Computer Assisted Structure Elucidation of Two Biflavonoids from the Leaves of Ochna Mauritiana

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Introduction

Ochna mauritiana is a small tree endemic to the island of Mauritius that is reported to have antioxidant activity.[1] 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-based linker.[2] 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_3OD 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 ^{13}C peaks are appearing very close together, which also limits the usefulness of the conventional 2D experiments. To address this, band-selective versions of the experiments were performed wherever it was needed (see Figure 1).

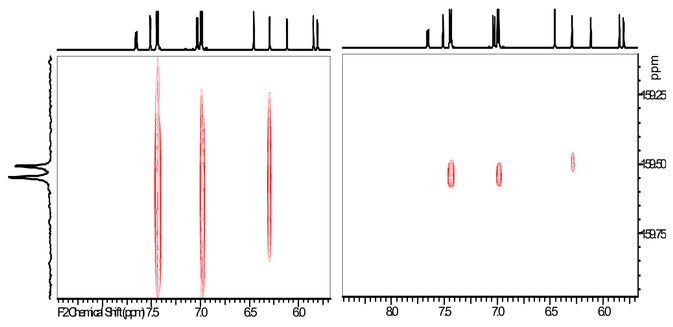

Figure 1: *Comparison between conventional (left) and band-selective (right) ^{1}H-{^{13}C} HMBC spectrum of compound **1** in the F1 region around 159.5 ppm.*

HR-ESI-MS recorded for compound **1** (m/z 539.0953 $[M-H]^-$, calcd. 539.0978) and compound **2** (m/z 553.1116 $[M-H]^-$, calcd. 553.1135) established molecular formulas of $C_{30}H_{20}O_{10}$ and $C_{31}H_{22}O_{10}$, respectively. Preliminary analysis of their 1D NMR data suggested that these compounds were flavonoid-type molecules.

In order to limit the possibility that the compounds were already known, a search was performed using the ^{13}C recorded spectra against the ACD/Structure Elucidator compound database which contains 95+ million ^{13}C predicted spectra of compounds from PubChem and ChemSpider.

The search for compound **1** took around 30 sec and produced 10 results. The molecule with PubChem ID 10437291, 2',3'-Dihydroochnaflavone, was the one with the lowest mean deviation between the experimental and the predicted ^{13}C chemical shift of 1.277 ppm. The literature reference cited by PubChem[3] as well as references therein[4,5] contained the ^{1}H and ^{13}C NMR spectra of the compound in DMSO-d_6 and acetone-d_6 which were in agreement with our results.

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[6] and the NMR data is listed in Table 1.

Table 1: Spectral data obtained for compound **2** dissolved in CD_3OD.

Atom	C Shift	H Shift	H Multiplicity	COSY	HMBC
1	55.056	3.855	s		
2	42.593	2.997	dd (16.91, 12.59)	2.63, 5.31	
2	42.593	2.628	m	3.00, 5.31	
3	78.322	5.314	dd (12.59, 3.01)	2.63, 3.00, 7.41	3.00, 7.41
4	91.913	6.58	d (2.25)	6.28	6.28
5	97.64	5.721	m		
6	97.775	6.285	d (2.25)	6.58	6.58
7	98.24	5.682	d (2.07)		
8	100.807	6.481	s		
9, 10	116.274	6.984	m	7.41	7.41
11	119.486	7.528	d (2.44)	7.65	6.89, 7.65
12	120.25	6.89	d (8.64)	7.65	
13	124.689	7.653	dd (8.64, 2.44)	6.89, 7.53	6.89, 7.53
14, 15	127.34	7.41	d (8.83)	5.31, 6.98	5.31
16	182.274				6.48, 6.58
17	193.495				2.63, 3.00, 5.31
18	99.724				5.68, 5.72
19	104.602				6.28, 6.48, 6.58
20	117.179				6.48, 6.89
21	132.917				3.00, 5.31, 6.98
22	145.569				6.89, 7.53, 7.65
23	157.828				6.58
24	158.832				6.98, 7.41
25	161.418				6.28, 6.48
26	162.641				6.89, 7.53, 7.65
27	163.186				5.72
28	164.225				5.68
29	165.72				6.48, 6.89, 7.53, 7.65
30	165.766				3.86, 6.28, 6.58
31	176.659				5.72

The Molecular 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.

