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Research Article

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Resolving structural ambiguity of Oroxylin synthesized by different approaches

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ABSTRACT

Oroxylin A (OA) is an O-methylated flavone, a chemical compound found in the medicinal plant Scutellaria baicalensis and the Oroxylum indicum tree. Limited reports are available for the synthesis of Oroxylin A. During synthesis of OA by known methods, ambiguity observed in chromatographic analysis. To resolve this vagueness and investigate the correct structure, OA is prepared using two different methods followed by spectroscopic and spectrometric analysis. The obtained information from LC-MS/MS[2] and 2D NMR revealed the difference in the reported structure is due to positional isomers. Principally reported chemical entity does not match the claimed structure when synthesized by reported method, consequently misleading. Hence, the correct structures were established.

Keywords: Flavonoid; Oroxylin A; Neglectein; De-protection; 2D NMR.

INTRODUCTION

Oroxylin A (5, 7-dihydroxy-6-methoxy-2-phenyl-4H-chromen-4-one) is an O-methylated flavone, a chemical compound that found in the medicinal plant Scutellaria baicalensis [1] and the Oroxylum indicum tree [2]. It is also found in the extract of *colebrookea oppositifolia* [3] and *gomphrena martiana*[4] has medicinal value whose anti gastric ulcer and anti-oxidant properties are reported [5]. Baicalein an intermediate of Oroxylin A (OA) has reported activity for its derivatives [6-8]. 7-O Acyl and glycoside derivative of OA has potent anti bacterial and anti gastric ulcer activity [9]

Various analytical and preparative methods are reported in the literature for isolation and purification of baicalein, wogonin and oroxylin A from the medicinal plant Scutellaria baicalensis by high-speed counter-current chromatography were reported. Reports are available on Identification and quantification of baicalein, wogonin, oroxylin A and their major glucuronide conjugated metabolites in rat plasma after oral administration of Radix scutellariae product [10].

Two approaches are reported in literature for synthesis of OA [11-16]. First approach involves Baicalein as starting materials. This approach engrosses selective benzylation at C_7 position and further methylation followed by deprotection of benzyl group gives OA, However resulted into poor yield. In second approach 5, *6, 7-trimethoxy-2-phenyl-4H-chromen-4-one* demethylated using acetic acid HBr gives OA. But product obtained by this scheme did

not match with chromatographic retention times with respect to former scheme. Hence there is need to investigate correct structure of Oroxylin A.

EXPERIMENTAL SECTION

Samples were analyzed on Alliance 2690 HPLC (Waters, Milford, MA, USA) system equipped with 2487 UV detector. A Kromasil C18 column (250 mm x 4.6 mm i.d. 5 µm akzo nobel, Bohus, Sweden) was used for chromatographic separation. The mobile phase consisting of A: water and B: acetonitrile, with timed gradient programme T (min)/ B (%): 0/50, 5/50, 15/20, 25/20, 30/50, 35/50 with flow rate of 1.0 ml per minute was used. The injection volume was 20µL and the detector wavelength was fixed at 232 nm.

The LC-ESI/MS and MS/MS analysis was carried out on LCQ-Advantage (Thermo Finnigan, San Jose, CA, USA) ion trap mass spectrometer. The LC part was consisted of an Agilent 1100 series quaternary gradient pump with a degasser and auto sampler. The chromatographic condition described in section 2.2 has been used for the analysis. The source voltage was kept at 3.0 kV and capillary temperature at 250 °C. Nitrogen was used as both sheath and auxiliary gas. Mass range was kept at m/z 50-500. MS/MS studies were carried out by maintaining normalized collision energy at 30% with the mass range m/z 50-500.

The ^{1}H, ^{13}C measurements of synthetically prepared impurity sample were recorded on a AVANCE 400 (Bruker, Fallanden, Switzerland) instrument at 300 K. Nuclear Overhauser effect spectroscopy (2D NOESY) was also performed using the same instrument. The ^{1}H and ^{13}C chemical shift values were reported on the δ scale in ppm relative to $CDCl_3$ (7.28 ppm) and (77.0 ppm) respectively.

