

# ISOLATION AND STRUCTURAL CHARACTERIZATION OF TWO SAPONINS FROM THE ROOTS OF *Sansevieria trifasciata* ‘Laurentii’

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## Abstract

*Sansevieria* is a genus consisting of about 60 species natively distributed in tropical and subtropical areas. Many *Sansevieria* species are used for ornamental purposes or in traditional medicine to treat human diseases. *Sansevieria trifasciata* ‘Laurentii,’ commonly known as snake plant, is an evergreen perennial plant used in traditional medicine. The phytochemical investigation of the roots of *S. trifasciata* ‘Laurentii’ resulted in the isolation and structural characterization of two previously reported saponins: (24*S*,25*R*)-1 $\beta$ -[( $\beta$ -*D*-fucopyranosyl)oxy]-3 $\beta$ -hydroxySpirost-5-en-24-yl  $\beta$ -*D*-glucopyranoside (**1**) and 26-[( $\beta$ -*D*-glucopyranosyl)oxy]-3 $\beta$ ,22 $\alpha$ -dihydroxyfurosta-5,25(27)-dien-1 $\beta$ -yl-*O*- $\alpha$ -*L*-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -*L*-arabinopyranoside (**2**) by analysis of their 1D and 2D NMR spectra and HR-ESI-MS, together with the comparison to data published in the literature.

**Keywords:** Asparagaceae; Furostane-type; *Sansevieria trifasciata* ‘Laurentii’; Saponin; Spirostane-type.

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## 1. INTRODUCTION

The *Sansevieria* genus consists of xerophytic perennial herbs with erect, stiff leaves and short, thick, stoloniferous rhizomes. This genus consists of 50 to 70 species, or even over 80, which are distributed in tropical and subtropical regions of the world. Many *Sansevieria* species are used for ornamental purposes or in traditional medicine to treat human diseases (Thu et al., 2021). *Sansevieria trifasciata* ‘Laurentii,’ a species of the *Sansevieria* genus commonly known as snake plant or mother-in-law’s tongue, is an evergreen perennial plant used in traditional medicine to treat asthma, cough, asthenia, hypertension, diarrhea, hemorrhoids, abdominal pain, eczema, edema, yellowing skin, anuria, viral hepatitis, malaria, and snake and insect bites (Đỗ, 2004; Giovannini & Howes, 2017; Said et al., 2015). Previous chemical investigations of *S. trifasciata* ‘Laurentii’ revealed the presence of alkaloids, flavonoids, terpenoids, and saponins (Andhare et al., 2012; González et al., 1972; Nguyen et al., 2022; Lontoc et al., 2018; Mimaki et al., 1996, 1997; Pettit et al., 2005; Tchegnitegni et al., 2015). Nevertheless, there are few studies in Vietnam on the isolation of saponins from the *Sansevieria* genus. To expand the chemotaxonomic knowledge of the *Sansevieria* genus, we have now conducted phytochemical screening of the roots of *S. trifasciata* ‘Laurentii.’ This resulted in the isolation of two steroidal saponins by analysis of their 1D and 2D nuclear magnetic resonance (NMR) spectra, by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), and by comparison with data published in the literature.

## 2. EXPERIMENTAL

### 2.1. General procedures

HR-ESI-MS experiments were conducted with a micrOTOF II mass spectrometer (Bruker®, Germany).  $^{13}\text{C}$ ,  $^1\text{H}$ , HSQC, COSY, ROESY, TOCSY, and HMBC NMR spectra were recorded with an NMR Inova spectrometer (Agilent Technologies®, USA) in pyridine-*d*<sub>5</sub> at ambient temperature with tetramethylsilane as the internal standard (in the appendix). An Elmasonic S10H ultrasound cleaner (Elma, Switzerland) was used for the extractions. Column chromatography was performed to isolate compounds of the extract using Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) as the stationary phase. The primary fractional separation was performed using vacuum liquid chromatography (VLC) using Silicycle silica gel RP-18 (Canada) of particle size 75–200  $\mu\text{m}$  as the stationary phase. The isolation and purification of saponins from the fractions were carried out by medium-pressure liquid chromatography (MPLC) using Merck silica gel 60 (Germany) of particle size 15–40  $\mu\text{m}$  as the stationary phase. Thin-layer chromatography (TLC) was performed with Merck silica gel 60F<sub>254</sub> (Germany) and developed with  $\text{CHCl}_3$ -MeOH-H<sub>2</sub>O-AcOH (70:30:5:1). Saponins were detected by spraying the TLC with 10% H<sub>2</sub>SO<sub>4</sub>, followed by heating.

### 2.2. Plant materials

Roots of *S. trifasciata* ‘Laurentii’ were collected in Thai Nguyen, Vietnam, in June 2022 (21°35'53" N, 105°50'17" E). The roots were identified by Dr. Thuong Sy Danh, Thai Nguyen University of Education, and a specimen is stored under code number

NDH202201TN in our laboratory at the Department of Biology, Thai Nguyen University of Education, Vietnam.

### 2.3. Extraction and isolation

The plant material (roots, dried weight 68.3 g) was extracted three times successively with a solvent system of EtOH-H<sub>2</sub>O (75:35, 600 mL each, 30 W, 50 °C, 30 min). The aqueous-ethanolic extract was concentrated to dryness under reduced pressure. The viscous concentrate (18.9 g) was dissolved in water (30 mL) and further partitioned by a VLC on RP-18 using the eluent system of H<sub>2</sub>O/EtOH (1/0, 1/1, 0/1; 500 mL each) to give three fractions (I–III). Fraction II was then partitioned again on a VLC on RP-18 using the same protocol as above to give three subfractions (II.1–II.3). Subfraction II.2 (0.53 g) mainly contained saponins and was separated by MPLC on silica gel 60 with an eluent system of CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 70/30/5 (v/v/v), resulting in five subfractions (II.2.1–II.2.5). Subfraction II.2.2 (39.3 mg) was chromatographed on a successive MPLC on silica gel 60 and eluted with CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 75/25/3 (v/v/v) to yield compound **1** (3.2 mg). Subfraction II.2.4 (18.6 mg) was purified by column chromatography on Sephadex LH-20 with EtOH 96%, yielding compound **2** (2.8 mg).

