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A Review on Structural Elucidation and Pharmacological Effects of Compound Auraptene from *Feronia Limonia*

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Abstract: ***Plant serve as vast source for many phytoconstituents which exhibit pharmacological effects. Identification of these types of potential plants is of significance in medicine. Therefore it is necessary to study the pharmacognostic characteristic of the plant before using in the field of research and pharmaceutical formulation. Feronia limonia plant is well known for its medicinal properties. The extracts and pure compounds derived from all parts of this plant show a wide spectrum of pharmacological activities, including hepatoprotective, snake bite, anti-tumour, antimicrobial, antidiabetic, anti-inflammatory, analgesic, antioxidant, antimutagenic, anti-malarial and other activities. Auraptene has been isolated from root and fruit of this plant. The main objective to study the chemical structure of isolated plant constituent auraptene is that it may be responsible for their pharmacological activity.***

Keywords: *F. limonia*, Auraptene, Spectral analysis, Pharmacological effects

1. Introduction

Human beings have been dependent on higher plants for their health care needs since the very beginning of human civilization. Auraptene is natural bioactive monoterpene coumarin ether and is consumed all over the world. Auraptene, also known as 7-geranyloxycoumarin, is a bioactive monoterpene coumarin from Rutaceae family, which is isolated from many plants like *Citrus aurantium*, *Aegle marmelos*, *Feronia limonia* etc. Auraptene is a highly pleiotropic molecule, which can modulate intracellular signaling pathways that control inflammation, cell growth and apoptosis. It has a potential therapeutic role in the prevention and treatment of various diseases due to its anti-inflammatory and antioxidant activities as well as its excellent safety profile. Different studies have demonstrated that auraptene possesses numerous pharmacological properties including anti-inflammatory, anti-oxidative, anti-diabetic, anti-hypertensive and anticancer as well as neuroprotective effects. Bahram Bibak et al.[1]

Feronia Limonia is a moderate-sized tree which belongs to the family Rutaceae. It is widely distributed in Asia particularly India, Sri Lanka and Myanmar. The extracts and pure compounds derived from all parts of this plant show a wide spectrum of pharmacological activities, including hepatoprotective, snake bite, anti-tumour, antimicrobial, antidiabetic, anti-inflammatory, analgesic, antioxidant, antimutagenic, anti-malarial and other activities. Takuji Tanaka.[2] Different components like esters, amino acids, tyramine derivatives, steroids, glycosides, flavanoids, fatty acids, alkaloids, flavanoids, tetranortriterpene, coumarins, triterpenoids has been isolated from different parts of this plant. Auraptene has been isolated from root and fruit of this plant. The main objective to study the chemical structure of isolated plant constituent auraptene is that it may be responsible for their pharmacological activity.

The procedure generally used for the isolation of the compounds was thin layer chromatography and column chromatography. Structure of the compound isolated, was established mainly on the basis of spectral evidences i.e. UV, IR, ^{1}H NMR, Mass and ^{13}C NMR.

2. Methodology

The air-dried and finely crushed plant material (5kg) of *Feronia limonia* was extracted with ethanol. The ethanolic extract on keeping over night, deposited a dirty white residue, which was separated by filteration. The filtrate was concentrated and poured into large excess of ice cold water with constant stirring. A reddish brown aqueous solution and a light brown water insoluble residue were separated.

The water insoluble fraction was extracted with different solvents in increasing order of polarity in soxhlet extractor, viz, hexane, benzene, ether and ethyl acetate, respectively. Benzene fraction was concentrated and subjected to preparative TLC using solvent, benzene : ethyl acetate (9:1, v/v) and methanol as eluting agent. Single compound has been isolated by means of preparative TLC from benzene soluble fraction, viz., FL-1 (m.p.66ºC) which was identified as 7-geranyloxycoumarin or auraptene.

