

Extraction of Ferulic Acid Ester from Piper Longum

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Abstract – Piper Longum is an important medicinal plant which belongs to family piperaceae is well described in Ayurved Charaksamihita etc. Long piper was purchase from the local market Jaipur, Rajasthan. The chemical investigation of the dried fruits afforded β -sitosterol in very small amount, piperine, episesamin, sesamin, N-methyl- (4'-methoxyphenyl octyl) - 3, 4-methylene dioxy cinnamoyl amide and 30-hydroxytriacontanylferulate.

Keywords – β -sitosterol, piperine, episesamin, sesamin, methylenedioxy cinnamoyl amide, hydroxytriacontanyl ferulate, spectral studies.

I. INTRODUCTION

Long piper is widely distributed that tropical and subtropical regions of the globe i.e. India, Sri Lanka, America and Eastern countries. Black piper has economical and commercial value all over the world. The plant cultivated in hotter parts of India. Indian long piper is mostly obtained from the wild plants i.e. from Assam, West Bengal, Uttar Pradesh and Uttranchal. The Medicinal use of long piper is reported mainly in Ayurvedic, Unani and Siddha preparation have been described by several researchers. Knowing the medicinal importance of piper longum chemical investigation was carried out [1-4]. Piper longum has been reported for a various Bio-chemicals is essential oils, piperine, phenolics, alkaloids, terpenes, flavonoids, Lignans, esters and amides [5] etc. Long piper has been reported for insecticidal and acaricidal [6,7], antifungal [8-11], antiamoebic [10-14], antimicrobial [15,16], antiasthmatic [17,18], anticancer [19,27], antioxidant [28], analgesic [29], antidiabetic [30-31], antiinflammatory [32], immunomodulatory [33-35], antidepressant [36-37], antiulcer [38-39] and Hepatoprotective activity [40-42].

II. MATERIAL AND METHODS

IR spectra were recorded on SIMADZU FTIR-8400S spectrometer using KBr pellets. ¹HNMR and ¹³CNMR spectra (300 MHz and 75 MHz respectively) were recorded on JEOL AL-300 spectrometer in part per million (S) in CDCl₃ with TMS as an internal reference. FAB mass spectra were recorded on JEOL SX 102/BA-600 spectrometer.

III. EXPERIMENTAL WORK

The dried fruits of piper longum was purchase about 1 kg from local market of Jaipur. Rajasthan, India. The dried piper longum (1 Kg) were powdered and extracted with ethanol on water bath for 24 hours. The extract was filtered off and solvent was removed under reduced pressure the crude extract so obtained was dissolved in chloroform. The chloroform soluble portion of the removal of solvent was chromatographed over silica gel column using solvents.

Chemical Investigation of the ethanolic extract of the dried fruits of piper longum afforded six compounds namely β -sitosterol, piperine, episesamine, sesamine, N-methyl-(4'-methoxyphenyloctyl)-3,4-methylene dioxycinnamoylamide and 30-hydroxy triacontanylferulate is being reported from this plant. Structures of these compounds have been elucidated on the basis of spectral studies (IR, NMR, MS).

A) Isolation of Compound 1

Elution of column with chloroform gave compound I as a colourless solid after removal of the solvent. The product obtained was crystallized with acetone; gave white small crystals. It showed melting point 118°C and Reference value was found 0.70 in chloroform and iodine vapour was used as developing agent, gave spectral data as: IR(KBr) 2900, 1510, 1260, 1065, 1045 cm⁻¹ and mass (m/z) 354(m⁺).

B) Isolation of Compound 2

Compound 2 was isolated by eluting the column with petroleum ether & ethyl acetate (9:1) yielded sesamin and purified from acetone gave cream coloured compound. The spectral studies on as UV λ_{\max} (CH₃OH) 230 and 285 nm, IR (KBr) 2845, 1520, 1270, 1070, 1030cm⁻¹ and mass (m/z) 354(m⁺).

C) Isolation of Compound 3

When the column was eluted with benzene and chloroforms in ratio of (3:1) a compound 3 was isolated. The m.p. was observed 178°C and Reference value was found 0.82 by using 6% methanol as a solvent. The spectral data on as IR (KBr) 2910, 2870, 1675, 1500, 1445, 1255, 1110, 1060 cm⁻¹ and mass (m/z) 395(m⁺).

D) Isolation of Compound 4

On eluting the column with petroleum ether and benzene in 1:3 ratio 30-hydroxytriaconyanyl ferulate was obtained as a brown solid compound, crystallized by methanol, m.p. 88°C. On the basis of spectral data as IR (KBr) 3538, 1280, 1724 and mass (m/z) 654(m⁺).

