A new β-hydroxydihydrochalcone from Tephrosia uniflora, and the revision of three β-hydroxydihydrochalcones to flavanones

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The CH2Cl2/MeOH (1:1) extract of the stems of Tephrosia uniflora yielded the new β-hydroxydihydrochalcone (S)-elatadihydrochalcone-2′-methyl ether (1) along with the three known compounds elongatin (2), (S)-elatadihydrochalcone (3), and tephrosin (4). The structures were elucidated by NMR spectroscopic and mass spectrometric data analyses. Elongatin (2) showed moderate antibacterial activity (EC50 of 25.3 μM and EC90 of 32.8 μM) against the Gram-positive bacterium Bacillus subtilis, and comparable toxicity against the MCF-7 human breast cancer cell line (EC50 of 41.3 μM). Based on the comparison of literature and predicted NMR data with that obtained experimentally, we propose the revision of the structures of three β-hydroxydihydrochalcones to flavanones.

1. Introduction


2. Results and discussion

Chromatographic separation of the CH2Cl2/MeOH (1:1) extract of the stems of Tephrosia uniflora led to the isolation of the new β-hydroxydihydrochalcone (S)-elatadihydrochalcone-2′-methyl ether (1) along with the known compounds elongatin (2) [6,16], (S)-elatadihydrochalcone (3) [17], and tephrosin (4) [18] (Fig. 1). The compounds were identified by NMR spectroscopic and mass spectrometric data analyses, and by comparison of their spectroscopic data with that in the literature.

Compound 1 was isolated as a colourless solid, and was assigned the molecular formula C22H24O5 based on ESIMS ([M + H]+ m/z 369.3) and NMR data (Table 1) analyses. The UV (λmax = 214, 263, 310 nm), 1H NMR (δH 3.25 (dd, J = 17.4, 2.8 Hz) and 3.15 (dd, J = 17.4, 9.5 Hz) for CH2-α, and 5.28 (dd, J = 9.5, 2.8 Hz), for H-β) and 13C NMR (δc 204.4 (C=O), 53.7 (C-α), and 70.6 (C-β)) data suggested the compound to have a β-hydroxydihydrochalcone skeleton [17]. The 1H NMR signals at δH 7.40 (H-2/6), 7.34 (H-3/5), and 7.26 (H-4) with the corresponding constituents.

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carbon resonances at $\delta_C$ 126.0 (C-2/6), 128.5 (C-3/5), 127.6 (C-4) did not provide strong, 6.46), and 6.19) did not provide strong, and the NOESY cross-peaks of 2 confirmed these assignments by the HMBC correlations of H-5' ($\delta_H$ 6.19) to C-3 ($\delta_C$ 156.6), and of H-3' ($\delta_H$ 5.54) to C-3 ($\delta_C$ 108.3) allowed the placement of the 2,2-dimethylpyran residue at C-3/C-4', with the oxygen attached to C-4' ($\delta_C$ 156.6). The HMBC correlations of 2'-O-Me ($\delta_H$ 3.75) to C-2' ($\delta_C$ 154.6), and 6'-OMe ($\delta_H$ 3.76) to C-6' ($\delta_C$ 157.9), and the NOESY cross-peaks of 2'-O-Me ($\delta_H$ 3.75) to H-4' ($\delta_H$ 6.46), and 6'-OMe ($\delta_H$ 3.76) to H-5' ($\delta_H$ 6.19), allowed the placement of two methoxy groups at C-2' ($\delta_C$ 154.6) and C-6' ($\delta_C$ 157.9). As H-5' ($\delta_H$ 6.19) did not provide strong, 3J cross peaks to any oxygenated aromatic carbons, these groups have to be two- and four-bonds away from it. The negative Cotton effect at 275 nm in the ECD spectrum of 1 suggested it to be S-configured at its $\beta$ carbon, similar to elatadihydrochalcone (3), a co-metabolite that has earlier been reported from T. elata with a negative Cotton effect at 290 nm [17], and to a synthetic $\beta$-hydroxydihydrochalcone that has been reported by Ferreira and co-workers to show a negative Cotton effect at 240 nm [21]. Due to the low concentration of the studied sample, the broad and less intense Cotton effect >300 nm reported for some related compounds was not detected. Based on the above spectroscopic evidence, the new compound 1 was characterized as (S)-2',6'-dimethoxy-3'/4'(2',2'-dimethyl-2H-chromen-6-yl)-$\beta$-hydroxydihydrochalcone, and was given the trivial name elatadihydrochalcone-2'-methyl ether.