(24*S*,25*R*)-1β-[(β-*D*-fucopyranosyl)oxy]-3β-hydroxyspirost-5-en-24-yl β-*D*-glucopyranoside (**1**): white amorphous powder; <sup>1</sup>H NMR (600 MHz in pyridine-*d*<sub>5</sub>) and <sup>13</sup>C NMR (150 MHz in pyridine-*d*<sub>5</sub>) spectral data are given in Table 1; HR-ESI-MS (positive-ion mode): *m/z* 777.4057 [M+Na]<sup>+</sup> (calculated for C<sub>39</sub>H<sub>62</sub>NaO<sub>14</sub>, 777.4037).

26-[(β-*D*-glucopyranosyl)oxy]-3β,22α-dihydroxyfurosta-5,25(27)-dien-1β-yl-*O*-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside (**2**): white amorphous powder; <sup>1</sup>H NMR (600 MHz in pyridine-*d*<sub>5</sub>) and <sup>13</sup>C NMR (150 MHz in pyridine-*d*<sub>5</sub>) spectral data are shown in Table 1; HR-ESI-MS (positive-ion mode): *m/z* 909.4471 [M+Na]<sup>+</sup> (calculated for C<sub>44</sub>H<sub>70</sub>NaO<sub>18</sub>, 909.4460).

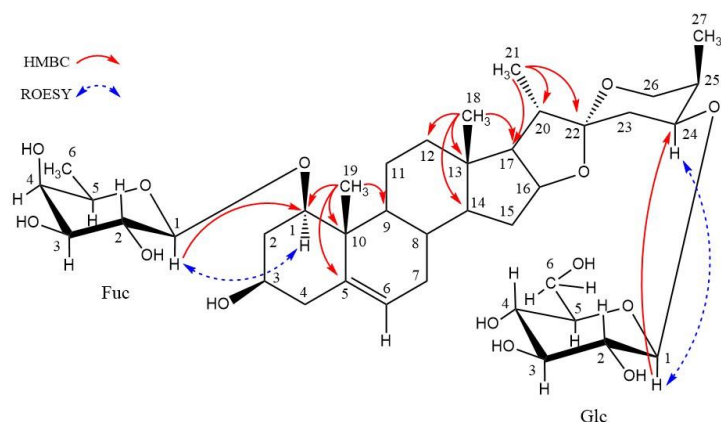
## 3. RESULTS AND DISCUSSION

The extract of the roots of *S. trifasciata* ‘Laurentii’ was fractionated by a combination of silica gel, Sephadex LH-20 column chromatography, and MPLC, resulting in the isolation of compounds **1** and **2** (Figures 1 and 2). The structural elucidation of the isolated compounds was performed by 1D and 2D NMR analysis (<sup>1</sup>H, <sup>13</sup>C, HSQC, HMBC, COSY, TOCSY, and ROESY) and mass spectrometry (HR-ESI-MS in positive-ion mode). The sugar moieties of isolated compounds were identified by extensive 2D NMR analysis as arabinose (Ara), rhamnose (Rha), and glucose (Glc). The absolute sugar configurations were determined to be L for Ara and Rha, and D for Glc (Nguyen et al., 2020). The relatively large <sup>3</sup>J<sub>H-1, H-2</sub> values of the Ara (7.6 Hz) indicated an α anomeric orientation for Ara and β anomeric orientation for Glc and Fuc. The large <sup>3</sup>J<sub>H-1, C-1</sub> values of Rha (166–168 Hz) confirmed that the anomeric protons were equatorial (α-pyranoid anomeric form) (Nguyen et al., 2021).

Compound **1** was obtained as a white amorphous powder with a molecular formula of C<sub>39</sub>H<sub>62</sub>O<sub>14</sub>, proved by HR-ESI-MS at *m/z* 777.4057 [M+Na]<sup>+</sup>. The <sup>1</sup>H NMR

of the aglycone portion of compound **1** showed the presence of two tertiary methyl signals at  $\delta_{\text{H}}$  0.88 (s, aglycone H-18), 1.08 (s, aglycone H-19), a secondary methyl group at  $\delta_{\text{H}}$  1.01 (d,  $J = 6.4$  Hz, aglycone H-21), an olefinic proton at  $\delta_{\text{H}}$  5.55 (d,  $J = 5.6$  Hz, aglycone H-6), and two anomeric protons at  $\delta_{\text{H}}$  4.81 (d,  $J = 7.6$  Hz) and 4.95 (d,  $J = 7.6$  Hz). These proton signals, together with a distinctive quaternary carbon signal at  $\delta_{\text{C}}$  111.8 (aglycone C-22), showed HMBC correlations with methylene protons at  $\delta_{\text{H}}$  3.86 (br d,  $J = 12.1$  Hz, aglycone H-26a) and  $\delta_{\text{H}}$  4.27 (br d,  $J = 12.1$  Hz, aglycone H-26b), and other carbon signals at  $\delta_{\text{C}}$  139.1 (aglycone C-5), 124.8 (aglycone C-6), 40.1 (aglycone C-25), 62.2 (aglycone C-26), and 15.5 (aglycone C-27), indicated the aglycone of compound **1** as a spirostanol type, which is in good agreement with the literature data (Rezgui et al., 2013). The chemical shift of the H-26a position of the aglycone differs from that of the H-26b position of the aglycone. The resulting  $\Delta = 0.41 > 0.35$  in pyridine- $d_5$  led to the identification of the orientation of the 27-methyl group as  $25S$  (Agrawal, 2003). Furthermore, the  $^{13}\text{C}$  NMR spectrum of compound **1** showed considerable structural similarity to that of ruscogenin (Mimaki et al., 2008). However, comparing the molecular formula of compound **1** with the published data of ruscogenin revealed different signals from C-22 to C-27 in the F ring of the aglycone. An oxygen atom located at the C-24 position of the aglycone was determined by observation of the structural fragment –C(23)H<sub>2</sub>–C(24)H(–O)–C(25)H(–C(26)H<sub>2</sub>–O)–Me(27) in the F ring of the aglycone. The configuration of the H-24 proton was determined by calculating the spin-coupling constants, including H-23 axial/H-24 = 11.6 Hz, H-23 equatorial/H-24 = 5.1 Hz, and H-24/H-25 = 5.1 Hz, resulting in the identification of the  $24S$  orientation for this position. Thus, the aglycone of compound **1** was identified as (24*S*,25*R*)-spirost-5-ene-1 $\beta$ ,3 $\beta$ ,24-triol, which was previously isolated from *Dracaena surculosa* and *Beaucarnea recurvata* (Eskander et al., 2011; Yokosuka et al., 2000).