Synthesis of OA as per scheme (I)

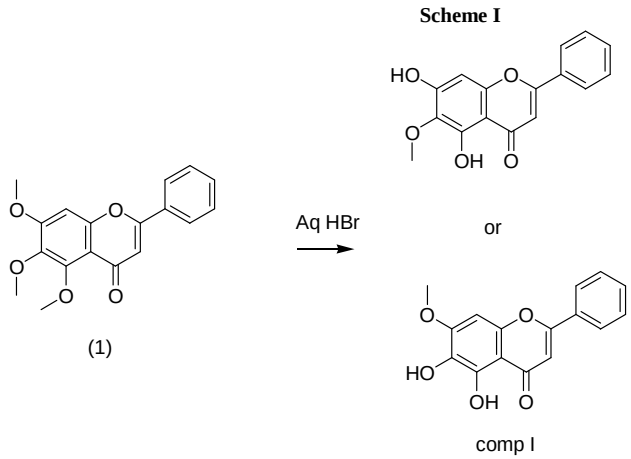
A solution of 5,6,7 trimethoxy flavones (1**)** (2 g, 6.4 mmol) in 47% HBr (50 ml) and glacial acetic acid (100 ml) was refluxed for 5-6 h, and then carefully poured onto crushed ice. The resulting yellow precipitate was filtered and collected. Recrystallization from ethanol afforded 1.5 g.

Synthesis of OA as per scheme (II)

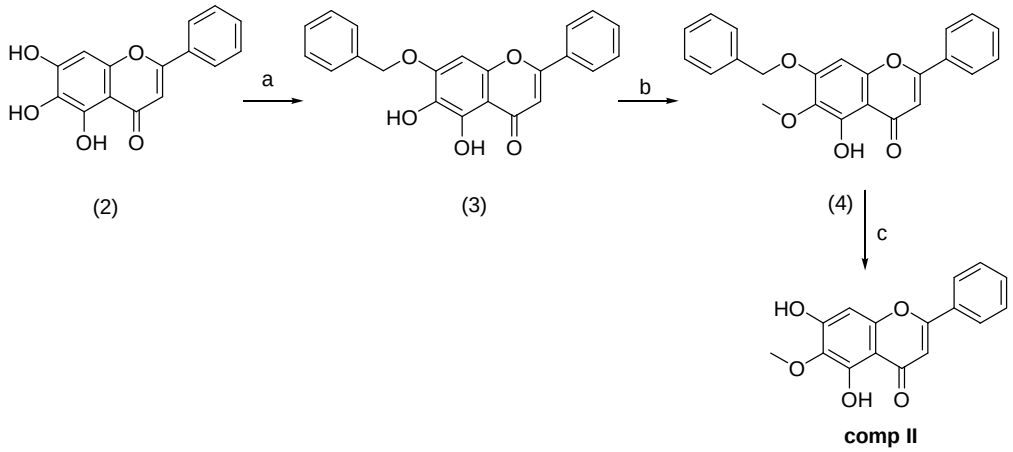
Preparation of 7-Benzyloxy-5,6-dihydoxy flavone

Baicalein (3 g) was dissolved in anhydrous acetone (300 ml) and treated with sodium iodide (1.5 g), sodium bicarbonate and benzyl chloride (1.26 ml).the mixture was refluxed for 24 hr, filtered hot. The inorganic salts washed with hot acetone and the solvent distilled off from the filtrate. Crystallized from alcohol. (Yield = 1.2 g)

Scheme I



Scheme II



Reaction condition: a) Benzyl chloride, sodium bicarbonate, Actone; b) Dimethyl sulphate, Potassium carbonate; c) Conc HCl.