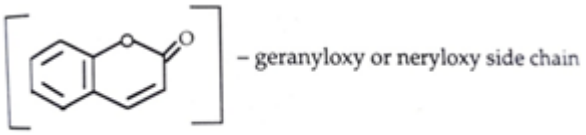
3. Result and Discussion

The light yellow coloured shining crystal, m.p. 66ºC, was analysed for $C_{19}H_{22}O_3$ on the basis of elemental analysis and molecular weight determination. The UV spectrum displayed absorption at 245, 254 and 324nm, similar to that of monoalkoxycoumarin. Chatterjee A et al.[3] The IR spectrum of the compound suggested the presence of α, β-unsaturated δ-lactone (1725cm^{-1}). Dyer R John.[4], gem-dimethyl group (2900-3000cm^{-1}) and the aromatic ring (1610cm^{-1}). Compound FL-1 as 7-alkoxycoumarin as confirmed by ^{1}H NMR. ^{1}H NMR spectrum of the compound showed the presence of five aromatic protons. This means that the coumarin nucleus in monosubstituted. The ^{1}H NMR data of the compound FL-1 are given in table I.

^{1}H NMR ($CDCl_3$, 60MHz):

Table 1:

Assignments	Chemical shifts (δ-ppm)	*J* values (Hz)
CH_3 \ C – / CH_3	1.6 and 1.65 (s, 3H each)	
3′-Me	1.75 (s, 3H)	
4′->CH_2 & 5′->CH_2	2.0-2.20 (m, 4H)	
1′-> CH_2	4.60 (d, 2H)	6.5
6′- ≥CH	5-5.2 (bm, 1H)	
2′- ≥CH	5.48 (t, 1H)	6.8
H-3	6.25 (d, 1H)	9.5
H-6 & H-8	6.85 (m, 2H)	
H-5	7.37 (d, 1H)	8.5
H-4	7.65 (d, 1H)	9.5



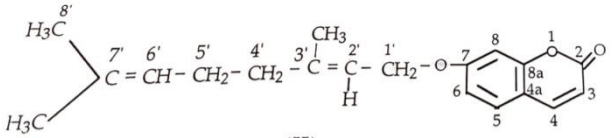
(I)

It showed the presence of either geranyloxy or neryloxy side chain. Talapatra S K et al.[5] The structure of the side chain was established on the basis of ^{1}H NMR spectrum. ^{1}H NMR spectrum of the coumarin displayed signals at δ 1.60 and 1.65 (s, 3H each, gem-dimethyl group at 7′), 1.75 (s, 3H, 3′-Me), 2.0-2.20 (m, 4H, 4′->CH_2 and 5′->CH_2), 4.60 (d, 2H, *J* = 6.5 Hz, 1′-> CH_2), 5-5.2 (bm, 1H, 6′-≥CH coupling with vicinal 5′->CH_2 and allylic 7′-Me′s) and 5.48 (t, 1H, *J* = 6.5 Hz, 2′-≥ CH coupling with 1′-CH_2 and also with allylic 4′-> CH_2 and 3′-Me) for either geranyloxy or neryloxy side chain. The structure of the compound, thus, could e represented as I

It′s ^{1}H NMR spectrum also revealed the presence of presence of two doublets at δ 6.25 (d, 1H, *J* = 9.5Hz) and 7.65 (d, 1H, *J* = 9.5Hz) ppm assignable to C-3 and C-4 protons, respectively. A doublet at δ 7.37 ppm (1H, *J* = 8.5 Hz) clearly indicated the presence of a proton at C-5. Due to the overlapping of the signals of C-6 and C-8 protons, a multiplet corresponding to two protons at δ 6.85 ppm has been observed. Murray R D H et al.[6] Thus, it clearly indicated that the side chain was located at the position C-7, in the coumarin nucleus. Silverstein R M et al.[7]

The nature and position of the side chain was evident by the study of high resolution ^{1}H NMR spectrum. Fisher J F et al.[8] The geometry about the double bond (2′-3′ position) has been suggested to be trans (geranyloxy form) rather than cis (neryloxy form). Coates R M et al.[9], on the basis of fine splitting of the 2′-vinyl hydrogen in the ^{1}H NMR spectrum. At 60 MHz, the vinyl proton at C-2′ appeared as a triplet at δ 5.48 ppm (1H, *J* = 6.8 Hz). Bhacca N C et al.[10] But at 300 MHz, this absorption appeared as a triplet (*J* = 6.8 Hz) with each peak further splitting into quartets (*J* = 1.2 Hz). This splitting and coupling constant along with stereochemical considerations and the reported trans arrangement of the –Me group to hydrogen in geraniol. Pinder A R. [11], suggested that the vinyl proton was trans to the –Me group.