IV. RESULT AND DISCUSSION

A) COMPOUND 1

In the mass spectrum the molecular ion peak was observed at m/z 354 (M⁺). On the basis of ¹H NMR, eighteen protons and by ¹³C NMR presence of twenty carbon atoms was confirmed. Thus the molecular formula for compound 1 was established as C₂₀H₁₈O₆.

In the IR spectrum The C-H stretching was observed at 2850. The absorptions at 1250, 1075 and 1040 were assigned for C-O-C stretching. An absorption band at 1500 confirmed the presence of >C=C< stretching of aromatic ring. The proton NMR spectrum (δ ppm, CDCl₃), of compound (1) showed a multiplet from 6.76-6.89 accounted for six protons of aromatic ring. The protons of two methylenedioxy group (OCH₂O) appeared as a singlet at 5.98. A doublet at 4.82(*J*=4.95Hz) was assigned for the proton present at C-2 position. The proton present at C-6 position was established as a doublet at 4.37(*J*=7.32Hz). The presence of oxymethylene protons present at C-4 position were observed as a doublet at 4.08 (*J*=9.33Hz)

and a multiplet at 3.80 for one proton respectively. Two multiplets at 3.29 and 3.80 were assigned for one proton each present at C-8 position. The presence of two protons appeared as a multiplet at 3.30 and 2.87 and established for C-1 and C-5 protons respectively. In the ¹³C NMR spectrum (δ ppm, CDCl₃) of Compound 1, the absorptions observed at 50.12 and 54.63 were assigned for C-1 and C-5 carbons respectively. The presence of two methylenedioxy groups was confirmed by the absorption at 101.04. The presence of aromatic carbons were assigned by the absorptions at 132.19 (C-1'), 135.04(C-1''), 106.37(C-2'), 106.54(C-2''), 147.61(C-3',3''), 147.91(C-4',4''), 108.15(C-5',5''), 118.67(C-6'), 119.61(C-6'') and their assignment have been shown in parentheses. Other signals located at 82.00(C-2), 70.88(C-4), 87.64(C-6), and 69.67(C-8) were established accordingly. The above ¹H NMR and ¹³C NMR spectral data of compound 1 were found similar to episesamin. On the basis of above discussion and spectral data compound 1 was identified as episesamin.

B) COMPOUND 2

On the basis of ¹H NMR the number of protons were calculated as 12 and ¹³C NMR showed the presence of twenty carbon atoms in the title compound. Thus on the basis of above observations the molecular formula for compound 2 was established as C₂₀H₁₈O₆. The IR spectrum (KBr, Cm⁻¹) showed the characteristic absorption 2850 showing the presence of carbon-hydrogen stretching. An absorption band at 1500 showed the presence of aromatic carbon-carbon double bond stretching. Besides this absorption other important peaks were observed at 1250, 1060 and 1020 for C-O-C stretching. In the proton NMR spectrum (δ ppm, CDCl₃), the presence of six aromatic protons was confirmed by observing a multiplet from 6.75 to 6.90. A sharp singlet observed at 5.94 was assigned for four protons of two methylenedioxy groups. The presence of two protons present at C-2 and C-6 positions were observed as a doublet at 4.70 (*J*=6.09Hz). The axial protons located at C-4 and C-8 positions were in the form of double doublet at 3.86(*J*=13.70, 4.89Hz) where as a multiplet at 4.22 confirmed the presence of two protons at C-4 and C-8 positions in equatorial configuration. The protons attached at C-1 and C-5

positions showed a sharp singlet at 3.06 for two protons. In the ^{13}C NMR spectrum (δ ppm, CDCl_3) of compound 4, absorptions at 54.75 was assigned for C-1 and C-5 carbon atoms. The methylenedioxy ($-\text{OCH}_2\text{O}-$) carbon showed absorption at 101.46. The presence of twelve aromatic carbons were observed at 135.48(C-1',1''), 106.89(C-2',2''), 147.52(C-3',3''), 148.38(C-4',4''), 108.58(C-5',5'') and 119.75(C-6',6'') and their assignment have been given in parenthesis. In 2,6-diaryl-3,7-dioxiabicyclooctane ring the signals were observed at 86.19 for C-2 and C-6 and 72.11 for C-4 and C-8 carbon atoms respectively. On the basis of these observations compound was identified as sesamin.

