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Asimafoetidnol: a New Sesquiterpenoid Coumarin from the Gum Resin of *Ferula assa-foetida*

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Dedicated to the memory of Professor (Mrs.) Asima Chatterjee

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Chemical investigation of the gum resin of *Ferula assa-foetida* L. resulted in the isolation of a new sesquiterpenoid coumarin, 7-(((*E*)-5-((1*S*,3*S*,6*S*)-3,6-dihydroxy-2,2,6-trimethylcyclohexyl)-3-methylpent-2-en-1-yl)oxy)-2*H*-chromen-2-one (asimafoetidnol), together with several other known compounds. The structure of asimafoetidnol was established on the basis of spectroscopic analyses. Geometry optimization of the compound has been carried out using a DFT/B3LYP/3-21G* method.

Keywords: Umbelliferae, *Ferula assa-foetida* L., sesquiterpenoid coumarin, NMR spectroscopy, asimafoetidnol.

Ferula assa-foetida (locally called "Hing") is a member of the family Umbelliferae. A native of Afghanistan, this plant also grows in the northern part of India (Kashmir) and Iran. It is herbaceous and perennial and grows up to two meters in height [1]. The gum resin of the plant enjoys a reputation as a folklore medicine. It is used as a sedative, anti-spasmodic, diuretic, and vermifuge [2,3], and is believed to increase sexual appetite [4]. The presence of asadisulfide and asasulfide are the key features for its characteristic aroma and taste [5]. Very recently, farnesiferol C, a compound isolated from *F. assa-foetida*, has been reported as an antiangiogenic as well as an anticancer agent [6]. The antioxidant activity of the methanol extract of *F. assa-foetida* has also been recently reported [7]. Besides these, several coumarins and sesquiterpenoid coumarins have been isolated from this species [8-12]. Recently, five compounds have been isolated from the ethyl acetate fraction of the ethereal extract of the gum resin of *F. assa-foetida* L. four of which *viz*. assafoetidnol [1], saradaferin [10], assafoetidin and ferocolicin [11] have already been reported. The present article deals with the isolation and characterization of the new sesquiterpenoid coumarin asimafoetidnol (**1**) (7-(((*E*)-5-((1*S*,3*S*,6*S*)-3,6-dihydroxy-2,2,6-trimethyl-cyclohexyl)-3-methylpent-2-en-1-yl) oxy)-2*H*-chromen-2-one).

The molecular formula of **1** was established as $C_{24}H_{32}O_5$ (*m/z* 423.492 $[M+Na]^+$, calcd. for $C_{24}H_{32}O_5Na$: *m/z* 423.511) by HREIMS. Absorption maxima at 215 and 324nm in the UV spectrum showed the presence of a typical 7-oxygenated coumarin [11]. The IR spectrum showed the presence of a hydroxyl group (3420 cm^{-1}), carbonyl (1705 cm^{-1}), olefinic moiety (1570 cm^{-1}) and gem dimethyl (1400 and 1380 cm^{-1}).

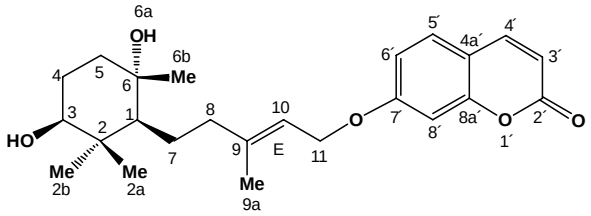

Figure 1: Structure of asimafoetidnol (**1**), a new sesquiterpenoid coumarin isolated from *Ferula assafoetida* L.

The presence of a coumarin moiety was confirmed by an AB system at C-4' (δ_H 7.66) and C-3' (δ_H 6.28, J = 9.5 Hz) in its ^{1}H NMR spectrum. The location of the oxygen substituent was also confirmed from the coupling pattern of position 6' and position 8' (Figure 1). The hydrogen at C-6' showed *ortho* as well as *meta* coupling (δ_H 6.85, J = 8.8, 2.4), while the hydrogen at C-8' (δ_H 6.88) underwent only *meta* coupling (J = 2.4 Hz) (Table 1). The ^{13}C NMR spectra, including DEPT-90° and 135° experiments clearly showed the presence of four methyl, five methylene, and eight methine signals, the rest being due to quaternary carbons. Analysis of the ^{1}H NMR as well as the ^{13}C NMR

Table 1: NMR spectroscopic data of asimafoetidnol (**1**).