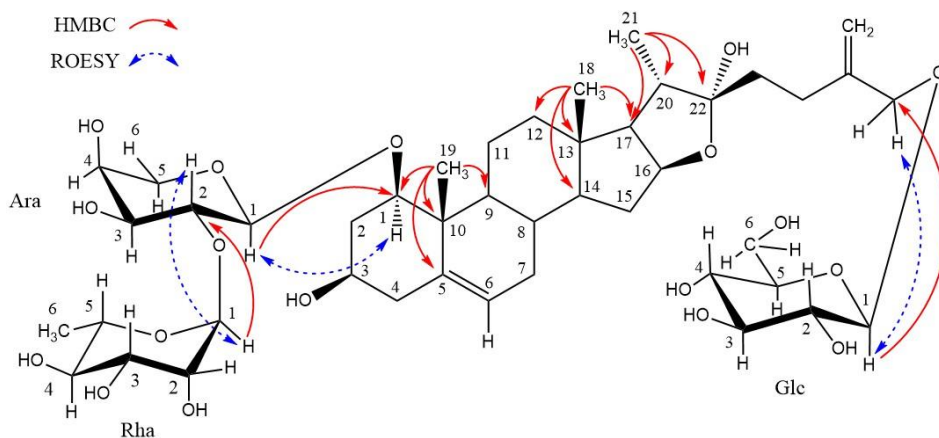
The study continued with observations in the sugar region. Analysis of the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum allowed the sequential assignment of the resonances for the two monosaccharides, starting from the anomeric proton signals at  $\delta_{\text{H}}$  4.81 (d,  $J = 7.6$  Hz) and 4.95 (d,  $J = 7.6$  Hz). Multiplet patterns and measurements of coupling constants gave evidence for the presence of a  $\beta$ -D-fucopyranosyl unit and a  $\beta$ -D-glucopyranosyl unit (Table 1). Complete assignments of the resonances of each sugar were achieved by 2D NMR analyses (COSY, TOCSY, HSQC, and HMBC). The structure of the saccharidic part of the molecule was established by HMBC and ROESY experiments. The HMBC correlation between  $\delta_{\text{H}}$  4.81 (d,  $J = 7.6$  Hz, Fuc H-1)/ $\delta_{\text{C}}$  and 81.5 (aglycone C-1), together with the ROESY correlation between  $\delta_{\text{H}}$  4.81 (d,  $J = 7.6$  Hz, Fuc H-1)/ $\delta_{\text{H}}$  4.00 (dd,  $J = 11.1$ , 2.9 Hz, aglycone H-1), indicated that the Fuc moiety was attached to the C-1 position of the aglycone. The glycosidic linkage of the Glc moiety to the C-24 position of the aglycone was determined by observing the HMBC correlation between  $\delta_{\text{H}}$  4.95 (d,  $J = 7.6$  Hz, Glc H-1) and  $\delta_{\text{C}}$  71.8 (aglycone C-24), and the ROESY correlation between  $\delta_{\text{H}}$  4.95 (d,  $J = 7.6$  Hz, Glc H-1) and  $\delta_{\text{H}}$  4.58 (aglycone H-24). The structure of compound **1** was thus determined as (24*S*,25*R*)-1 $\beta$ -[( $\beta$ -D-fucopyranosyl)oxy]-3 $\beta$ -hydroxyspirost-5-en-24-yl  $\beta$ -D-glucopyranoside (Figure 1). This compound has previously been isolated from the whole plant of *Dracaena surculosa* (Yokosuka et al., 2000).



**Figure 1. The structure of compound 1 with the keys of HMBC (red arrow) and ROESY (blue arrow)**

Compound **2** was obtained as a white amorphous powder with a molecular formula of  $C_{44}H_{70}O_{18}$ , proved by HR-ESI-MS at  $m/z$  909.4471  $[M+Na]^+$ . For the aglycone part of the molecule, the  $^1H$  NMR spectrum of compound **2** showed signals for two tertiary methyl groups at  $\delta_H$  0.85 (s, aglycone H-18) and 1.28 (s, aglycone H-19), a secondary methyl group at  $\delta_H$  1.03 (d,  $J = 6.4$  Hz, aglycone H-21), exomethylene protons at  $\delta_H$  5.09 and 5.38 (each 1H, br s, aglycone H-27), an olefinic proton at  $\delta_H$  5.57 (br d,  $J = 5.6$  Hz, aglycone H-6), two methine proton signals at  $\delta_H$  3.98 (dd,  $J = 11.1, 3.2$  Hz, aglycone H-1),  $\delta_H$  3.85 (aglycone H-3), indicative of secondary alcoholic functions, and two methylene proton signals at  $\delta_H$  4.55 and  $\delta_H$  4.12 (each 1H, br d,  $J = 12.3$  Hz, H-26), along with three anomeric protons at  $\delta_H$  4.72 (d,  $J = 7.6$  Hz), 4.98 (d,  $J = 7.6$  Hz) and 6.32 (br s). The  $^{13}C$  NMR spectrum showed a hemiacetal function at  $\delta_C$  112.5, three secondary alcoholic functions at  $\delta_C$  81.2 (aglycone C-1), 71.2 (aglycone C-3), and 81.4 (aglycone C-16), and one primary alcoholic function at  $\delta_C$  73.9 (aglycone C-26), suggesting the occurrence of a furostanol skeleton. Comparing the NMR signals of compound **2** with those of the aglycone of compound **1** led to the determination of the same molecule of A-ring hydroxylated at C-1 and C-3, but the difference was revealed at the F ring, where the spirostane form of compound **1** was replaced by a furostane in compound **2**. This aglycone of compound **2** was identified as furosta-5,(25)27-diene-1 $\beta$ ,3 $\beta$ ,22 $\alpha$ ,26-tetrol (Mimaki et al., 1999; Tu et al., 2023). In the sugar region, signals of three correlation peaks in the HSQC spectrum of compound **2** were seen at  $\delta_H$  4.72 (d,  $J = 7.6$  Hz)/ $\delta_C$  100.6,  $\delta_H$  4.98 (d,  $J = 7.6$  Hz)/ $\delta_C$  104.8, and  $\delta_H$  6.32 (br s)/ $\delta_C$  101.3, which were further identified as Ara, Glc, and Rha sugar moieties, respectively. The linkage positions between the substituents and the aglycone were established by the following spectral data. The correlation peak at  $\delta_H$  4.72 (d,  $J = 7.6$  Hz, Ara H-1)/ $\delta_C$  and 81.2 (aglycone C-1) observed in the HMBC spectrum, along with the correlation peak at  $\delta_H$  4.72 (d,  $J = 7.6$  Hz, Ara H-1)/ $\delta_H$  3.98 (dd,  $J = 11.1, 3.2$  Hz, aglycone H-1) observed in the ROESY spectrum, indicated that the Ara moiety was attached to the C-1 position of the aglycone. The HMBC correlation peak at  $\delta_H$  6.32 (br s, Rha H-1)/ $\delta_C$  73.7 (Ara C-2), together with the ROESY correlation peak at  $\delta_H$  6.32 (br s, Rha H-1)/ $\delta_H$  4.53 (Ara H-2), determined that the Rha unit was linked to the C-2 position of the Ara unit. Finally, the Glc unit was found to link to the C-26 position of the aglycone by observation of the correlation peaks at  $\delta_H$  4.98 (d,  $J = 7.6$  Hz, Glc H-1)/ $\delta_C$

