Ochna mauritiana is a small tree endemic to the island of Mauritius that is reported to have antioxidant activity. The plant genus Ochna is known to produce a plethora of secondary metabolites belonging to the chemical classes anthranoids, triterpenoids, steroids, fatty acids, and flavonoids. Of these, the most abundant metabolites are the structurally diverse biflavonoids—polyphenolic dimers of flavonoid units connected through an alkyl or alkoxy-linked linker. As part of a phytochemical investigation of the leaves of O. Mauritiana, we have isolated, using silica gel flash chromatography and preparative layer chromatography (PLC), two biflavonoid compounds (1 and 2) from an ethyl acetate portion of the organic extract of this plant. Approximately 12.5 mg of 1 and 2.5 mg of 2 were placed in NMR tubes and dissolved with CD$_3$OD in order to elucidate their structures. High-Resolution Electrospray Ionization Mass Spectra (HR-ESI-MS) were also recorded in order to establish the molecular formulae of these two natural product compounds.

## Results and Discussion

Both compounds were relatively proton deficient resulting in few correlations in the long range 2D NMR experiments. Due to the repeated ring structure some of the 13C peaks are appearing very close together, which also limits the usefulness of the conventional 2D experiments. To address this, bond-selective versions of the experiments were performed wherever it was needed (see Figure 1).

The search for compound 2 did not produce any results which further reinforced the belief that this was a novel molecule. In this respect, a complete Computer Assisted Structure Elucidation was initiated using ACD/Structure Elucidator Suite and the NMR data is listed in Table 1.

### Table 1: Spectral data obtained for compound 2 dissolved in CD$_3$OD

<table>
<thead>
<tr>
<th>Peak</th>
<th>δ (ppm)</th>
<th>J (Hz)</th>
<th>Intensity</th>
<th>2D Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.85</td>
<td>s</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.48</td>
<td>d (2.25)</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.53</td>
<td>d (2.25)</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

The Molecule Correlation Diagram (MCD) produced from these data is shown in Figure 2. Some obvious bonds were drawn manually (e.g., the two carbonyls and the unsaturated ring). The MCD was then checked for errors and discrete structure elucidation was initiated.

The search for compound 1 took around 30 sec and produced 10 results. The molecule with PubChem ID 10432911, 7,3’-Dihydroxyflavone, was the one with the lowest mean deviation between the experimental and the predicted 13C chemical shift of 1.27 ppm. The literature reference cited by PubChem$^3$ as well as references therein$^4$ contained the 1H and 13C NMR spectra of the compound in DMSO-$d_6$ and acetone-$d_6$, which were in agreement with our results.

## Conclusions

The structures of the two compounds isolated were unequivocally elucidated using a CASE system and a series of 1D and 2D NMR experiments. The structure of 1 was very quickly found to be the one of the known compound 7,3’-dihydroxyflavone,3,5 based on a database search of its 13C NMR spectrum whilst compound 2 was found to be the new 7-O-methyl biflavonoid derivative of 1. Both compounds showed similar poor antimycobacterial activity (MIC$_{90}$ > 50 µg/ml).

### References