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Isolation and Characterization of New Compound from Zanthoxylumalatum

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***Abstract-* The chloroform extract of the dried powdered stem bark of *Zanthoxylumalatum* afforded five compounds ie., α-amyrin (1), lupeol (2), episesamin (3), sesamin (4) and N-methyl-(4'-methoxyphenylhexyl)-3,4-methylenedioxycinnamoylamide (new compound) (5). The column chromatography, crystallization, PTLC and Co-TLC were used for isolation and purification of the compounds. Structures of these compounds were established on the basis of IR, ^{1}HNMR, ^{13}CNMR and Mass spectral studies.**

***Keywords-* *Zanthoxylumalatum,* N-methyl-(4'-methoxyphenylhexyl)-3,4-methylenedioxycinnamoylamide, spectral studies.**

I. INTRODUCTION

Zanthoxylum is a genus of the family rutaceae. It is distributed worldwide mostly in tropical and subtropical areas. It is an aromatic, picklydioecious evergreen tree or shrub mostly grows in the hilly tracks. *Zanthoxylumalatum* is commonly known as Tumbru, Tejfal in Garhwal hills (Uttrakhand) India. The bark, fruits and seeds of the plant are extensively used in the indigenous system of medicine as anthelmintic, carminative, stomachic and an aromatic tonic in fever. The local inhabitants of Garhwal (Uttrakhand) region chew the fragrant twigs for cleaning teeth and bad breath. *Zanthoxylum* fruit extract is used as an antiobesity [1], antimicrobial [2] agents and also in dental cream preparation [3]. Methanolic extract of the stem of Z. *simulans* have been reported for anti-platelet aggregation [4] activities. Whereas extract of Z. *armatum* and Z. *piperitum* have been reported for significant mosquitoes repellent [5-6] activity. Antileukamic[7], anti-inflammatory [8], antimicrobial [9-11], antithypertensive [12-15], antioxidant[16-17], cyctotoxic [18-20], antitumor[21-22] activities have also been reported in *Zanthoxylum* species.

II. MATERIALS AND METHODS

IR spectra were recorded on SIMADZU FTIR-8400S spectrometer using KBr pellets. ^{1}HNMR and ^{13}CNMR spectra (300 MHz and 75 MHz respectively) were recorded on JEOL AL-300 spectrometer in part per millions (S) in $CDCL_3$ with TMS as an internal reference. FAB mass spectra were recorded on JEOL SX 102/BA-600 spectrometer.

III. EXPERIMENTAL

The stem bark of *Zanthoxylumalatum* was collected about 10 kg and the authenticity of the plant was confirmed by Department of Botany, HNB Garhwal University (Central University), Srinagar, Garhwal (Uttarakhand) India.

IV. EXTRACTION AND ISOLATION

The stem barks were dried without exposed to direct sunlight for 30 days. About 1.750 kg shady dried powdered stem barks extracted with methanol over water bath for (12 x 3) hrs. The hot extract was filtered and concentrated by rotavapour where 78g of brownish mass was obtained. The extract after removal of solvent was redissolved in chloroform and then treated with acetonitrile to remove fats. The fat free chloroform soluble portion of extract was concentrated by rotavapour and chromatographed over column (Length 120 & diameter 2.5 cm), silica gel (60-120 mesh).The compounds 1 to 5 were isolated, purified as.

ISOLATION OF COMPOUND 1: The column was eluted by petroleum ether and benzene in the ratio of 3:1, The compound was isolated as a solid, crystallized from methanol to obtained as colourless crystals of m.p. 185°C, gave spectral data as : IR (KBr) 3350, 1650, 1590, 1384, 1372, 1060 cm^{-1} and Mass (m/z) 427 (M^++H), 426 (M^+).

ISOLATION OF COMPOUND 2: When column was eluted with benzene, compound 2 was isolated. After removal of solvent under reduced pressure solid product was obtained which was crystallized from methanol yielded colourless powder which showed m.p. 220°C and gave spectral data as : IR (KBr) 3635, 1650, 1390, 1365 cm^{-1} and Mass (m/z) 427 (M^++H), 426 (M^+).