Position	^{13}C	^{1}H (*J* in Hz)	^{1}H-^{1}H COSY	HMBC
1	53.28	1.34 (s)	H-7	C-7,8,6
2	40.07			
3	79.10	3.31 (dd;8.8,2.7)	H-4	
4a	25.82	1.73 (m)	H-3,5	C-3,5,6
4b		1.52 (m)		
5a	39.64	1.68 (m)	H-4	C-3,6
5b		1.43 (m)		
6	75.10			
7	23.60	2.04 (m)	H-1,8	C-1
8	39.84	2.11 (m)	H-7	C-1,7,9a
9	142.63			
10	118.51	5.48 (t; 5.4)	H-11, 9a	C-8,9a,11
11	65.41	4.62 (d; 6.6)	H-10	C-9a,10
2'	162.05			
3'	113.27	6.28 (d; 9.5)	H-4'	C-4',4'a
4'	143.20	7.66 (d; 9.5)	H-3',5'	C-3',4'a,5'
5'	128.69	7.38 (d; 8.5)	H-4',6'	C-4',4'a,6',7'
6'	123.00	6.85 (dd;8.8,2.4)	H-5',8'	C-4'a,5',8'
7'	161.25			
8'	101.50	6.88 (d; 2.4)	H-6'	C-6',8'a
2a	18.69	1.03 (s; CH_3)		
2b	16.74	1.07 (s; CH_3)		
3a		3.56 (s; OH)		
6a		3.74 (s; OH)		
6b	22.08	1.35 (s; CH_3)		
9a	16.30	1.80 (broad s)		
4'a	112.45			
8'a	155.86			

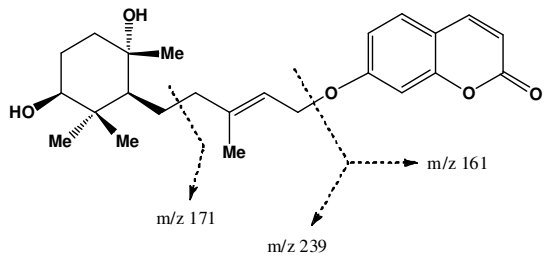

Figure 2: Mass spectral fragmentation of **1**.

spectra (Table 1) indicate four methyl singlets from their chemical shift values at position 2a (δ_H: 1.03, δ_C 18.69); position 2b (δ_H: 1.07, δ_C: 16.74); position 6b (δ_H: 1.35, δ_C: 22.08); and position 9a (δ_H: 1.80, δ_C: 16.30). Moreover, an olefinic double bond and secondary alcoholic methine resonated at position 10 (δ_H: 5.48, δ_C: 118.51) and position 3 (δ_H: 3.31, δ_C: 79.1), respectively.

From the EI mass spectrum of **1**, the fragment at *m/z* 239 ($C_{15}H_{27}O_2$) indicates the presence of a sesquiterpenoid moiety attached to hydroxy coumarin (Figure 2).

Correlation studies indicate a coupling of the C–10 proton to the most downfield methylene attached to oxygen and also to a methyl at 9a, along with methylene at position 8. So the structure of this part was: -O–CH_2–CH=C(CH_3)–CH_2-. Looking at the mass spectrum of this compound, the fragment at *m/z* 171 ($C_{10}H_{19}O_2$) was due to the rest of the

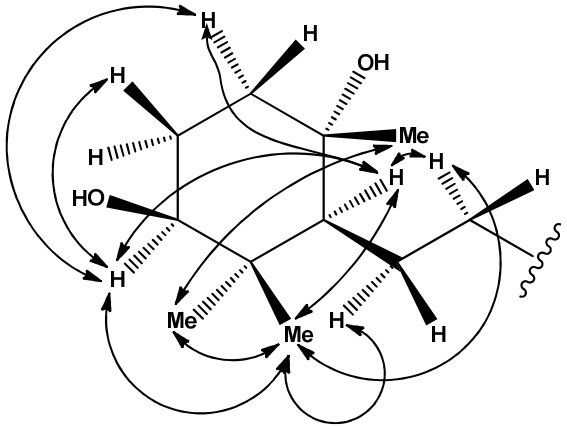

Figure 3: Important 1D NOE enhancements for asimafoetidnol (**1**).

sesquiterpenoid moiety and the formula indicates the presence of a six-membered ring. Linking of the six-membered ring to the rest of the linear parts of the sesquiterpenoid moiety was established by HMBC (Table 1). This showed correlation of the methylenes at 7 and 8 with position 1 of the six-membered ring. Stereochemical disposition of the double bond as the (*E*) isomer was established from the vinyl methyl resonating position in the ^{13}C NMR spectrum (δ_C: 16.3) [13-15]. Besides this, the ^{13}C NMR resonance of C–6b (δ_C: 22.08) indicates a shielding to some extent and it was concluded that it had a *trans* geometry with respect to H–1 [13, 16]. The relative configuration was supported by a single irradiation NOE experiment. The key NOE enhancements (Figure 3) determined the orientation of the methyl and hydroxyl groups at position 6. The coupling constant in the ^{1}H NMR spectrum of the hydroxyl methine proton at position 3 suggests an equilateral assignment in asimafoetidnol (**1**).

