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An unambiguous and practical synthesis of Oroxylin A: a commonly misidentified flavone

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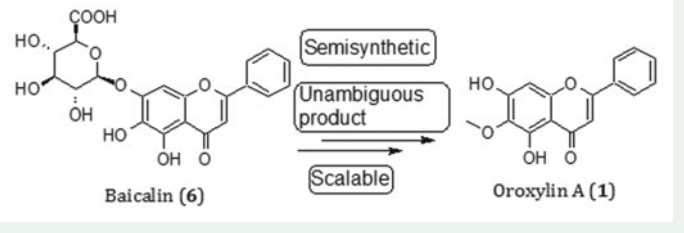
ABSTRACT

Oroxylin A, a major flavonoid in the extracts of *Oroxylum indicum* as well as *Scutellaria baicalensis* possesses useful medicinal properties. Many of the published routes claiming the synthesis of Oroxylin A (**1**) have in fact led to a regioisomer Negletein that was misinterpreted as Oroxylin A. In the present work, we describe a novel, straight-forward and scalable semi-synthetic approach for rapid access to the title compound, the structure of which is unambiguously secured by NMR and X-ray crystallographic analysis of a derivative. This work also encompasses the synthesis of a glycosylated derivative of Oroxylin A *viz* OAGME (**2**), which has marked pharmacological importance.



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1. Introduction

Oroxylin A (**1**) (5,7-dihydroxy-6-methoxy flavone, Figure 1) a mono-*O*-methylated flavone found in the traditional medicinal plants of India and China such as *Oroxylum indicum* (Deka et al. 2013; Dinda et al. 2015) and *Scutellaria baicalensis* (Li and Chen 2005) respectively, exhibits pleiotropic pharmacological activities such as anti-cancer, anti-oxidant, and anti-inflammatory, obesity and memory enhancement, creating an intense interest on this molecule (Kim et al. 2007; Komulainen et al. 2008; Lu et al. 2016; Song et al. 2012; Sun et al. 2009; Yoon et al. 2008). The low concentration of this compound in these traditional medicinal plants has led to a resurgence of activity in synthetic as well as bio-transformative approaches to access this molecule. Several

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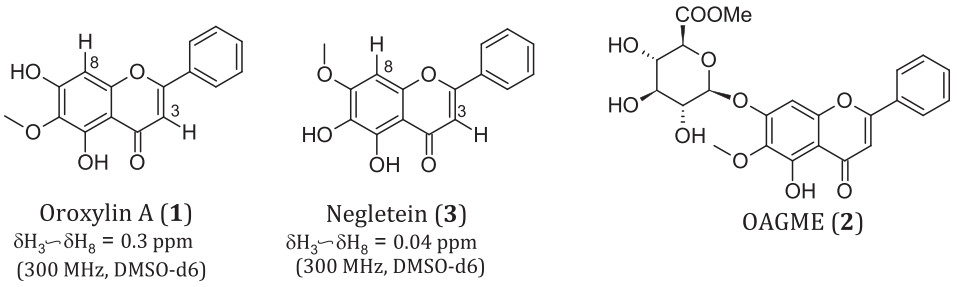