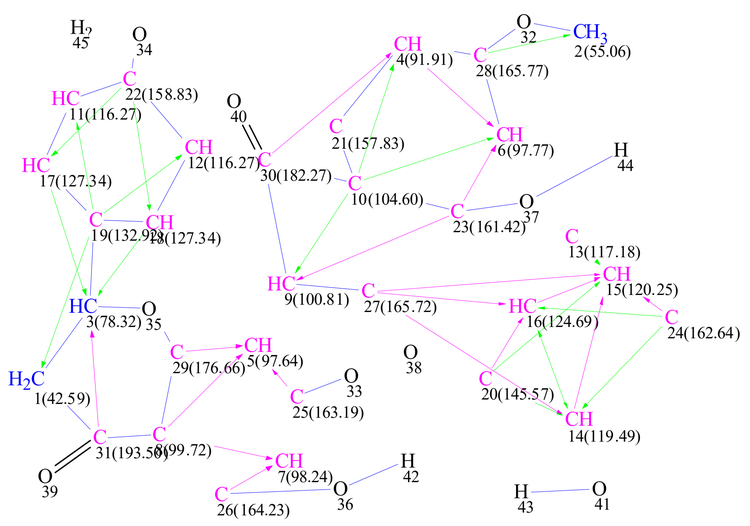

Figure 2: MCD for compound **2**, automatically generated from data in Table 1.

ACD/Structure Elucidator Suite needed 14 minutes to generate ca. 25 unique structures for compound **2**. The structures were then ranked according to the predicted ^{13}C and ^{1}H chemical shift difference to the observed spectra. The top 3 structures are shown in Figure 3.

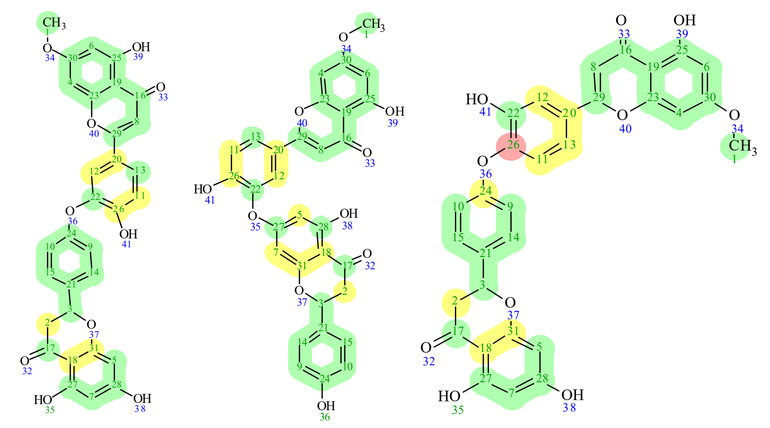

Figure 3: *The top 3 structures predicted for compound **2**. The colouring indicates the quality of the match between the predicted and experimental ^{13}C and ^{1}H chemical shifts— green: a deviation less than 3 ppm; yellow: less than 15 ppm and red indicating more than 15 ppm deviation of ^{13}C chemical shift units. The mean combined (^{13}C+^{1}H) chemical shift deviation for the shown structures is 3.874, 4.291 and 4.45 ppm respectively.*

The first and second structure appear to have similar predicted chemical shift deviations however they are significantly different. The first one was confirmed to be the correct one using NOESY spectra. The observed key NOESY correlations confirming that the first structure is the correct one are shown in Figure 4.

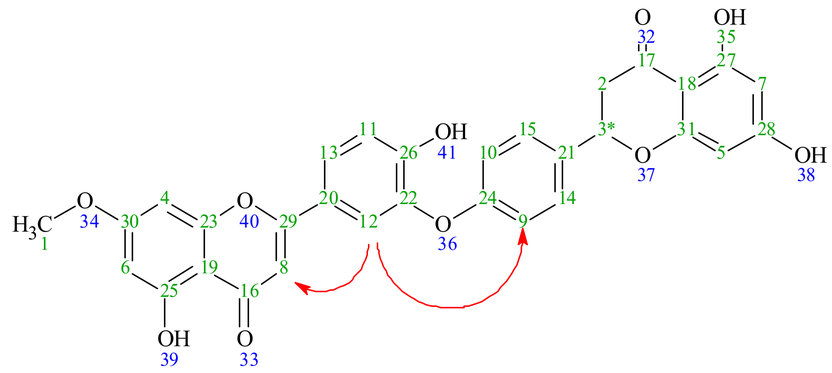

Figure 4: Important NOESY correlations of atom 12 to atoms 8 and 9, confirming the validity of the first structure from Figure 3.

Both compounds were evaluated for their *in vitro* activity against the H37Rv strain of *Mycobacterium tuberculosis* and exhibited MIC_{90} values of 72.8 µg/ml (compound **1**) and 69.2 µg/ml (compound **2**) and MIC_{99} values of 84.3 µg/ml (compound **1**) and >125 µg/ml (compound **2**).

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 2'', 3''-dihydroochnaflavone,3-5 based on a database search of its ^{13}C 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 ($MIC_{90/99}$ > 50 µg/ml).

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