$\beta$-Hydroxydihydrochalcones are a rare subclass of chalcones [17]. Some compounds that have originally been reported as $\beta$-hydroxydihydrochalcones were later revised to flavanones [17]. A review of the recent literature suggests that further flavanones may have mistakenly been reported as $\beta$-hydroxydihydrochalcones [22–24]. To clarify the spectroscopic difference between these two compound classes, herein we compare the $^1$H and $^{13}$C NMR data of the $\beta$-hydroxydihydrochalcones (S)-elatadihydrochalcone-2'-methyl ether (1), (S)-elatadihydrochalcone (3) [17], ziganin (5) [23], 3-(S)-hydroxy-3-phenyl-1-(2',4',6'-trihydroxyphenyl)propan-1-one (6) [22], and balanochalcone (7) [24] with those of the corresponding flavanones (5a, 6a and 7a). We further give the predicted chemical shifts of the $\beta$-hydroxydihydrochalcone and flavanone skeletons, and note that the chemical shifts of the $\alpha$- and $\beta$-protons (Table 2), and of the corresponding carbon atoms (Table 3) provide a helpful tool for differentiation between these compound classes.

In $\beta$-hydroxydihydrochalcone 3 (Table 2), H-$\beta$ resonates at $\delta_H$ 5.28, and CH$_2$-$\alpha$ at $\delta_H$ 3.45 and $\delta_H$ 3.34, whereas the H-2 of flavanone 5a resonates at $\delta_H$ 5.40, its CH$_2$-$\alpha$ at $\delta_H$ 3.14 and its CH$_2$-$\beta$ at $\delta_H$ 3.17. A systematic comparison of the chemical shifts (Table 2) suggest that the values reported for the H-$\beta$ and H-2a of 5 [23], 6 [22] and 7 [24] that were originally suggested to be $\beta$-hydroxydihydrochalcones better fit to the H-2 and CH$_2$-$\beta$-3 chemical shifts of flavanones 5a [24], 6a [26] and 7a [27], respectively. The C-$\alpha$, C-$\beta$ and C=O of $\beta$-hydroxydihydrochalcone 3 resonate at $\delta_C$ 52.7, 70.2 and 204.2, respectively (Table 3), whereas the C-2, C-3 and C-4 of flavone 5a resonate at $\delta_C$ 42.9, 79.3 and 196.5, respectively. The chemical shifts of the corresponding carbons of compounds 5, 6 and 7 resemble those of C-2, C-3 and C=O of flavanones 5a, 6a and 7a rather than the chemical shifts of C-$\alpha$-C-$\beta$ and C=O of $\beta$-hydroxydihydrochalcones. Based on this spectroscopic evidence (Tables 2 and 3), we propose the revision of 5, 6 and 7 from $\beta$-hydroxydihydrochalcones to flavanones 5a, 6a and 7a, respectively (Fig. 1).

We also note that the oxygenation pattern of ring A in compound 7 [24] and (ring B in compound 7a) do not correspond to the reported NMR data (Table 2 and Table 3). Ring A in 7 (ring B in 7a) is symmetrical and hence C-2-C-6 (C-2' and C-6') in compound 7a are chemically equivalent and should resonate at the same frequency. In contrast, different chemical shifts have been reported [24] for these positions (Tables 2 and 3) suggesting that the originally assigned substitution pattern of this ring in this compound is erroneous. The reported NMR data is not in agreement with the above spectroscopic evidence.