In ^{1}H NMR spectrum at 300 MHz, the signal for C-6 proton ortho-coupled to C-5 proton and meta-coupled to C-8 proton, appeared as a double doublet at δ 6.85 ppm (1H, *J* = 8.5 and 2.4Hz) and the signal for C-8 proton which was only meta-coupled to C-6 proton, appeared as a doublet at δ 6.82 ppm (1H, *J* = 2.4Hz). The above high resolution ^{1}H NMR data confirmed the presence of a geranyloxy side chain C-7 position of the coumarin nucleus. The structure of the compound can, thus, be represented as 7-geranyloxycoumarin (II):

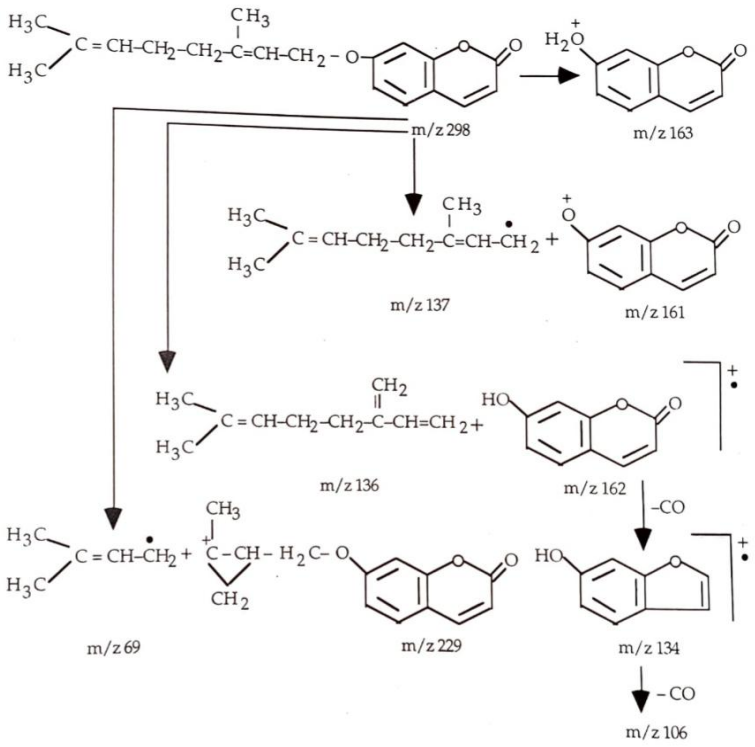


(II)

The structure of the compound FL-1 as 7-geranyloxycoumarin or auraptene was further confrmed by ^{13}C NMR data. Patra A et al.[12] The chemical shifts of all the carbons of coumarin nuclei of 7-geranyloxycoumarin were very much similar to those of 7-hydroxycoumarin. Cussans N J et al.[13] The signal for C-7, when compared with that of simple coumarin, appeared appreciably downfield (about 30 ppm), indicating its linkage with oxygen function and the signals for C-6 and C-8 appeared upfield. The effect on C-5 and C-8a (meta-carbons) was not much, usually 0.5-2 ppm downfield. The chemical shifts of the side chain carbons of 7-geranyloxycoumarin were very similar to those of geraniol. Bohlmann F et al.[14] except C-1′, C-2′ and C-3′. C-1′ and C-3′ underwent slight downfield shift while C-2′ underwent upfield shift. The assignments of the chemical shifts of all the carbons are given in experimental section.

The mass spectrum exhibited molecular ion peak at m/z 298. The mass spectrum was consistent with structure II and was characterized by the extremely facile fragmentation of the allylic ether bond with hydrogen transfer from the 3′- Me to the ether oxygen in a 6-membered cyclic transition state, resulting in a 7-hydroxycoumarin radical ion, appearing as a base peak at m/z 162 and consequently, the parent ion at m/z 298 was extremely weak. Barnes C S et al. & Adesogan E K.[15-16] The other diagnostically important peaks were at m/z 229 (M-69, 4′-5′ cleavage), 163 (1′-O cleavage with intra-molecular capture of 2H), 161 (M-137, 1′-O cleavage), 134 (a-CO to give benzofuran type ion), 136 and 137 (side chain after 1′-O cleavage, with or without H-loss to the ether oxygen), 106 (loss of CO from 134), 68 and 69 (5′-4′ cleavage with or without H-loss). Fragmentation pattern has been represented in scheme I.