ISOLATION OF COMPOUND 3: The elution of the column with benzene gave compound 3 as a colourless solid after removal of the solvent. The product obtained was crystallized with acetone and petroleum ether, gave white needle shape crystals. It showed melting point 120°C and R_f value was found 0.75 in chloroform and iodine vapour was used as developing agent, gave spectral data as : IR (KBr) 2850, 1500, 1250, 1075, 1040 cm^{-1} and Mass (m/z) 354 (M^+).

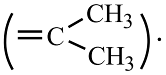
ISOLATION OF COMPOUND 4: The column was eluted with benzene and chloroform in the ratio of 3:1, afforded a compound 4. The solvent was removed under reduced pressure and the solid so obtained was recrystallized from acetone as a white needles. The m.p. was observed 123°C and R_f value was calculated as 0.72 by using chloroform as mobile phase. The developing agent used was iodine vapour and 10% H_2SO_4 in ethanol separately, gave spectral data as : IR (KBr) 2850, 1500, 1250, 1060, 1020 cm^{-1} and Mass (m/z) 354 (M^+).

ISOLATION OF COMPOUND 5: When the column was eluted with benzene and chloroform in the ratio of 1:1, a compound 5 was isolated. The solvent was removed under reduced pressure and the product so obtained was crystallized with acetone as a colourless crystals. The m.p. was observed 180°C and *Rf* value was found 0.83 by using 6% methanol in chloroform as a solvent, gave spectral data as : IR (KBr) 2920, 2875, 1680,1510, 1500, 1450, 1250, 1100, 1050 cm^{-1} and Mass (m/z) 395 (M^+).

V. RESULT AND DISCUSSION

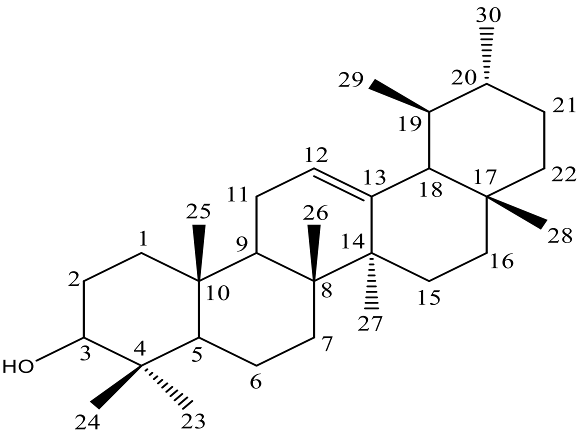
COMPOUND 1: Gave positive tests with Liebermann-Burchardand Noller's reagents, which suggested its triterpenoid nature. In mass spectrum the molecular ion peak was observed at m/z 426 (M^+). The molecular formula of compound 1 was assigned as $C_{30}H_{50}O$. The spectral studies i.e. IR, ^{1}HNMR and ^{13}CNMR of compound 1 was found same with the values reported for α-amyrin. On the basis of these observations the structure of compound 1 was confirmed as α-amyrin.

COMPOUND 2 : The mass spectrum of compound 2 showed molecular ion peak at m/z 426 (M^+). The molecular formula for compound 2 was assigned as $C_{30}H_{50}O$. It gave positive tests with Liebermann-Burchard and Noller's reagents, thus confirmed its triterpenoid nature. The IR spectrum showed strong absorption at 3635 suggested the presence of –OH group. The presence of >C=C< was confirmed by the characteristic absorption at 1650. The sharp absorptions observed at 1390 and 1365 indicate bending vibrations of gem-dimethyl group

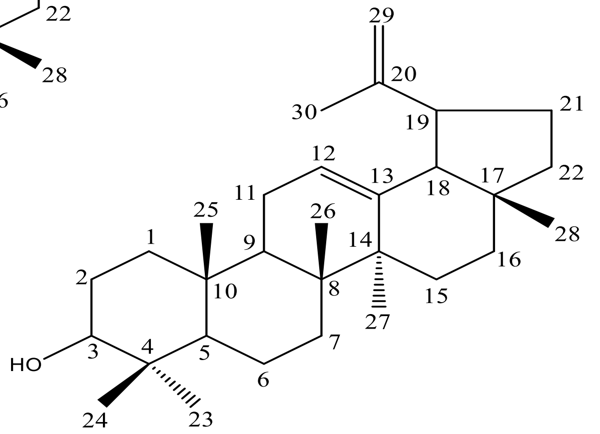


$\left(=C\begin{matrix}CH_3\\CH_3\end{matrix}\right)$.

