Pakistan Journal of Pharmaceutical Sciences
Vol. 8(1), January 1995, pp.69-71

SHORT COMMUNICATION

STUDIES ON PUNICA GRANATUM-I ISOLATION AND IDENTIFICATION OF SOME CONSTITUENTS FROM THE SEEDS OF PUNICA GRANATUM

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ABSTRACT

Initial phytochemical screening of the seeds of *Punica granatum* has revealed the presence of Ursolic acid and fl-Sitosterol alongwith a long straightchain hydrocarbon - nonacosene. Presence of estrogens and glycosides have also been detected.

Introduction

Pomegranate or *Punica granawn* is a small tree or shrub, belonging to Punicaceae family. The tree is found to grow wild in Persia, Arabia, Afghanistan and parts of western Pakistan. The tree is much valued for its fruit and its various parts are reputed for their medicinal properties. Leaf juice is considered to be astringent, while bark and rind of the fruits are valuable in chronic diarrhea and early stages of dysentery (Nadkarani, 1976). From the literature *survey* it has been observed that most of the work has been carried out on the constituents of leaves, bark, stem and roots. Active constituents isolated from the plant are the alkaloids pellentrine, isopellentrine, pseudopcllinterine and methyl isopellentrine. These compounds are known to possess anthelmintic properties (Wibaut 1957). β-Sitoslerol, friedelin, estrogens and D-mannitol have also been reported to be present in stem, roots and leaves (Fayez, Negam and Sharaf, 1963). In view of its reputed healing properties and reported isolation of estrogens we have also initiated a study of the isolation of bioactive constituents from the seeds of *P. granatum.* In our present communication we wish to report the presence of some sterols and other constituents from Pomegranate seeds.

Results and Discussion

Pomegranate seeds (2Kg) were extracted with methanol using soxhlet apparatus. The methanol extract was concentrated under reduced pressure to 500ml, water l00ml added and extracted successively with pet. ether (40-60) and ethyl acetate.

The pct. ether extract (Ext. 1) on concentration yielded a yellow gum which was found to be a mixture of several compounds on examination by TLC. The gum was, therefore, chromatographed over silica gel column using pct. ether, pet. ether/benzene (1:1), benzene, benzene/ethyl acetate (9:1). Three compounds A, B and C were obtained and which were identified as nonacosene $_{(C29H58);}$ ursolic acid (C3oH4803) and fl-sitosterol (C29HSO0) respectively.

Nonacosene:

The neat pet. ether fraction on concentration yielded a light buff coloured waxy substance, Compound-A, which was identified to be a saturated hydrocarbon, C29H59. M` = 406; m.p. 60-66°C. Its mass and ir spectrum were typical of a straight chain hydrocarbon.

Ursolic Acid:

The pet. ether/benzene fraction on concentration yielded a off- white crystalline material, Compound-B; m.pt. 280^0-282 which on recrystallisation from methanol gave white shiny crystals melting at 286-288°C[Lit. 291° (Thomson, 1956)1. This compound was identified as ursolic acid through spectroscopic studies and by derivatisation. Its I.R. shows strong absorption at 3400-3500 cm^{-1} due to -OH, and 1680 cm^{-1} due to - CO =of carboxylic acid. Molecular ion peak appears at m/e 456 (M^+). Peak at rink 248 is due to Retrodiel-Alder fragment (Budzikicwicz, et al., 1964). Its acetate, m.p. 288-290°) was prepared by treating it with acetic anhydride and pyridine.

β-Sitosterol:

Compound-C, eluted with benzene:ethyl acetate (9:1), was initially a waxy solid compound and was crystallised from $CHCl_3$:MeOH melting at 139-140°. Its infrared spectrum (KBr disc) showed strong absorption at 3500-3400 cm^{-1} due to -OH group. The mass spectrum showed M^+ at m/e = 414. Other peaks were found to be present at m/e 399 (M^+-CH_3), 396 (M^+-H_2O), 381, 329, 303 and 273. A prominent peak at m/e 149 also indicated a steroidal nature of the hydrocarbon. The compound gave a positive Libermann-Burchard test and was identified a *β*-sitosterol.

The ethyl acetate extract (Ext. 2) obtained as mentioned above from aq. methonolic concentrate on tic examination in various solvent systems [cyclohexane/ethyl acetate

(1:1); dichloromethane/acetone (47:3); and dichloromethane/methanol (24:1)] revealed the presence of several steroids when sprayed with Liebermann-Burchard reagent and 50% sulphuric acid. These were probably the estrogens as reported previously (Dean, et al., 1971; Heftmann, et al., 1966). Their exact identity could, however, not be established due to non-availability of reference compounds. A portion of the original alcoholic extract of the seeds also showed the presence of glycosides the details of which will be communicated in our next report.

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