

Phytochemical Constituents of the Bark of *Vitex negundo* L.

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Abstract

p-Hydroxybenzoic acid (**1**) and β -sitosterol (**2**) were isolated, and identified from the methanol and hexane extracts of *Vitex negundo*. The structure of the compounds were established on the basis of spectral analysis.

Keywords: *p*-hydroxybenzoic acid, β -sitosterol, *vitex negundo*.

Introduction

Vitex negundo L. belongs to the family Verbenaceae is a much branched shrub with quadrangular tomentose and densely whitish tomentose branchlets, up to 5 m tall or sometimes a small, slender tree. The different parts of *Vitex negundo* are used as medicinal plant by ethnic people for the treatment of various diseases. Its roots and leaves have been used as a tonic and in various ailments.^{1,2} Its leaves are used as aroma, tonic, vermifused for relief headache and catarrh, discutient, useful in dispersing swellings of joints from acute rheumatism and of testes from suppressed gonorrhea. Previous studies on the steam barks of *Vitex negundo* have resulted in the isolation of many terpenes, sterols, phenolic compounds, flavanoids, alkaloids, organic acids, glucosides, and anthocyanines.³⁻¹² Most of them had reported to have antifeedant, antibacterial activities, antiarthritic activity and anti-inflammatory activity.^{13,14} This paper deals with the isolation and identification of the constituents like *p*-hydroxybenzoic acid (**1**) and β -sitosterol (**2**). The structures of the isolated compounds were established by detailed analysis of their ¹H-NMR, ¹³C-NMR

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spectra. The melting point and Co-TLC results are also used for comparison with the authentic samples.

Experimental Methods

The melting points were uncorrected. IR spectra were recorded in KBr disc. ^1H -NMR and ^{13}C -NMR spectra were recorded in 500 MHz NMR using CD_3OD and TMS as a solvent and internal standard, respectively. The purity of the compound was checked on TLC (silica-gel G_{254} pre-coated) plates.

The steam bark of the *V. negundo* was collected from Bansbari (at 1500 m. altitude), Sindhupalchok district of Nepal.

The air dried and powdered stem bark (1 Kg) of the *V. negundo* (Verbenaceae) were extracted with hexane (2.7 L, 13 h), dichloromethane (3 L, 14 h) and methanol (2.5 L, 12 h) in a soxhlet extractor to get hexane, dichloromethane and methanol extracts, respectively.

The methanol extract (15 g) was subjected to column chromatography on silica gel column. The column was eluted initially with hexane and then hexane-ethyl acetate and finally with methanol. The sub-fractions were pooled into 21 major fractions on the basis TLC character. The fractions 209-240, 241-270 and 271-303 were obtained by eluting with 1%, 2% and 3% methanol in ethyl acetate, respectively, showed one major spot with minor impurities on TLC. These fractions were combined and concentrated to get white needle shaped crystals. It was purified by washing with hexane, chloroform followed by re-crystallization with methanol. The obtained 2 g of the crystal was designated as compound (1).

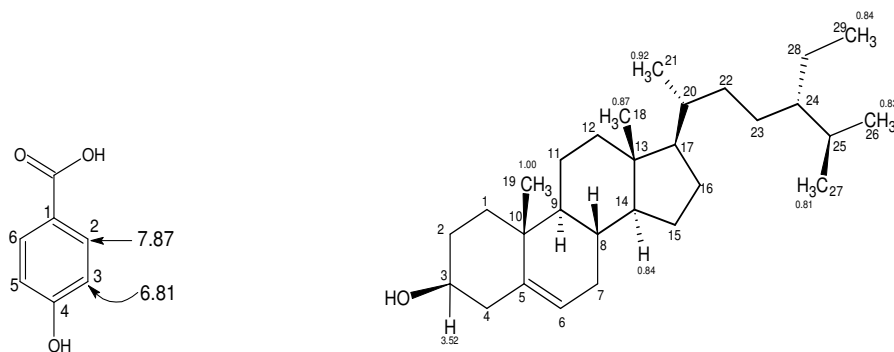
The hexane extract (5.0 g) was subjected to column chromatography on silica gel column. The column was eluted initially with hexane and then hexane-ethyl acetate and finally with methanol. Fractions of 107 to 134 eluted with 3% ethyl acetate in hexane were found similar on TLC with a major spot and a minor spot. The concentrated solution was kept for twenty-four hours at room temperature when needle shaped crystals appeared. The crystals were separated from mother liquid and washed successively with pure hexane and 1, 2 and 5 % ethyl acetate in hexane. The white substance was re-crystallized from methanol and which showed a single spot on TLC (R_f 0.52 in 15% ethyl acetate in hexane system). The obtained 45 mg of crystals are designated as compound (2).

