhing. However, this activity was less potent as compared to aspirin.

CONCLUSION

The compound taraxerol obtained from the petroleum ether (40°–60°C) extract of the fruits of *Dregea volubilis* has shown analgesic activity in swiss albino mice.

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REFERENCES

1. Chopra R. N., Nayar S. L., Chopra I. C. Glossary of Indian Medicinal Plants. *Council of Indian and Scientific Research, New Delhi*. **3**: 393–396 (1956).
2. Panda N., Banerjee S., Mandal N. B. and Sahu P. Pregnan Glycosides. *Natural Product Communications*, **3**: 665–669 (2006).
3. Sahu N.P., Panda N., Mandal N.B., Banejee S, Koike K. and Nikadio T. Polyoxy Pregnane Glycosides from the Flowers of Dregea Volubilis. *Phytochemistry*, **61**: 383–388 (2002).
4. Panda N., Mandal N.B., Banerjee S., Sahu N. P., Koike K., Nikaidio T., Weber M. and Luger P. Polyhydroxy Pregnanes from Dregea volubilis. *Phytochemistry*, **61**: 8400–8403 (2003).
5. Mahato S. B., Nandy A. K. and Roy G. Triterpenoids,. *Phytochemistry*, **31**: 2199–2205 (1992).
6. Sakural N., katsu Y. and Inoue T., Triterpenoids from Myrica Rubra. *Phytochemistry*, **26**: 217–219 (1987).
7. Morais G., Watanabe M., Mataka S., Ideta K. and Thiemann T. Areno annelated Estranes by Intermolecular Cycloaddition. *9th International Conference on Synthetic Organic Chemistry*, ECSOC-9: (2005).
8. Mukherjee P. K. *Quality Control of Herbal Drugs*. 2ND Edition: 554–555 (2007).
9. Parkes M.W. and Pickens J.T. Conditions Influencing the Inhibition of Analgesic Drugs of the Response to Intraperitoneal Injections of Phenylbenzoquine in Mice. *British Journal of Pharmacology*, 25: 81–87 (1957).
10. Gupta M., Mazumder U. K., Kumar R. S. and Kumar T. S. Studies on Anti-inflammatory, Analgesic & Anti-pyretic Properties of Methanol Extract of Caesalpinia bonducella Leaves in Experimental Animal Models. *Indian Journal of Pharmacology & Therapeutics*. **2**: 30–34 (2003).
11. Sutradhar R. K., Rahman M., Ahmed M., Bachhar S. C., Saha A., and Guha S. K. Bioactive Alkaloid from Sida cordifolia Linn. With Analgesic and Anti-inflammatory Activity. *Indian Journal of Pharmacology and Therapeutics*, **5**: 175–178 (2006).
12. Krishnaveni M., Suja V., Vasanth S. and Shyamaladevi C. S. Anti-inflammatory and Analgesic Actions of 4',5,6- Trihydroxy-3',7-Dimethoxy Flavone from Vicoa indica DC. *Indian Journal of Pharmacology*, **29**: 178–181 (1997).
13. Khanna A. T., Sivaraman R. and Vohora S.B. Analgesic Activity of Silver Preparations Used in Indian Systems of Medicine. *Indian Journal of Pharmacology*, **29**: 393–398 (1997).