and 73.9 (aglycone C-26) in the HMBC spectrum, and at  $\delta_{\text{H}}$  4.98 (d,  $J = 7.6$  Hz, Glc H-1)/ $\delta_{\text{H}}$  4.55 (br d,  $J = 12.3$  Hz, aglycone H-26b) in the ROESY spectrum. Thus, the structure of compound **2** was proved to be 26-[( $\beta$ -D-glucopyranosyl)oxy]- $\beta$ 3,22 $\alpha$ -dihydroxyfurosta-5,25(27)-dien-1 $\beta$ -yl- $O$ - $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranoside (Figure 2). This compound has previously been isolated from the aerial parts of *Sansevieria trifasciata* collected in Cameroon (Teponno et al., 2016).



**Figure 2.** The structure of compound **2** with the keys of HMBC (red arrow) and ROESY (blue arrow)

**Table 1.**  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectral data of compounds **1** and **2** ( $\text{C}_5\text{D}_5\text{N}$ ,  $\delta$  in ppm,  $J$  in Hz)

C-position	<b>1</b>		<b>2</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	81.5	4.00 dd (11.1, 2.9)	81.2	3.98 dd (11.1, 3.2)
2	35.1	2.03, 2.87	36.2	2.00, 2.91
3	73.2	3.91	71.2	3.85
4	39.4	1.71, 1.78	39.8	2.32, 2.69
5	139.1	–	139.3	–
6	124.8	5.55 d (5.6)	125.1	5.57 d (5.6)
7	32.3	0.89, 1.68	32.1	1.93, 1.99
8	36.5	1.53	36.3	1.51
9	55.2	1.10	50.5	1.46
10	42.5	–	42.8	–
11	23.3	1.39, 3.20	23.8	1.36, 3.18
12	40.3	1.22, 1.59	40.1	1.19, 1.53
13	40.2	–	40.3	–
14	56.5	1.08	56.1	1.16
15	32.4	1.43, 2.05	32.1	1.39, 2.10
16	81.6	4.59 q (7.0)	81.4	4.63 q (7.0)
17	62.9	1.79	62.3	1.83

**Table 1.**  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectral data of compounds **1** and **2** ( $\text{C}_5\text{D}_5\text{N}$ ,  $\delta$  in ppm,  $J$  in Hz) (cont.)

C-position	<b>1</b>		<b>2</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
18	16.6	0.88 s	16.8	0.85 s
19	14.1	1.08 s	13.6	1.28 s
20	41.5	1.98 t (6.4)	40.8	2.05 t (6.4)
21	14.9	1.01 d (6.4)	14.7	1.03 d (6.4)
22	111.8	–	112.5	–
23	32.7	1.82 dd (13.1, 5.1), 1.86 dd (13.1, 11.6)	32.5	2.05, 2.18
24	71.8	4.58 ddd (11.6, 5.1, 5.1)	29.2	2.15, 2.28
25	40.1	2.25	146.6	–
26	62.2	3.86 br d (12.1), 4.27 br d (12.1)	73.9	4.12 br d (12.3), 4.55 br d (12.3)
27	15.5	1.15 d (7.0)	109.2	5.09 br s, 5.38 br s
Fuc-1	101.3	4.81 d (7.6)		
2	72.2	4.35 dd (9.3, 7.6)		
3	74.8	4.15 dd (9.3, 3.1)		
4	72.4	4.10 br d (3.1)		
5	71.2	3.75 br q (6.4)		
6	17.3	1.58 d (6.4)		
Ara-1			100.6	4.72 d (7.6)
2			73.7	4.53
3			75.5	4.11
4			69.8	4.25
5			67.2	3.71, 4.29
Rha-1			101.3	6.32 br s
2			72.5	4.81 br s
3			72.2	4.68 dd (9.3, 2.9)
4			74.5	4.55 dd (9.9, 9.3)
5			69.7	4.88 dq (9.9, 6.4)
6			18.9	1.71 d (6.4)
Glc-1	103.9	4.95 d (7.6)	104.8	4.98 d (7.6)
2	74.8	4.08	74.7	4.08
3	78.2	4.29	77.8	4.36
4	71.3	4.16	71.4	4.21
5	78.2	3.98	77.9	3.99
6	62.6	4.31, 4.56	62.5	4.35, 4.58

The spectrum of biological effects tested for the saponins isolated from *Sansevieria* species is rather limited, and *in vitro* evaluations have been performed in most cases. Previously, a study of the biological activity of compound **1** isolated from *Dracaena surculosa* revealed no cytotoxic activity against HL-60 leukemia cells (Yokosuka et al., 2000). The same biological result was achieved in the case of compound **2** on Hela cell lines that were previously isolated from *S. trifasciata* collected in Cameroon (Teponno et al., 2016). Most saponins have powerful hemolytic activities because steroids have high affinities for cholesterol on erythrocyte membranes, thereby altering membrane permeability (Thu et al., 2021). In this context, it is noteworthy that compounds **1** and **2** showed no hemolytic effects *in vitro*. This could be explained by the linkage of sugar units around the aglycone moiety of the saponin, reducing its hydrophobicity, and the loss of the amphipathic features. The characterization of these saponins gives a better understanding of the phytochemistry and biological activities of the *Sansevieria* genus and suggests more evaluations of biological activity should be performed to establish structural activity related to these compounds.