Results and Discussion

The compound-1 was a crystalline solid having melting point at 215°C ; single spot on TLC with R_f 0.57 (50: 50: 0.5, CH_2Cl_2 - EtOAc - AcOH) and 0.67 (95 : 5 : 0.5, CHCl_3 - MeOH - AcOH). It gave positive test for carboxylic acids with 5%

NaHCO₃ solution indicating the compound to be carboxylic acid. It gave violet color with ferric chloride solution indicating the presence of phenolic group. The ¹H-NMR spectrum of the compound (**1**) exhibited two doublet peaks at δ 6.81 (2H, J = 8.76) and δ 7.87 (2H, J = 8.80) equivalent to four protons due to the presence of disubstituted benzene ring. The ¹³C-NMR spectra of the compound (**1**) exhibited total five signals including a signal (δ 170.1) assigned for the carbon of carboxylic acid. The four different signals at δ 122.6, δ 132.9, δ 116.0 and δ 163.3 of carbons were assigned for C-1, C-2 and C-6, C-3 and C-5 and C-4 respectively of a benzene ring. On the basis of spectral analysis and by direct comparison with the authentic sample (Co-TLC & Melting Point), the compound (**1**) is identified as 4-hydroxybenzoic acid (*p*-hydroxybenzoic acid).

The compound (**2**) was a crystalline solid having melting point at 136°C, single spot on TLC with R_f 0.47 (2:8, ethyl acetate/hexane). It gave positive Libermann-Burchard test indicating the compound to be sterol. The mass spectrum showed M⁺ at m/z at 414 corresponding to the molecular formula (C₂₉H₅₀O). The IR Spectrum showed presence of –OH group (3434 cm⁻¹). The ¹H-NMR spectrum exhibited two singlets at δ 1.00 and 0.67 were assigned for the methyl group of C-19 and C-18, respectively. The doublets at δ 0.92 (d, J = 6.1 Hz); 0.81 (d, J = 6.9 Hz); 0.83 and (d, J = 6.9 Hz) and 0.84 (t, J = 7.3 Hz) were accounted for methyl group at C-21, C-26 and C-27. A signal at δ 5.35 in ¹H-NMR can be accounted for an olefinic proton at C-6. Other multiplet at δ 3.52 equivalents to a singlet proton was assigned for the proton of C-3. The low field signal may be due to the attachment of β -OH group at C-3 carbon. Thus, the assignment of hydroxyl group at C-3 and the double bond at C-5 were assigned accordingly. Three multiplets equivalent to two protons each appeared at δ 1.83, 2.00 and 2.27 were assigned for three CH₂ groups. The remaining protons were appeared as multiplets at δ 1.05-1.65. From all the spectral analysis and Co- TLC with authentic sample the compound (**2**) was identified as β sitosterol.



Compound (1)
(¹H-NMR of *p*-hydroxybenzoic acid)

Compound (2)
(¹H-NMR of β -sitosterol)

Conclusions

The methanol and hexane extracts of the air dried and powdered steam bark of *V. negunda* resulted in the isolation of *p*-hydroxybenzoic acid (**1**) and β -sitosterol (**2**). The structures of the isolated compounds were established on the basis of their spectral data.

Acknowledgments

The authors wish to thank Prof. Dr. R. P. Choudhary, Central Department of Botany, Tribhuvan University for the identification of the plant. The research facilities providers; Central Department of Chemistry and Research Centre for Applied Science and Technology, Tribhuvan University, Kirtipur, Nepal are gratefully acknowledged. The research project is partially supported V. Wagen Research Foundation, Hannover, Germany.

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