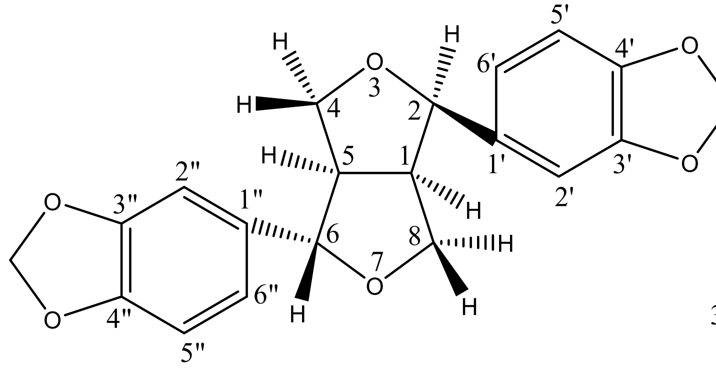
In the ^{1}HNMR spectrum (δ ppm, $CDCl_3$) sharp signals at 0.76(s,3H,C-27), 0.77(s,3H,C-25), 0.82(s,3H,C-24), 0.85(s,3H,C-26), 0.92(s,3H,C-28), and 0.96(s,3H,C-23) were observed and confirmed the presence of six tertiary methyl groups. The methyl group attached to olefinic carbon was observed as a singlet for three protons at 1.68. The two vinylic protons attached at position C-29 were observed as a pair of broad singlets at 4.60 and 4.50 for one proton each. The proton present at C-3 position was located at 3.20 as a triplet due to presence of hydroxyl group at this position. A multiplet at 2.36 was assigned for two protons present at C-21 position in pentacyclic ring. The remaining twenty three protons were observed in the region from 1.24 to 1.78. The ^{13}CNMR spectrum (δ ppm, $CDCl_3$) showed characteristic absorptions for olefinic carbon atoms at 109.74 and 151.33 for C-20 and C-29 respectively. The attachment of hydroxyl group at C-3 position was confirmed by a signal observed at 79.41. The signals for other carbon atoms were observed at 38.70(C-1), 27.45(C-2), 39.00(C-4), 55.23(C-5), 18.37(C-6), 34.38(C-7), 41.02(C-8), 50.45(C-9), 37.22(C-10), 21.03(C-11), 24.98(C-12), 38.10(C-13), 43.08(C-14), 27.50(C-15), 35.67(C-16), 42.86(C-17), 48.30(C-18), 48.02(C-19), 29.05(C-21), 40.06(C-22), 28.00(C-23), 15.50(C-24), 16.02(C-25), 16.15(C-26), 14.56(C-27), 18.01(C-28), and 19.37(C-30). The assignment for all the carbon atoms has been done accordingly and their position is given in parentheses. The above spectral data are in good agreement with reported values for lupeol. On the basis of above findings compound 2 was characterized as lupeol.