#### 4. CONCLUSION

The phytochemical investigation of the roots of *S. trifasciata* ‘Laurentii’ resulted in the isolation and structural characterization of two saponins. These compounds have been isolated previously in species of the *Dracaena*, *Beaucarnea*, and *Sansevieria* genera of the Asparagaceae family. Further experiments on the extract from the roots of *S. trifasciata* ‘Laurentii’ are proposed to evaluate the biological activity of these compounds and to complete the chemotaxonomic data of this species and the *Sansevieria* genus.

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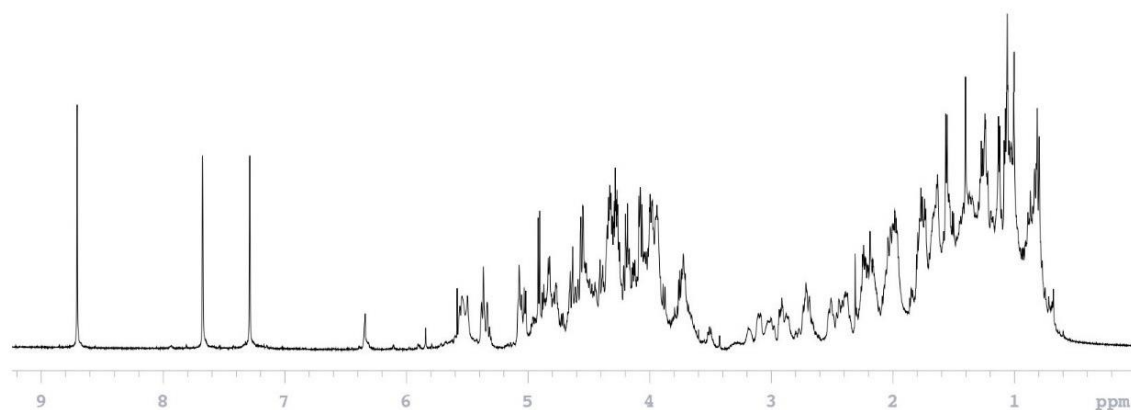
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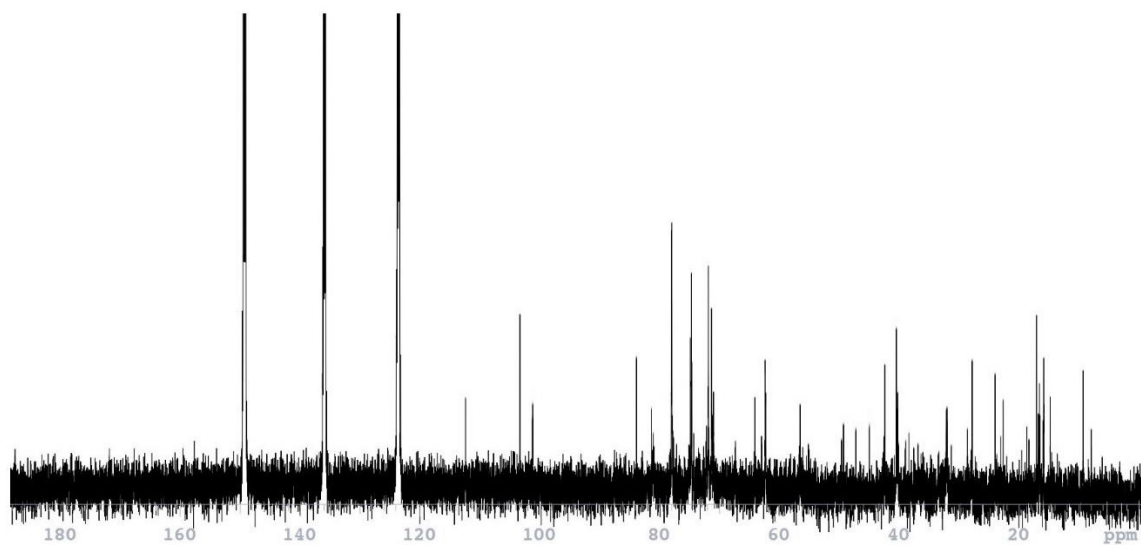
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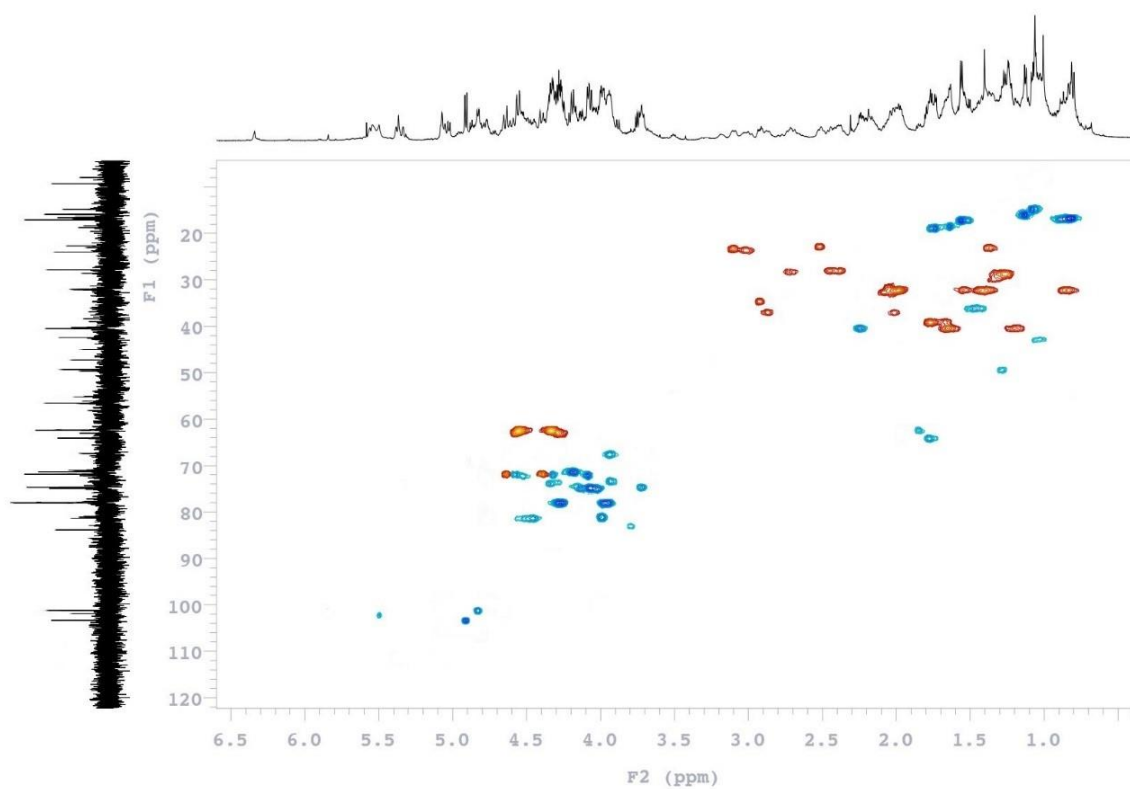
#### APPENDIX: Supplementary data