7-Benzyloxy-5-hydroxy-6-methoxy flavones

The above benzyl ether (1 g) in anhydrous acetone (200 ml) was refluxed with anhydrous potassium carbonate (5 g) and dimethyl sulphate (0.22) for 6 hr. and product worked up as given in above experiments. Crystallized from alcohol yield =0.6 g.

Oroxylin-A

The *7-Benzyloxy-5-hydroxy-6-methoxy flavones* were dissolved in glacial acetic acid (30 ml) concentrated HCl (4 ml) added and the mixture heated on a boiling water bath for 2 hrs. Acetic acid and benzyl chloride where removed under reduced pressure and residue treated with crushed ice (100 g) the solid product was filtered and washed with water. Product crystallized with alcohol yield =0.2 g

RESULTS AND DISCUSSION

Detection of OA by HPLC and LC/MS

HPLC of compound synthesized by scheme (I) was designated as comp-I showed the peak at retention time 12.87 min. while compound synthesized by scheme (II), designated as comp-II, eluted at retention time 16.62 min. Which corroborate that comp-I and comp-II are structurally different. Both the samples obtained from scheme (I) and scheme (II) were further used for DI-Mass spectrometry analysis. Mass analysis showed m/z 285 for both comp-I and comp-II (Fig. 3a, 4a), which suggested both comp-I & II to be isomer.

Structural elucidation of OA by MS^2 and NMR spectroscopy

Being even mass for both comp-I & II in DI-Mass, it was postulated that none of the compounds contain any nitrogen atom in the structure. Further MS^2 study of comp-I and comp-II was undertaken. Interestingly both compounds gave similar MS^2 fragment ions at m/z 270 and 285 (Fig. 3b, 4b). ^1H NMR of comp-I showed singlet at 8.8 and 12.5 ppm while comp-II showed singlet at 10.8 and 12.9 ppm. All these protons correspond to one proton each. These protons were exchangeable with D_2O which confirms the presence of labile hydroxyl proton. Being downfield, comp-I peak at 12.5 ppm and comp-II peak at 12.9 is basis for them to be involved in hydrogen bonding (Fig 1). ^1H, ^{13}C and DEPT NMR spectra confirmed presence of methoxy group in both compounds, but variation in their position defined them to be accountable for isomerism.

2D NOESY experiment of comp-I showed cross peak signal for spatial correlation of methoxy proton and H6 proton, confirming the vicinity while both the hydroxy groups are in vicinity with ortho to each other. However, methoxy proton of comp-II did not show any such spatial correlation with aromatic proton (Fig 2). In combination with 2D NOESY other 2D NMR ^1H-^1H (DQF) and ^1H-^{13}C (HMBC and HMQC) assigned all the proton and carbon of comp-I and comp-II precisely. The collective data confirmed comp-I to be Negletein (5,6-dihydroxy-7-methoxy-2-phenyl-4H-chromen-4-one) and comp-II is genuine OA (5,7-dihydroxy-6-methoxy-2-phenyl-4H-chromen-4-one).