C) COMPOUND 3

The mass spectrum exhibited the molecular ion peak at m/z 395 (M^+). The proton NMR spectrum indicated the presence of twenty nine protons and ^{13}C NMR indicated the presence of twenty-four carbons in the title compound. On the basis of above spectral data the molecular formula of Compound 3 was calculated as $\text{C}_{24}\text{H}_{29}\text{NO}_4$. In the IR spectrum (KBr , cm^{-1}) absorption at 2920 and 2875 showed carbon-hydrogen stretching, at 1680 for carbonyl group of amide ring, at 1510 for carbon-carbon double bond stretching of aromatic skelton, at 1450 (C-H stretching) indicated the presence of N-methyl(4'-methoxyphenyl octyl)-3-4-methylenedioxy cinnamoylamide group. The aryl alkyl ethers display an asymmetrical C-O-C stretching bond at 1250 with symmetrical stretching at 1050. The absorption at 1100 confirmed the presence of methylenedioxy group in the compound. In the proton NMR spectrum (δ ppm, CDCl_3), two doublets for one proton each was observed at 7.55 ($J=14.82$ Hz) and 6.16 ($J=15.00$ Hz) and assigned to the protons present on carbon-carbon double bond i.e. C-7 and C-8 position respectively. On the basis of coupling constant (i.e. 15.00 Hz), it is clear that the protons present at C-7 and C-8 are in trans configuration. The aromatic protons present at C-2' and C-6' positions showed a doublet at 7.15 ($J=8.58$ Hz) where are the protons present at C-3' and C-5' positions also showed a doublet at 6.88, having coupling constant ($J=8.25$ Hz). Aromatic protons present at C-6 and C-5 that is two protons were observed as a doublet at 6.98 ($J=6.93$ Hz) and

6.80($J=8.22$ Hz) respectively. The C-2 proton was observed at 7.26 and was overlapping with NMR solvent (CDCl_3) signal. A sharp singlet at 6.00 confirmed the presence of methylenedioxy group in the compound 3. A singlet for three protons observed at 3.80 for three protons and assigned for methoxy group. The proton at C-10 position appeared as a quartet at 3.61 for two protons. A singlet for three protons observed at 3.49 confirmed the presence of N- CH_3 group. Presence of two methylene protons at C-11 and C-15 positions was observed as a triplet at 2.82($J=6.24$ Hz). A broad singlet observed at 1.62 showed the presence of six protons of three methylene groups i.e. C-12, C-13 and C-14 were established. In the ^{13}C NMR (δ ppm, CDCl_3), spectrum a signal at 163.00 was assigned for the carbon atom of amido group present at C-9 position. The two signals observed at 140.78 and 130.84 confirmed the presence of olefinic carbon atoms i.e. C-7 and C-8 positions respectively. The signals observed at 130.84(C-1), 108.52(C-2), 148.90(C-3), 152.00(C-4), 114.10(C-5), 123.86(C-6), 129.77(C-1'), 108.52(C-2'), 106.32(C-3'), 146.96(C-4'), 109.01(C-5') and 119.00(C-6') showed the presence of twelve carbon atoms of two benzene nucleus in compound 3 and their assignment have been shown in presentheses. The methyl group attached to nitrogen atom appeared at 33.00. The carbon atom of methoxy ($-\text{OCH}_3$) group was observed at 55.28. The signals observed at 40.93 and 34.77 were assigned for C-10 and C-15 Carbon atoms respectively. A signal located at 101.43 was due to the presence of the carbon atom of methylenedioxy group. The remaining signals for four carbon atoms were ascertained at 29.10 for C-11 to C-14 carbons. On the basis of above spectral studies and discussion compound 3 was identified as N-methyl-(4'-methoxyphenyl Octyl-3,4-methylenedioxy cinnamoyl amide.

D) COMPOUND 4

Elution of column with ethyl acetate-methanol (95:5) afforded triacontanylferulate, m.p. 93°C, IR (KBr) ν_{max} (Cm^{-1}) 3450, 2920, 2840, 1715, 1690, 1620, 1600, 1590, 1500, 1050, 955 etc. Compound 4 showed M^+ ion at m/z 654. Corresponding to molecular formula $\text{C}_{42}\text{H}_{72}\text{O}_5$ which was also supported by ^1H NMR and ^{13}C NMR spectra. The IR spectrum of compound

confirmed the presence of hydroxyl and ester group by observing the absorptions at 3450 and 1715 cm⁻¹ respectively. The presence of ester group conjugated with carbon-carbon double bond was established by the sharp absorption at 1620 and 955 cm⁻¹. The presence of phenyl ring was supported by the absorptions at 1600, 1590 and 1500 cm⁻¹. The ¹H NMR spectrum showed prominent signals for trans-ferulate moiety at δ 7.60, 7.57 and δ 6.30, 6.26 with a coupling constant J=15 Hz, confirming the trans geometry at C-7' and C-8' positions. The aromatic protons were observed at 7.06 (dd), 7.00 (d) and 6.88 (d), while in the aliphatic region broad singlet at 5.82 confirmed the presence of phenolic hydroxyl group. A sharp singlet at δ 3.92 for three protons confirmed the presence of methoxy group. Two triplets at 4.18 and 3.64 for two protons each were observed and assigned for the methylene protons attached to ester and hydroxyl group respectively. The ¹³C NMR spectrum is also in agreement with the above values [43-45]. On the basis of above spectral data compound 4 was characterized as 30-hydroxytriacontanylferulate.

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