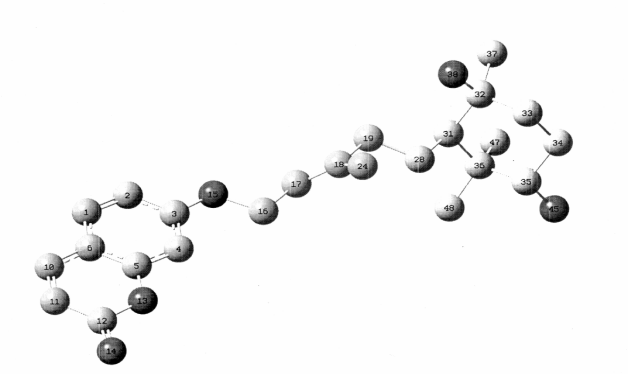

Figure 4: Geometry optimization of **1** by DFT/B3LYP/3-21G* method.

Our proposed stereochemistry gains support from the geometry optimization of the molecule by the DFT/B3LYP/3-21G* method, shown in Figure 4, in which the hydrogen atoms of the structure have been suppressed in order to view the skeleton clearly.

Obviously, the hydrogen atoms are very much present in their usual positions by valency. The atoms are numbered sequentially. Important distances for considering interaction of the cyclohexane substituent groups with the first side chain atom (28) are the following:

38(O) – 28 (C) = 2.74Å, and 37(methyl C) – 28 (C) = 3.92 Å. Clearly the –OH oxygen is closer to the side chain compared with the methyl group. The essential results from the calculation have been presented in Table 2.

The predicted change in energy was -5.393303D-06 and E(RBLYP) = -1302.75500227 A.U. after 11 cycles.

Table 2: Geometry optimization of **1** by a DFT/B3LYP/3-21G* method.

Item	Value	Threshold	Converged?
Maximum Force	0.000165	0.000450	YES
RMS Force	0.000035	0.000300	YES
Maximum Displacement	0.000818	0.001800	YES
RMS Displacement	0.000201	0.001200	YES

Experimental

General: Melting point, uncorrected, was recorded on an electrically heated Köfler Block apparatus. Column and thin layer chromatography were carried out using silica gel (Qualigens 60–120 mesh, Spectrochem 60–120, and 100–200 mesh) and silica gel G (Spectrochem and SRL, India), respectively. Anhydrous sodium sulfate was used for drying solvents. The analytical samples were routinely dried over $CaCl_2$ *in vacuo* at room temperature. The IR spectrum was recorded in a KBr disc on a Perkin-Elmer RXI FT-IR spectrometer. The UV spectrum was measured on a Hitachi U-3501 spectrophotometer and the optical rotation on a Perkin Elmer (Model 341) polarimeter. Elemental analysis was conducted using the Perkin-Elmer 2400 Series II elemental analyzer and results were found to be in good agreement (± 0.2%) with the calculated values for the elements. NMR spectra were recorded with a Bruker DRX 500 NMR spectrometer (11.7 Tesla), equipped with a Silicon Graphics INDY computer. These spectra were recorded with a BBI (broad band inverse) probe with a 5 mm NMR sample tube at 27°C. The actual spectrometer frequencies were 500.134 MHz and 125.770 MHz, respectively, for ^1H and ^{13}C NMR spectra. HREIMS was carried out on an Agilent G1969A LC time-of-flight (TOF) system equipped with an Agilent 1100 Series LC solvent delivery module.

Plant material: The gum resin obtained (150 g) from the root or rhizome of *Ferula assa-foetlda* L. was submerged in diethyl ether at room temperature for 28 days. After the stipulated period, the solvent was removed from the extract under reduced pressure. The resultant gummy mass was stirred with *n*-hexane and ethyl acetate, sequentially. The concentrated ethyl acetate fraction (18 g) was chromatographed over silica gel and eluted with *n*-hexane/ethyl acetate mixtures by increasing the polarity gradually. The *n*-hexane/ethyl acetate (25:75) mixture afforded a white solid, which on repeated recrystallization over methanol—*n*-hexane gave white needle shaped crystals (33 mg) of asimafoetidnol (**1**).