Fig. 1. The structures of the compounds isolated from the stem of *Tephrosia uniflora*, and of the compounds revised herein.
with oxygenation at C-2' and C-6' but that of C-2' and C-5'. As we only have access to pdf copies of the NMR data of this compound, we recommend the re-analysis of 7a to ensure the accuracy of our suggestion for correction.

Having a β-hydroxydihydrochalcone skeleton as a working structure, the interpretation of the mass spectrometric data may easily lead to the misassignment of flavanones to β-hydroxydihydrochalcones, despite the molecular weight of flavanones being 18 amu lower. For compound 5, the HRESIMS data showing the m/z 469.26742 signal corresponding to the protonated molecular ion has allegedly been used to establish its molecular formula; however, the spectrum has not been attached as supplementary material [23] and hence this information cannot be confirmed. For compound 6 [22], the HRESIMS signal m/z 297.07603 [M + Na]⁺ was used to establish the molecular formula, corresponding to the β-hydroxydihydrochalcone structure 7. However, the spectrum shown in the supplementary material of this report does not show this signal [24], but instead m/z 289.0696 that was (erroneously) interpreted as "a quasi-molecular ion peak [M + H-H₂O]⁺" of β-hydroxydihydrochalcone 7. This mass signal corresponds to the protonated molecular ion of flavanone 7a, supporting the NMR-based revision of 7 to 7a. This MS-based misassignment is analogous to that of 4,2'-OMe 3.75 and C-6'-OMe 3.76, which showed m/z 286.0823 (HREIMS) that was reported as [M – H₂O]⁺ instead of recognizing it as the molecular peak of the corresponding flavanone, 4',7-dihydroxy-5-methoxyflavanone [17].

Elongatin (2) isolated from T. uniflora was evaluated for activity against the model bacteria B. subtilis and E. coli as well as for cytotoxicity against the MCF-7 human breast cancer cell line. It showed moderate antibacterial activity (EC₅₀ of 25.3 μM, EC₅₀ of 32.8 μM) against B. subtilis and cytotoxicity (EC₅₀ 41.3 μM), which is in agreement with a previous report on related flavonoids [12].

In conclusion, we report the isolation of (S)-elatadihydrochalcone-2'-methyl ether (1), a new β-hydroxydihydrochalcone, along with three known compounds from the stem of Tephrosia uniflora. In addition, we suggest the revision of the structures of the previously reported β-hydroxydihydrochalcones ziganin (5) [23], 3-(S)-hydroxy-3-phenyl-1-(2',4',6'-trihydroxyphenyl)propan-1-one (6) [22], and balanochalcone (7) [24] to the flavanones eriodictyol-7′-OMe 3.76 and C-6'-OMe 3.75, supporting the NMR-based revision of flavanones is typically <200 ppm, whereas those of β-hydroxydihydrochalcones is >200 ppm.

3. Materials and methods

3.1. Plant material

The stem of Tephrosia uniflora was collected along the Emali-Loitokitok road, Makueni County, in July 2016. The plant was authenticated by Mr. Pattrick C. Mutiso of the University Herbarium, Department of Biology, University of Nairobi, where a voucher specimen (PCM-2016/010) was deposited.

3.2. General experimental procedure

ECD experiments were run on a JASCO J-810 spectropolarimeter. UV spectroscopy was performed on a Shimadzu UV-1650 spectrophotometer. NMR spectra were acquired on a Bruker Avance N500 MHz spectrometer equipped with a TOF cryogenic probe. MestreNova (v14.0.0) software was used to process the spectra. TLC analyses was performed on Merck pre-coated silica gel 60 F₂₅₄ aluminium plates. Preparative reversed-phase HPLC chromatography was carried out using a Waters 600E HPLC system using the Chromulan software (v0.88, Piktron Ltd) and an RP-C8 Kromasil® column (250 mm × 25 mm, 5 μm). Column chromatography was done using silica gel 60 (mesh 230–400) and Sephadex LH-20 (GE Healthcare).