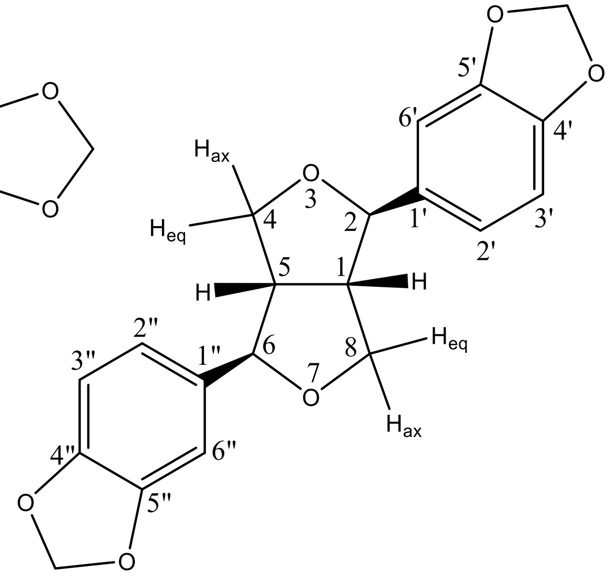
compound 1 as α-amyrin



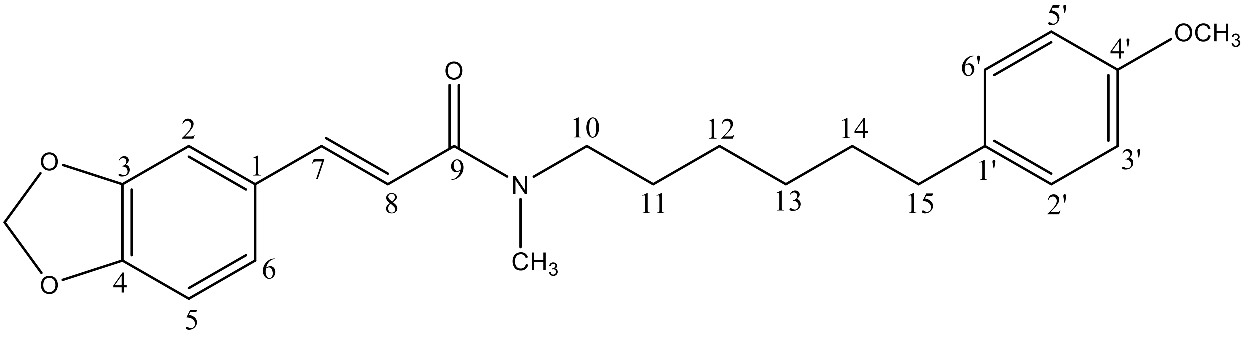
compound 2 as lupeol



compound 3 as episisamin



compound 4 as sesamin



compound 5 as N-methyl-(4'-methoxyphenyl hexyl)-3,4-methylenedioxy cinnamoyl amide

COMPOUND 3: In the mass spectrum the molecular ion peak was observed at m/z 354 (M^+). On the basis of ^{1}HNMR, eighteen protons and by ^{13}CNMR presence of twenty carbon atoms was confirmed. Thus the molecular formula for compound 3 was established as $C_{20}H_{18}O_6$.

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, $CDCl_3$), of compound (3) showed a multiplet from 6.76-6.89 accounted for six protons of aromatic ring. The protons of two methylenedioxy group (OCH_2O) 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 ^{13}C NMR spectrum (δ ppm, $CDCl_3$) of compound 3, 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 ^{1}H NMR and ^{13}C NMR spectral data of compound 3 were found similar to episisamin. On the basis of above discussion and spectral data compound 3 was identified as episesamin.

COMPOUND 4: On the basis of ^{1}H NMR the number of protons were calculated as 12 and ^{13}C NMR showed the presence of twenty carbon atoms in the title compound. Thus on the basis of above observations the molecular formula for compound 4 was established as $C_{20}H_{18}O_6$.

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 these absorptions other important peaks were observed at 1250, 1060, and 1020 for C-O-C stretching. In the proton NMR spectrum (δ ppm, $CDCl_3$) the presence of six aromatic protons was confirmed by observing a muiltplet 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*=6.09 Hz). The axial protons located at C-4 and C-8 positions were in the form of a 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 ($-OCH_2O-$) carbon showed an 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 paranthesis. In 2,6-diaryl-3,7-dioxabicyclo octane 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 4 was identified as sesamin.

COMPOUND 5: 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 5 was calculated as $C_{24}H_{29}NO_4$. In the IR spectrum (KBr, cm^{-1}) absorptions 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 for carbon-nitrogen stretching. Further absorption at 1500 and 1450 (C-H stretching) indicated the presence of N-methyl(4'-methoxyphenyl hexyl)-3-4-methylanedioxy cinnamoylamide) group. The aryl alkyl ethers display an asymmetrical C-O-C stretching band 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 positions 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 as 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 5. A singlet for three protons observed at 3.80 for three protons and assigned for methoxy group. The protons 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 5 and their assignment have been shown in parentheses. The methyl group attached to nitrogen atom appeared at 33.00. The carbon atom of methoxy ($-OCH_3$) 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 5 was identified as N-methyl-(4'-methoxyphenyl hexyl)-3,4-methylenedioxy cinnamoyl amide and reported for the first time hence it is new compound.

VI. ACKNOWLEDGEMENT

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