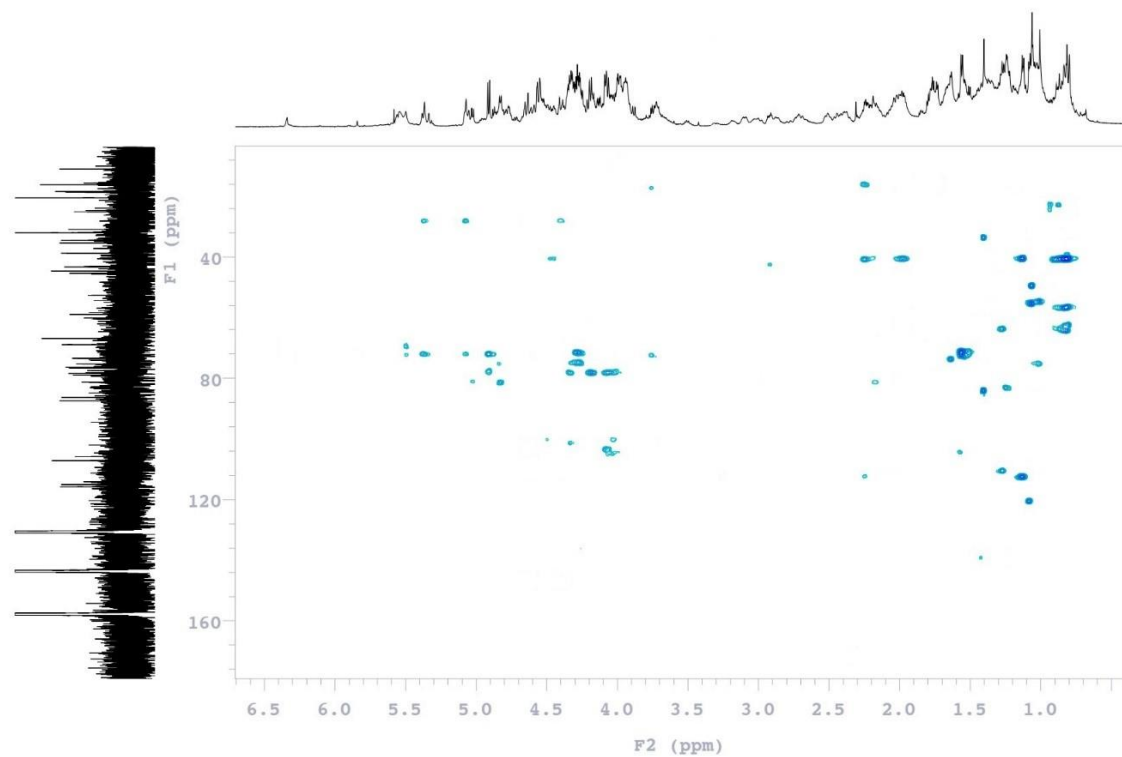
**Figure 1.**  $^1\text{H}$  NMR spectrum of compound 1 in  $\text{C}_5\text{H}_5\text{N}$  at 600 MHz



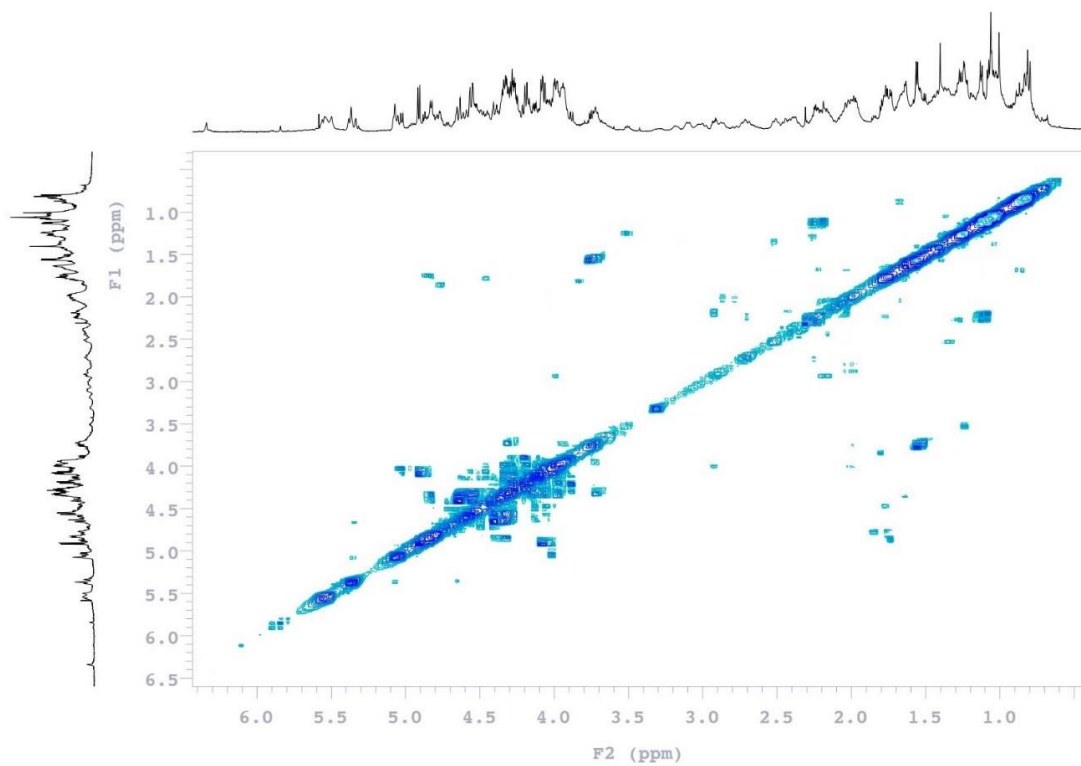
**Figure 2.**  $^{13}\text{C}$  NMR spectrum of compound 1 in  $\text{C}_5\text{H}_5\text{N}$  at 150 MHz