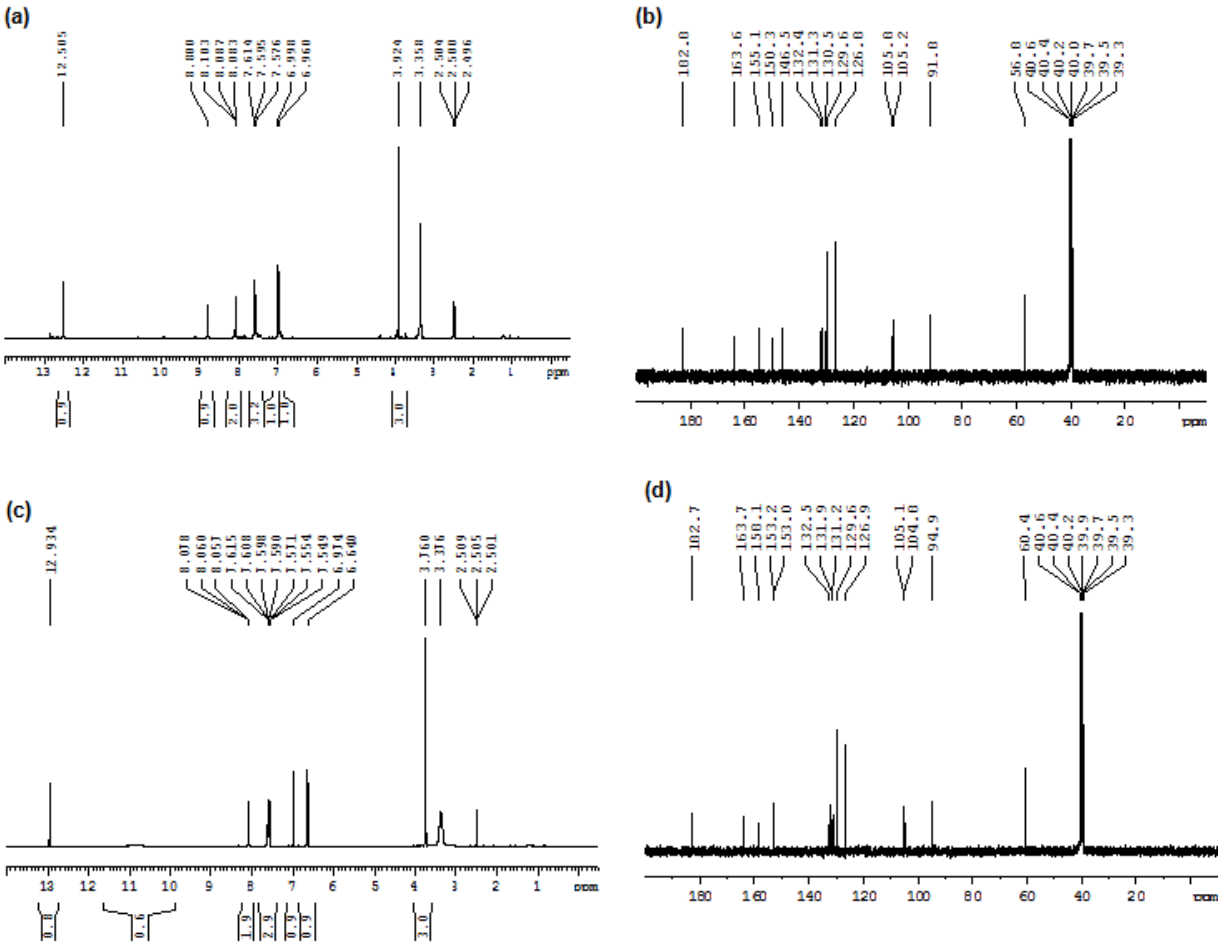


Fig. 1 1H, 13C NMR of product obtained by scheme-I; (a) and (b) and scheme-II (c) and (d)

