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Betulonic Acid and Moronic Acid from the Stem Bark of *Evodia Meliafolia* Benth

E. VENKATA RAO*, RAO V. S. V. VADLAMUDI** AND P. SRIDHAR.
Dept. of Pharmaceutical Sciences, Andhra University, Visakhapatnam - 530 003.

The isolation and identification of isobaurenol, betulonic acid, moronic acid, limonin and diosmin have been described from the stem bark of *Evodia meliafolia* Benth. This is the first report of occurrence of betulonic acid, moronic acid and diosmin in *E. meliafolia*.

EVODIA meliafolia Benth (Rutacea) is a medicinal plant growing at an altitude of 1000 ft. in Assam and Eastern Himalayas, Orissa and Bihar[1]. it was known to Chinese from ancient times as Hwang-peti and its bark was used both in medicine and in dyeing industry[2]. Limonin was isolated from the seeds of *E. meliafolia*[3]. Lengyall and Gellert[4] examined its root bark and isolated isobaurenol, limonin and limonindiosphenol besides a sterol mixture. We report here the results of our chemical examination of the stem bark of this plant.

EXPERIMENTAL

Powdered stem bark (750 g) was extracted successively with petroleum ether, chloroform, methanol and 75% ethanol. The petroleum ether extract (950 mg) was chromatographed on a silica gel column. The petroleum ether-benzyene (1:1) eluates afforded a pentacyclic triterpene alcohol (120 mg), m.p. 164-66°, $[\alpha]_D$ + 39.7° ($CHCl_3$), identified as isobaurenol. Pure benzene eluates yielded a low melting neutral substance (65 mg) m.p. 79- 81°, which is yet to be characterized. The $CHCl_3$ - MeOH (98:2) eluates gave triterpene A (150 mg) while the $CHCl_3$ - MeOH (85:15) eluates afforded triterpene B (250 mg).

***For correspondence**
****Present Address: Hoechst Marion Roussel, Research Centre, Lalbahadur Shastri Marg Mumbai - 400 080.**

The chloroform extract (2.3 g) on column chromatography over silica gel gave limonin (350 mg), m.p. 285-89°, $[\alpha]_D$ - 127.3° (pyridine) and + 33.7° (1 N alc. KOH). The methanol extract on concentration deposited a flavonoid (680 mg).

The identity of isobaurenol and limonin was established by their physical and chemical properties, spectral data and comparison with authentic samples isolated from *E. fraxinifolia*[5,6]. The identification of triterpenoids A and B and the flavonoid is described below.

RESULTS AND DISCUSSION

Triterpene A, m.p. 250-52°, $[\alpha]_D$ + 37.3° ($CHCL_3$), gave a positive LB reaction. The compound, formula $C_{30}H_{46}O_3$, M^+ (454), showed IR bands at 1715, 1700, 1650 and 895 cm^{-1} characteristic of a six membered ring ketone, a carboxylic acid and an exocyclic double bond respectively. With diazomethane it formed a methyl ester, m.p. 162-64°, $[\alpha]_D$ + 34.2° ($CHCl_3$) HRMS (EI) found: M^+, 468.3602 ($C_{31}H_{48}O_3$ requires 468.3605). The ^1H NMR of the acid showed six CH_3 groups between δ 0.92 to 1.02, one CH_3 adjacent to double bond at δ 1.68, a two proton multiplet at δ 2.4 for α- ketomethylene group and two proton doublets at δ 4.58 and 4.76 (J = 2Hz) for vinylic protons of exocyclic double bond. These data correspond to betulonic acid[7,8] and the conclusion was

supported by mass spectral fragmentation pattern[9]. The identity was confirmed by comparison with an authentic sample of betulonic acid obtained by Jones' oxidation of betulinic acid.

Triterpene B, needles from chloroform-methanol, m.p. 224-26°, $[\alpha]_D$ + 51.2° ($CHCl_3$), formula $C_{30}H_{46}O_3$, M^+ (454), also gave a positive LB reaction and was found to be slower than betulonic acid in t.l.c. ($CHCl_3$-MeOH. 99:1). Its IR bands (1715, 1695 and 1630 cm^{-1}) revealed it to be a ketocarboxylic acid with an olefinic bond. It formed a methyl ester with diazomethane, m.p. 166-68°, $[\alpha]_D$ + 57.3° ($CHCl_3$, HRMS (EI) found: M^+, 468.3609 ($C_{31}H_{48}O_3$ requires 468.3605). The ^1H NMR spectrum of the acid showed seven CH_3 groups between δ 0.78 to 1.07, a two proton multiplet at δ 2.48 for α-ketomethylene group and one proton singlet at δ 5.15 for trisubstituted double bond. These data are in close agreement with that of moronic acid (Δ^{18} oleanonic acid)[10]. The identity was confirmed by comparison of the MS of the acid[10] and its methyl ester[9]. Further the important mass fragments of diagnostic importance in the methyl ester[9] were identified by accurate mass measurements: 409.3481 ($C_{29}H_{45}O$, M-$COOCH_3$), 262.1924 ($C_{17}H_{26}O_2$), 248.1772 ($C_{16}H_{24}O_2$), 203.1800 ($C_{15}H_{23}$) and 189.1627 ($C_{14}H_{21}$, base peak).

The flavonoid obtained from the methanol extract, m.p. 275.79°, was sparingly soluble in ethanol, insoluble in dil. HCl and soluble in warm dil. NaOH. It gave positive tests for a flavonoid glycoside. Acid hydrolysis gave an aglycone, identified as diosmetin and the sugars glucose and rhamnose in equimolar proportion. These properties suggested that the compound could be diosmin. The identity was confirmed by preparation of the glycoside acetate with pyridine-Ac_2O, m.p. 150-60°, and comparison of its ^1H NMR spectrum with that of diosmin octaacetate[11].

The 75% ethanolic extract gave a blue colour with $FeCl_3$ for the presence of phenolics (tannins). The extract gave negative tests for alkaloids. Berberine was found to be specifically absent in the plant part examined. (cf. Ref. 2).

Betulonic acid, moronic acid and diosmin are reported here for the first time in *E. meliafolia*. While betulonic acid has been reported to occur in several plant species, moronic acid was found to be present only in a few species. Moronic acid was first known as its methyl ester having been prepared by Barton and Brookes[12] from morolic acid isolated from *Mora excelsa*. Subsequently moronic acid was isolated from *Roylea elegans*[10], *Salvia pomifera*[13] and *Boronia inornata*[14]. The occurrence of betulonic acid in medicinal plants is of interest in that it has been shown to have remarkable protective activity against chemically induced cytotoxicity in primary cultured rat hepatocytes[15]

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