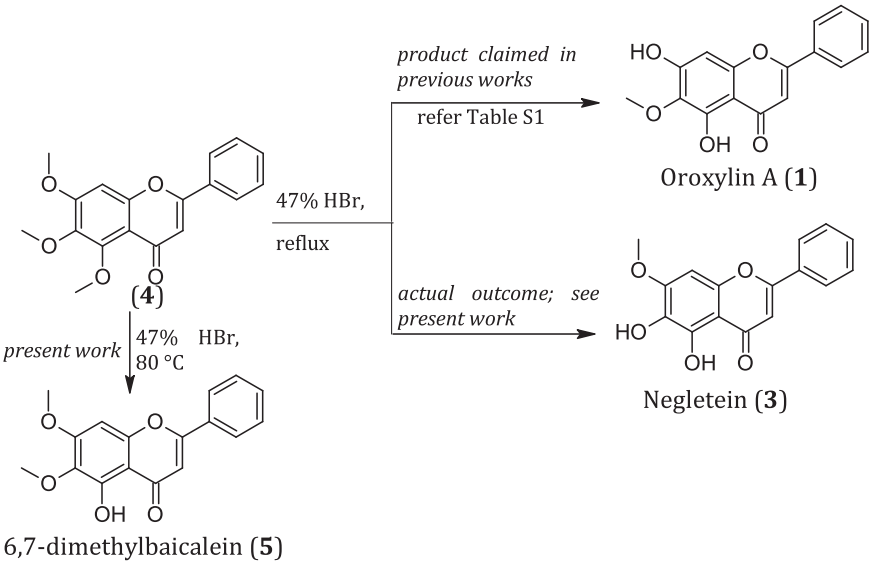
Figure 1. Structures of Oroxylin A (**1**) and OAGME (**2**) and Negletein (**3**).

impugned analogs of Oroxylin A have been synthesised and tested in anticipation of enhanced activity (Pham et al. 2012). Oroxyloside methyl ester (Oroxylin-A glucuronide methyl ester, OAGME (**2**, Figure 1) is another micro-component in *Oroxylum indicum* that has excellent anti-ulcer and alpha-glucosidase inhibition properties (Rao et al. 2017). Its ability to protect against aspirin, as well as ethanol-induced gastric ulcers has been proven superior to the marketed drugs such as Ranitidine and Omeprazole (Rao et al. 2007; Rao et al. 2010) garnering further interest on OAGME (**2**).

2. Results and discussion

Several published reports (Huang et al. 2003; Shaw et al. 2004) claiming the synthesis of Oroxylin A (**1**) have often actually ended up with its regioisomer- Negletein (**3**). Failure to recognise this error is further accentuated by the fact that the so-called synthesis and biological characterisation of *putative* Oroxylin A analogs are actually Negletein analogs (Pham et al. 2012). Misidentification of Negletein (**3**) as Oroxylin A (**1**) has also occurred in reports on isolation from plant sources such as *Scutellaria oblanga*. In particular, in a report by Rajendran et al. (2016), the authors actually isolated Oroxylin A (**1**), but misidentified it as Negletein (**3**). In a microbial biotransformation study of Baicalin (**6**) and Baicalein (**8**) to other flavones reported by Kostrzewa-Susłow et al. (2007), the authors profess to have obtained Oroxylin A (**1**) whereas their NMR spectral data is in excellent accordance with Negletein (**3**) and not with Oroxylin A (**1**). In yet another work claiming to convert Baicalein (**8**) to monomethylated Baicalein derivatives using *Escherichia coli*, the authors mis-identified the products by interchanging the NMR data of Oroxylin A (**1**) and Negletein (**3**) (Han et al. 2016) (Supplementary information Table S1).

Careful ^{1}H NMR interpretation is necessary to distinguish Negletein (**3**) and Oroxylin A (**1**). Specifically, H3 and H8 protons appear at 6.97 and 6.63 ppm (DMSO-d6) respectively, for Oroxylin A (**1**), (Fuentes et al. 2015; Joshi and Gawad 1977; Kim et al. 2007) whereas for Negletein (**3**), H3 and H8 NMR signals (DMSO-d6) are very close and appear at 6.96 and 6.92 ppm (Figure 1; He et al. 2016) respectively. Thus, the outcome of Huang's work must be Negletein (**3**) rather than impugned Oroxylin A (**1**) as interpreted in the report (Huang et al. 2003; Shaw et al. 2004). Since Pham et al. (2012) followed Huang's work for the preparation of Oroxylin A (**1**) and related analogs with no spectral data in the paper, it is presumable that the outcome of Pham's work might also have been Negletein analogs rather than Oroxylin A analogs. Similar