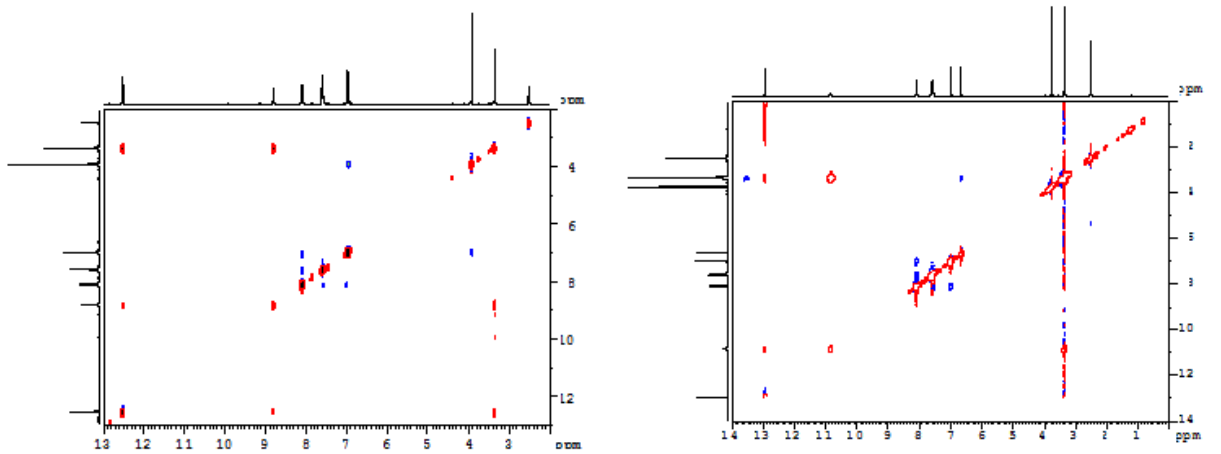


Fig.2 2D NOESY NMR of comp I and comp II

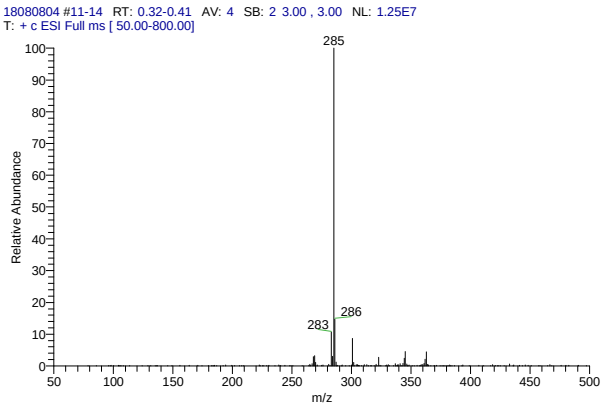


Fig. 3a DI-Mass spectrometry analysis of comp I

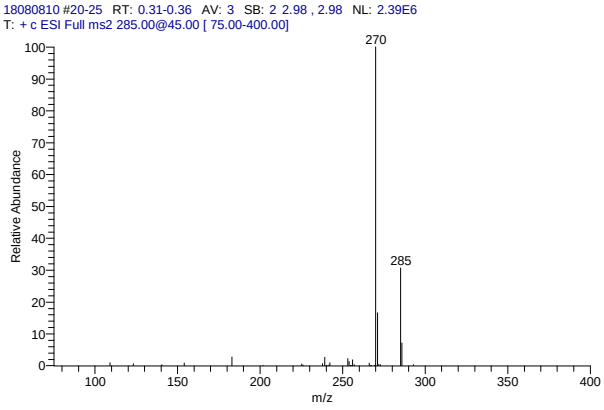


Fig. 3b ms2 study of comp-I

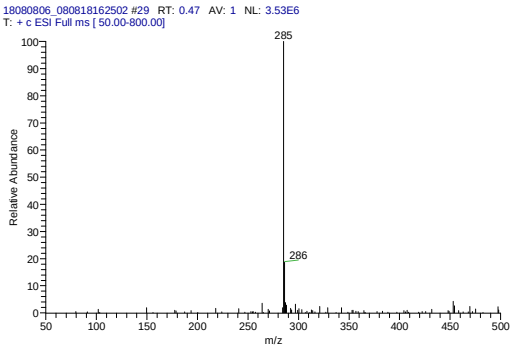


Fig. 4a DI-Mass spectrometry analysis of comp II

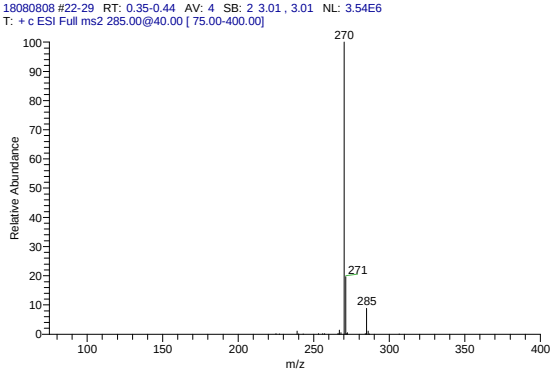
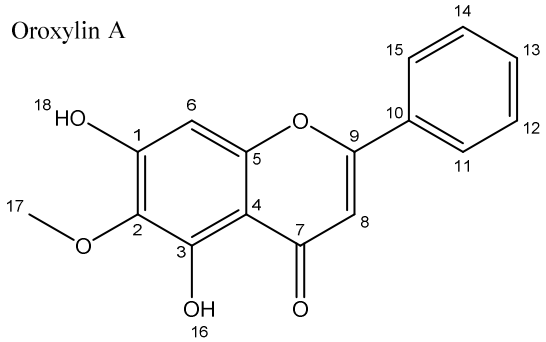


Fig. 4b ms2 study of comp-II

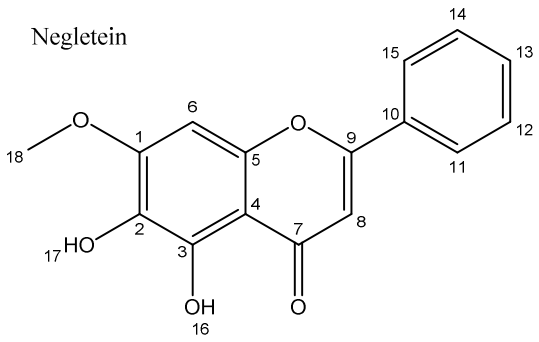
Table 1 ^{1}H and ^{13}C NMR assignment for comp-II (Oroxylin A) and comp-I (Neglectein) obtained different routes

Oroxylin A



Position	Integration	^{1}H (chemical shift in ppm)	Multiplicity, (J,Hz)[a]	^{13}C (chemical shift in ppm)
1	-	-	-	155.1
2	-	-	-	132.4
3	-	-	-	150.3
4	-	-	-	105.8
5	-	-	-	146.5
6	1H	6.99	s	91.8
7	-	-	-	182.8
8	-	7.59	s	105.2
9	-	-	-	163.6
10	-	-	-	131.3
11,15	2H	8.07-8.10	m	126.8
12,14	2H	7.54-7.60	m	129.6
13	1H	7.54-7.60	m	130.5
16	1H	12.50	s	-
17	3H	3.92	s	56.8
18	1H	8.80	s	-

Negletein



Position	Integration	^{1}H (chemical shift in ppm)	Multiplicity, (J,Hz)[a]	^{13}C (chemical shift in ppm)
1	-	-	-	158.1
2	-	-	-	131.9
3	-	-	-	153.2
4	-	-	-	105.1
5	-	-	-	153.0
6	1H	6.64	s	94.9
7	-	-	-	182.7
8	-	6.97	s	104.8
9	-	-	-	163.7
10	-	-	-	132.5
11,15	2H	8.05-8.07	m	129.6
12,14	2H	7.54-7.61	m	126.9
13	1H	7.54-7.61	m	131.2
16	1H	12.93	s	-
17	1H	10.80	s	-
18	3H	3.76	s	60.4

[a] ^{1}H-^{1}H coupling constants.

CONCLUSION

The present research objective was to provide un-ambiguous synthesis and structural characterization of Oroxylin A. When two prominent schemes were attempted to reproduce Oroxylin A, they gave different product than claimed. One of the scheme [Scheme II] resulted into Oroxylin A but with poor yield. In another scheme [Scheme I] the obtained product was Negletein, an isomer of OA. Present article is solving the lacuna of structural profiling of OA & Negletein which will be helpful for further researchers.

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