Scheme - I



The compound FL-3 was confirmed as 7-geranyloxycoumarin (aurapten) by direct comparison (m.p., Co-TLC) with an authentic sample. Patra A.[12]

Experimental

Solubility	Benzene, chloroform, acetone and methanol
Chromatography	Preparative TLC was carried on silica gel 'g' plates using following solvent system, Benzene : Ethyl acetate (9:1, v/v) (Rf = 0.90).
m.p.	66ºC
R_f	0.90 (benzene : ethyl acetate, 9 : 1, v/v)
Elemental analysis	
Found	C : 75.91%, H : 6.01%
Calculated for $C_{19}H_{22}O_3$	C : 75.82%, H : 6.3%
Spectral studies	
UV λ_{max}^{MeOH}	245, 254, 324 nm
IR ν_{max}^{KBr}	3000-3100, 2900-3000, 1725, 1610, 1400, 1350, 1235, 1200, 1125 cm^{-1}
^{1}H NMR [$CDCl_3$, 300MHz]	δ1.6 and 1.65 (s, 3H each, CH_3-C-CH_3), 1.76 (s, 3H, 3′-CH_3), 2.0-2.20 (m, 4H, 4′->CH_2 and 5′->CH_2), 4.60 (d, 2H, *J* = 6.5 Hz, 1′->CH_2), 5-5.2 (bm, 1H, 6′-≥CH), 5.48 (tq, 1H, *J* = 6.8 and 1.2Hz, 2′-≥CH), 6.25 (d,1H, *J* = 9.5Hz, H-3), 6.82 (d, 1H, *J* = 2.4Hz, H-8), 6.85 (dd, 1H, *J* = 8.5 and 2.4Hz, H-6), 7.37 (d, 1H, *J* = 8.5, H-5) and 7.65 (d, 1H, *J* = 9.5Hz, H-4) ppm.
^{13}C NMR ($CDCl_3$)	δ161.2 (C-2),112.4 (C-3), 143.4 (C-4), 112.3 (C-4a), 128.6 (C-5), 113.2 (C-6), 162.2 (C-7), 101.6 (C-8), 155.9 (C-8a), 65.5 (C-1′), 118.4(C-2′), 142.3 (C-3′), 39.5 (C-4′), 26.2 (C-5′), 123.6 (C-6′), 131.9 (C-7′), 25.6 (C-8′), 17.7 (3′-Me), 16.7 (7′-CH_3) ppm.
Mass spectra, m/z	M^+ 298, 229, 163, 162, 161, 137, 136, 106, 69, 68.

Pharmacological Effects

There is growing awareness in herbal medication as they are usually safe and devoid of significant adverse effects. Various interesting pharmacological effects have been reported for this bioactive monoterpene coumarin ether and are consumed all over the world. It is used with regards to the prevention of generative diseases. The most abundant prenyloxycoumarin found in nature is 7-geranyloxycoumarin, best known as auraptene which has been isolated by root bark, root, and fruit pericarp of *Feronia limonia*. Talapatra et al. 1973.[5] .Agarwal et al. 1989.[17]

Auraptene has excellent anti-inflammatory and antioxidant activities and exerts superior anticancer effects by modulating cell signaling pathways that control cytokines, growth factors, transcription factors, proliferation and apoptosis. Auraptene has been suggested to be effective in the treatment of a broad range of disorders including inflammatory disorders, dysentery, wounds, scars, keloids and pain. It has pharmacological properties including cardio protective, anti-diabetic, anti-hypertensive as well as neuroprotective effects. It has moderate hypotensive activity.

The pharmacological effects of *F. Limonia* fruit and root have been studied extensively in recent years. However, the

active ingredients which exert respective pharmacological actions need to be identified and isolated so that the fruit and root may be used in medical treatment in the future.

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