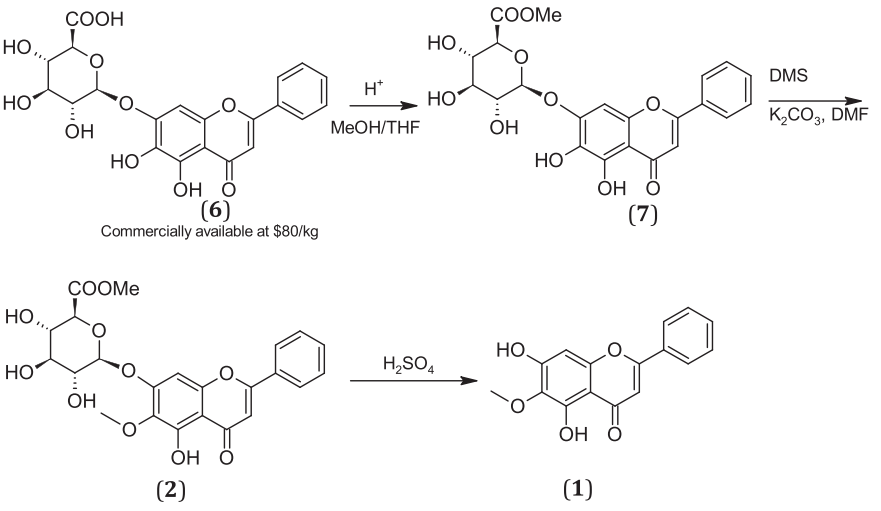
Scheme 1. Stepwise *O*-demethylation of 5,6,7-trimethylbaicalein (**4**).

chemistry was adopted by Tan and co-workers (2015) and once again, the ^{1}H NMR data furnished for Oroxylin A (**1**) is not in agreement with the authentic one.

Many of the erroneous claims (Huang et al. 2003; Pham et al. 2012; Shaw et al. 2004; Tan et al. 2015) on the synthesis of Oroxylin A (**1**) are predicated on the unfounded expectation that selective 5,7-*O*-demethylations would occur by the treatment of 5,6,7-trimethylbaicalein (**4**) with refluxing 47% HBr-acetic acid (Scheme 1). Instead, under these conditions 5,6-*O*-demethylations occur in a selective and stepwise fashion (5-*O*-demethylation occurs first followed by 6-*O*-demethylation) to produce Negletein (**3**). The 5-*O*-demethylated product (**5**) was isolated and characterised fully by us (Scheme 1, see supplementary file).

In a control experiment, when 5,6,7-trimethylbaicalein (**4**) in 47% HBr/AcOH was carried out at 80 °C for 3 h, we could see only a selective mono-*O*-demethylation at 5th position yielding 6,7-dimethylbaicalein (**5**) as a predominant product (Scheme 1). The compound (**5**) was isolated and characterised by ^{1}H NMR where a characteristic singlet at 12.76 ppm (DMSO-d6) was seen for the 5-OH proton (see supplementary file for NMR data) and the ^{1}H NMR analysis matched with the reported data for the compound (Righi et al. 2010). When the reaction was run at a higher temp at 110 °C for 3 h, we could see formation of a polar spot in TLC ($CHCl_3$:MeOH-9:1) *via* bis-*O*-demethylation, giving rise to Negletein (**3**) (Scheme 1) (see supplementary file for NMR data). This is in agreement with the NMR data of Negletein (**3**) published by He et al. (2016). Similar step-wise demethylation was also reported by Righi et al. (2010) in accordance with our assignment of the structure.

While some syntheses of Oroxylin-A (**1**) are reported (Fujita et al. 2018; Gao et al. 2004; Panhekar et al. 2015; Qidong et al. 2009; Várady 1965), they are not practical because of their lengthy sequences involving protection/deprotection sequences. We found that Baicalin (**6**) is available in large quantities from a sustainable plant source (*Scutellaria baicalensis*) at very economical prices in kilogram quantities ($80/kg). This served as our



Scheme 2. Preparation of Oroxylin A (**1**) from Baicalin (**6**).

starting material. The first step involved acid catalysed esterification of the carboxy group of the sugar moiety with methanol. This showed a highly selective base catalysed methylation of the phenolic group at C-6 position without affecting the phenolic group at C-5 position. The latter phenolic group is more acidic but much less nucleophilic than the former. This approach incidentally yielded OAGME (**2**) in multi hundreds of gram scale. To the best of our knowledge, this is the first report on the synthesis of OAGME (**2**) (Scheme 2).