Asimafoetidnol (**1**)

(0.033 g, 0.18%)
MP: 143ºC.
$[\alpha]^{25}_D$: +17.7 (*c* 0.03, $CHCl_3$).
Rf: 0.56 ($CHCl_3$-MeOH, 4:1).
IR (KBr): 3420,1705,1570,1400, 1380 cm^{-1}.
UV/Vis λ_{max} (MeOH) nm (log ε): 215 (3.64), 324 (3.86).
^1H NMR (500 MHz, $CDCl_3$): Table 1.
^{13}C NMR (125 MHz $CDCl_3$): Table 1.
HREIMS: *m/z* $[M+Na]^+$ calcd for $C_{24}H_{32}O_5Na$: *m/z* 423.511; found: *m/z* 423.492.
Anal. Calcd for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found C, 71.89; H, 7.97.

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References

[1] Abd El-Razek MH, Ohta S, Ahmed AA, Hirata T. (**2001**) Sesquiterpene coumarins from the roots of *Ferula assafoetida*. *Phytochemistry*, ***58***, 1289-1295.
[2] Boulas L. (**1983**) *Medicinal Plants of North Africa.* Reference Publication, Algoma, ML, Canada. 183.
[3] Chatterjee A, Pakrashi SC. (**1999**) *The Treatise of Indian Medicinal Plants.* Vol. ***4***, National Institute of Science Communication, New Delhi, India. 41.
[4] Eigner D, Scholz D. (**1990**) The magic book of *Gyani Dolma*. *Pharmazie in unserer Zeit*, ***19***, 141-152.
[5] Kojimoto T, Yahiro K, Nohara T. (**1989**) Sesquiterpenoid and disulfide derivatives from *Ferula assa-foetida*. *Phytochemistry*, ***28***, 1761-1763.
[6] Lee J-H, Choi S, Lee Y, Lee H-J, Kim K-H, Ahn K-S, Bae H, Lee H-J, Lee E-O, Ahn K-S, Ryu SY, Lue J, Kim S-H. (**2010**) Herbal compound farnesiferol C exerts antiangiogenic and antitumor activity and targets multiple aspects of VEGFR1 (Flt1) or VEGFR2 (Flk1) signaling cascades. *Molecular Cancer Therapeutics*, ***9***, 389-399.
[7] Dehpour AA, Ebrahimzadeh MA, Fazel NS, Mohammad NS. (**2009**) Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas y Aceites (Sevilla, Spain)*, ***60***, 405-412.
[8] Nassar MI, Abu-Mustafa EA, Ahmed AA. (**1995**) Sesquiterpene coumarins from *Ferula assafoetida* L. *Pharmazie*, ***50***, 766-767.

[9] Appendino G, Tagliapietra S, Nano GM, Jakupovic J. (**1994**) Sesquiterpene coumarin ethers from asafetida. *Phytochemistry*, ***35***, 183-186.
[10] Bandyopadhyay D, Basak B, Chatterjee A, Lai TK, Banerji A, Banerji J, Neuman A, Prange T. (**2006**) Saradaferin, a new sesquiterpenoid coumarin from *Ferula assafoetida*. *Natural Product Research, Part A: Structure and Synthesis*, ***20***, 961-965.
[11] Banerji A, Mallick B, Chatterjee A, Budzikiewiez H, Breuer M. (**1988**) Assafoetidin and ferocolicin, two sesquiterpenoid coumarins from *Ferula assafoetida* Regel. *Tetrahedron Letters*, ***29***, 1557-1560.
[12] Ghosh A, Banerji A, Mandal S, Banerji J. (**2009**) A new sesquiterpenoid coumarin from *Ferula assafoetida*. *Natural Product Communications*, ***4***, 1023-1024.
[13] Reddy P, Urban S. (**2008**) Linear and cyclic C18 terpenoids from the southern Australian marine brown alga *Cystophora moniliformis*. *Journal of Natural Products*, ***71***, 1441-1446.
[14] Kasparek S. (**1980**) Chemistry of tocopherols tocotrienols. In *Vitamin E: A Comprehensive Treatise*. Chapter 2, Machlin LJ (Ed.). Marcel Dekkar, New York. 7-65.
[15] Wehrli FW, Nishida T. (**1979**) The use of carbon-13 nuclear magnetic resonance spectroscopy in natural products chemistry. *Fortschritte der Chemie Organischer Naturstoffe*, ***36***, 1-229.
[16] Wu H, Nakamura H, Kobayashi J, Kobayashi, M, Ohizumi Y, Hirata Y. (**1986**) Physiologically active marine natural products from Porifera. XII. Structures of agelasines, diterpenes having a 9-methyladeninium chromophore isolated from the Okinawan marine sponge *Agelas nakamurai* Hoshino. *Bulletin of the Chemical Society of Japan*, ***59***, 2495-2504.