3.3. Extraction and isolation from the stem of Tephrosia uniflora

The air-dried stems of Tephrosia uniflora (500 g) were extracted (4 × 1 L) with CH₂Cl₂/MeOH (1:1) at room temperature. The crude extract (60 g) was partitioned between EtOAc and H₂O. The EtOAc layer was concentrated on a rotary evaporator to yield 30 g of crude extract. The crude EtOAc extract was adsorbed on silica gel, loaded onto a 300 g silica gel column, and eluted with isohexane containing increasing amounts of EtOAc (1% to 99% v/v). Based on their TLC profiles, the eluates were then pooled into 15 fractions. The fraction that was eluted with 3% EtOAc in isohexane was purified by column chromatography over Sephadex LH-20 (CH₂Cl₂/MeOH, 1:1), followed by further purification on Preparative TLC (isohexane/EtOAc, 7:3) to yield compound 2 (20 mg) as a white amorphous solid. The fraction that was eluted with

<table>
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<th>Table 1</th>
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<tr>
<td>1H (500 MHz) and 13C (125 MHz) NMR data for compound 1, in CDCl₃.</td>
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4% EtOAc in isoxeane was further purified by preparative HPLC (MeOH-H₂O, gradient elution 5%-90% H₂O) to give compound 1 (8 mg) as a colourless solid. The fraction that was eluted with 5% EtOAc was further purified on preparative HPLC (MeOH-H₂O, gradient elution 5%-90% H₂O) to give compound 3 (10 mg) and compound 4 (12 mg) as colourless solids.

3.4. (S)-Elatadihydrochalcone-2-methyl ether (1)

Colourless solid. UV (λmax: MeOH): 214, 263, 310 nm, ECD (MeOH, c 0.01): [θ]304 +91.1, [θ]275 +31.1, [θ]266 +22.1 and 13C NMR (Table 1). ESI-MS (m/z): 369.3 ([3, M + H]+), 263.3 (8), 258 (9), 247 (100), [C₁₄H₁₅O₄]⁺, 102 (5).

3.5. Antibacterial assays

Antibacterial activity of the isolated compound 2 was determined against the Gram-positive B. subtilis and Gram-negative E. coli bacteria following standard procedures [32]. In summary, compound 2 was dissolved in 100% DMSO to 10 mg/ml concentration and stored at −20 °C. Bacterial cultures of B. subtilis and E. coli were grown in Mueller-Hinton (HiMedia Laboratories Pvt. Limited, Mumbai, India) broth for 24 h until reaching the optical density (O-D = 0.5) [33], then diluted 10 times in pre-warmed medium and the compound added to a final concentration range as the compounds under testing. Effective concentration (EC50 and EC90) values were calculated from three independent replicate experiments using 2-fold dilution intervals. Non-linear regression dose-response inhibition following a log(agonist) vs. response was performed using GraphPad Prism version 9.2.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

3.6. Cytotoxicity assays

MCF-7 cells were used to assess the cytotoxic effect of the isolated compounds, as published elsewhere [35]. Briefly, the cells were cultured...
and kept in exponential growth in Dulbecco’s Eagle’s medium (Modified Medium) supplemented with 10% foetal calf serum and resseeded into 96-well microtiter plates to settle for 24 h as pre-assay preparation. The stock solution of the compound was added to a final concentration of 0.35% v/v of DMSO in the culture medium. The cell viability was determined using PrestoBlue (ThermoFisher) cell viability reagent kit and kept in exponential growth in Dulbecco’s Eagle’s medium. Mr. Patrick Mutiso is acknowledged for identification of the plant Tephrosia purpurea (Linn.) Pers. in rats, J. Ethnopharmacol. 108 (2006) 204–210.


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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2022.105166.

References