Finally, the sugar (glucuronide) part was cleaved from the aglycone using an acid mediated hydrolysis. Use of conc. HCl, dry HCl in THF or dry HCl in MeOH was not successful. These reactions were unfavourable due to one or more of the following reasons: low conversion, long reaction time, unidentified side products and formation of tar like base materials. Gratifyingly, a smooth reaction was observed when compound (**2**) was refluxed in dilute sulphuric acid (~30%) for 2 h. In this case, the colour of the reaction mixture did not turn dark and no formation of tar like material was observed. After the completion of reaction (checked using TLC), the reaction mixture was diluted with excess cold water, and the precipitated material was filtered and sucked dry to get crude sample of Oroxylin A. This was passed through a pad of silica gel to obtain analytically pure Oroxylin A (**1**) as a yellowish solid (70–75% yield).

The molecular integrity of the semi-synthetic Oroxylin A (**1**) prepared by the present method was confirmed by mass and NMR data which were both in agreement with the reported data, on Oroxylin A isolated from a natural source (Kim et al. 2007). Unfortunately, our attempts to prepare a X-ray analysis-suitable crystalline material of Oroxylin A (**1**) was not successful. Hence, to further substantiate the structure of the synthetic Oroxylin A prepared by the present method, it was mono-acetylated selectively at the 7-OH position under K_2CO_3/acetyl chloride/DMF conditions to obtain 7-*O*-acetyl Oroxylin A (**9**) (see supplementary file). This molecule was analysed by ^{1}H NMR and confirmed to be identical with the sample of 7-*O*-acetyl Oroxylin A prepared from natural Oroxylin A (see supplementary file). Further, the sample of 7-*O*-acetyl Oroxylin A (**9**) prepared from the synthetic Oroxylin A (**1**) was able to be crystallized; upon

conduction of X-ray crystallography, the structure of 7-*O*-acetyl Oroxylin A (**9**) was proven to be as previously expected.

Certain interesting features on the crystal structure of 7-*O*-acetyl Oroxylin A (**9**) were noted. The 6-OMe group and 7-acetyl groups in (**9**) were rotated perpendicular to the plane of the aromatic ring but away from each other. Interestingly, the unit cell contained molecular pairs of (**9**) in which the rotation of 6-OMe and 7-acetyl group in one pair is exactly opposite to the other pair (Supplementary information Figure S1; CCDC No. 1861116).

3. Experimental

3.1. General

Baicalin was purchased from Zhucheng Haotian Pharm Co. Ltd., China. All of the solvents and chemicals were of commercial grade and used as such. NMR was recorded on Varian 300 MHz instrument, and DMSO-d6 was used as the solvent unless mentioned otherwise. The chemical shifts are reported in δ units (ppm) relative to tetramethylsilane (TMS). Reaction progress was monitored by thin-layer chromatographic (TLC) analysis. TLC spots were visualised by UV light (254 nm) exposure. Flash chromatography was performed using 100–200 mesh silica gel.

3.2. Preparation of baicalin methyl ester (7)

Baicalin (**6**) (5.0 g, 11.2 mmol) was suspended in MeOH:THF (150 mL, 2:1) to which 2-3 drops of conc. H_2SO_4 was added. The reaction was stirred at 80 °C for 6 h. Progress of the reaction was followed by TLC. Upon completion of the reaction, solvent was evaporated, and the residue was triturated in hexane, filtered, and sucked dry to obtain baicalin methyl ester (**7**) (4.9 g, 95%). NMR: ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 8.11 (m, 2 H, Ar), 7.5 (m, 3 H, Ar), 7.0 (s, 1 H, H-8), 6.9 (s, 1 H, H-3), 5.25 (d, 1 H, sugar H-1), 4.25 (d, 1 H, sugar H-5), 3.65 (s, 3 H, OMe), 3.28–3.50 (m, 3 H, sugar H-2, H-3, H-4); ^{13}C NMR (75 MHz, DMSO-d6) δ ppm: 182.97, 169.65, 163.96, 151.66, 149.63, 147.24, 132.48, 131.25, 131.01, 129.59, 126.81, 106.57, 105.20, 100.20, 94.07, 75.70, 75.47, 73.18, 71.84, 52.47; mass: expected- 460.387 Da; observed- 461.107 $(M+H)^{+}$.