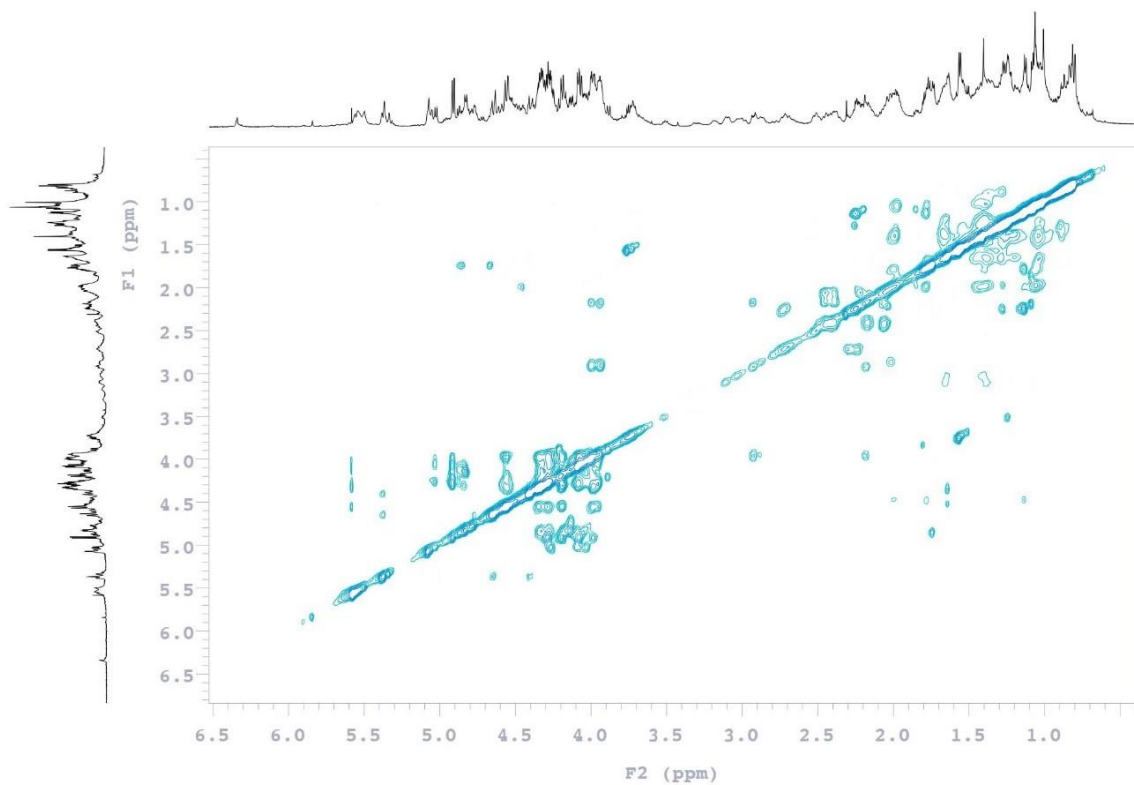
**Figure 3.** HSQC spectrum of compound 1 in  $\text{C}_5\text{H}_5\text{N}$  at 600 MHz



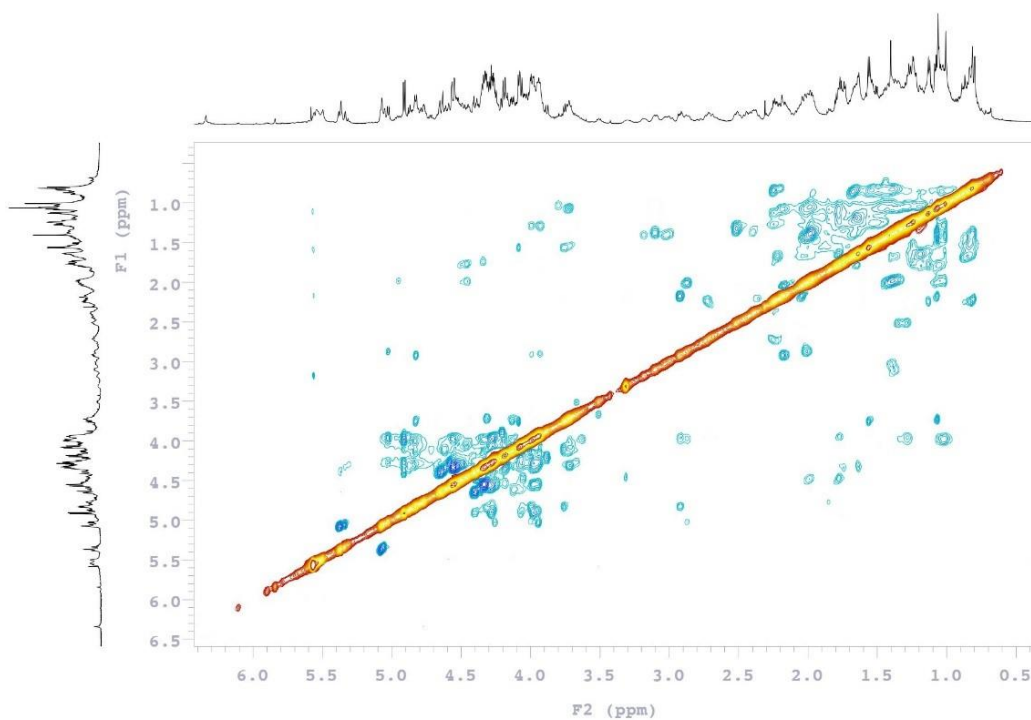
**Figure 4. HMBC spectrum of compound 1 in C<sub>5</sub>H<sub>5</sub>N at 600 MHz**