3.3. Preparation of OAGME (2)

Baicalin methyl ester (**7**) (4.9 g, 10.65 mmol) was dissolved in 35 mL of DMF to which K_2CO_3 (2.94 g, 2.0 eq) was added. It was cooled in an ice bath, dimethyl sulphate (1.5 mL, 1.5 eq) was added, and the reaction was allowed to stir over night at room temperature. After the completion of reaction (TLC), the mixture was poured, with stirring, into cold dilute HCl, and the precipitate was filtered, sucked dry to get OAGME (**2**) (4.6 g, 91%). NMR: ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 12.95 (1 H, s, 5-OH), 8.19 (m, 2 H, Ar), 7.73 (m, 3 H, Ar), 7.15 (s, 1 H, H-8), 7.07 (s, 1 H, H-3), 5.25 (d, 1 H, sugar H-1), 5.30–5.67 (m, 3 H, Sugar-OH protons), 4.27 (d, 1 H, sugar H-5), 3.80 (s, 3 H, Ar-OMe), 3.70 (s, 3 H, OMe), 3.34-3.48 (m, 3 H, sugar H-2, H-3, H-4); ^{13}C NMR (75 MHz, DMSO-d6) δ ppm: 182.96, 169.63, 164.19, 156.62, 153.03, 152.74, 133.02, 132.65,

131.08, 129.62, 126.89, 106.60, 105.47, 99.74, 94.45, 76.09, 75.70, 73.21, 71.75, 60.77, 52.45; mass: expected - 474.414 Da; observed - 475.123 $(M+H)^+$; m.p. 201–203 °C (lit: 201 °C).

3.4. Preparation of oroxylin A (1)

OAGME (**2**) (2.0 g, 4.21 mmol) was suspended in 10 mL water to which 5 mL conc. H_2SO_4 was added drop-wise. The resulting exothermic reaction mixture was heated to 100 °C for about 3 h by which time the reaction was complete as judged by TLC. Excess water was added to the reaction mixture and the product was filtered under suction. It was dried, and the crude was passed through a short column of silica gel using MDC as eluent to obtain Oroxylin A (**1**) as yellowish solid (0.86 g, 73%). NMR: ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 12.91 (s, 1 H, 5-OH), 10.79 (s, 1 H, 7-OH), 8.05 (m, 2 H, Ar), 7.58 (m, 3 H, Ar), 6.96 (s, 1 H, H-3), 6.62 (s, 1 H, H-8), 3.74 (s, 3 H, OMe); ^{13}C NMR (75 MHz, DMSO-d6) δ ppm: 182.70, 163.64, 158.00, 153.17, 152.97, 132.44, 131.89, 131.14,129.56, 126.81, 105.07, 104.79,94.83, 60.41; mass: expected - 284.263 Da; observed - 285.075 $(M+H)^+$; m.p. 197–198 °C (lit: 198–198.5 °C).

4. Conclusions

A straight-forward, scalable and rapid synthesis of pharmacologically useful Oroxylin A (**1**) and OAGME (**2**) is reported. Further, the process described here abides by several of the green chemistry criteria such as high yielding individual steps, natural raw materials, safe reaction conditions and non-toxic useful byproducts (e.g. Glucuronic acid). Several earlier works purportedly reporting the synthesis of Oroxylin A (**1**) or through isolation from natural resource or through biotransformation are corrected by our unambiguous synthesis and X-ray studies.

Disclosure statement

Portions of the results in this manuscript form part of a patent application by the authors.

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