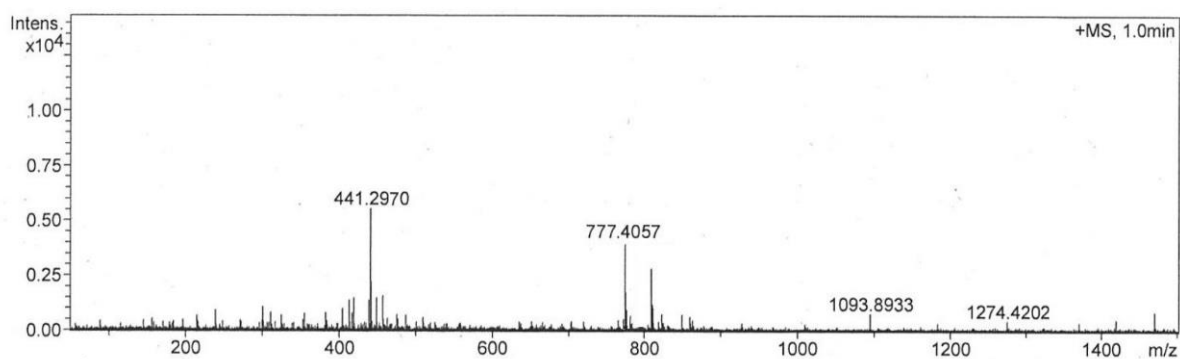
**Figure 5. <sup>1</sup>H-<sup>1</sup>H COSY spectrum of compound 1 in C<sub>5</sub>H<sub>5</sub>N at 600 MHz**



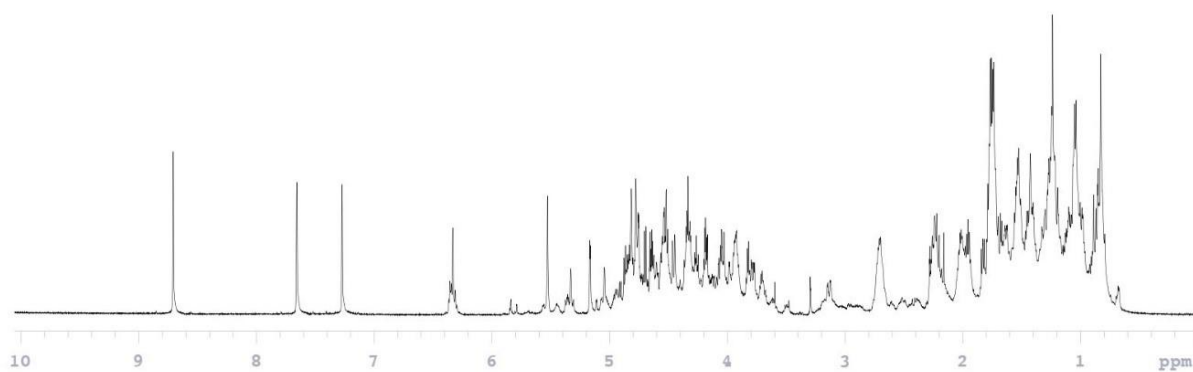
**Figure 6.** TOCSY spectrum of compound 1 in  $C_5H_5N$  at 600 MHz



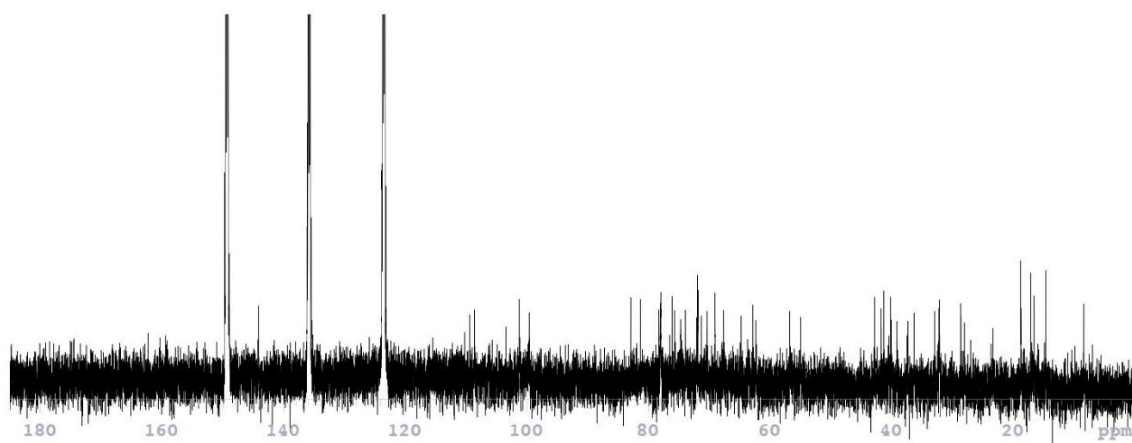
**Figure 7.** ROESY spectrum of compound 1 in  $C_5H_5N$  at 600 MHz



**Figure 8. Mass spectrum (HR-ESI-MS in positive-ion mode) of compound 1**



**Figure 9. <sup>1</sup>H NMR spectrum of compound 2 in C<sub>5</sub>H<sub>5</sub>N at 600 MHz**



**Figure 10. <sup>13</sup>C NMR spectrum of compound 2 in C<sub>5</sub>H<sub>5</sub>N at 150 MHz**

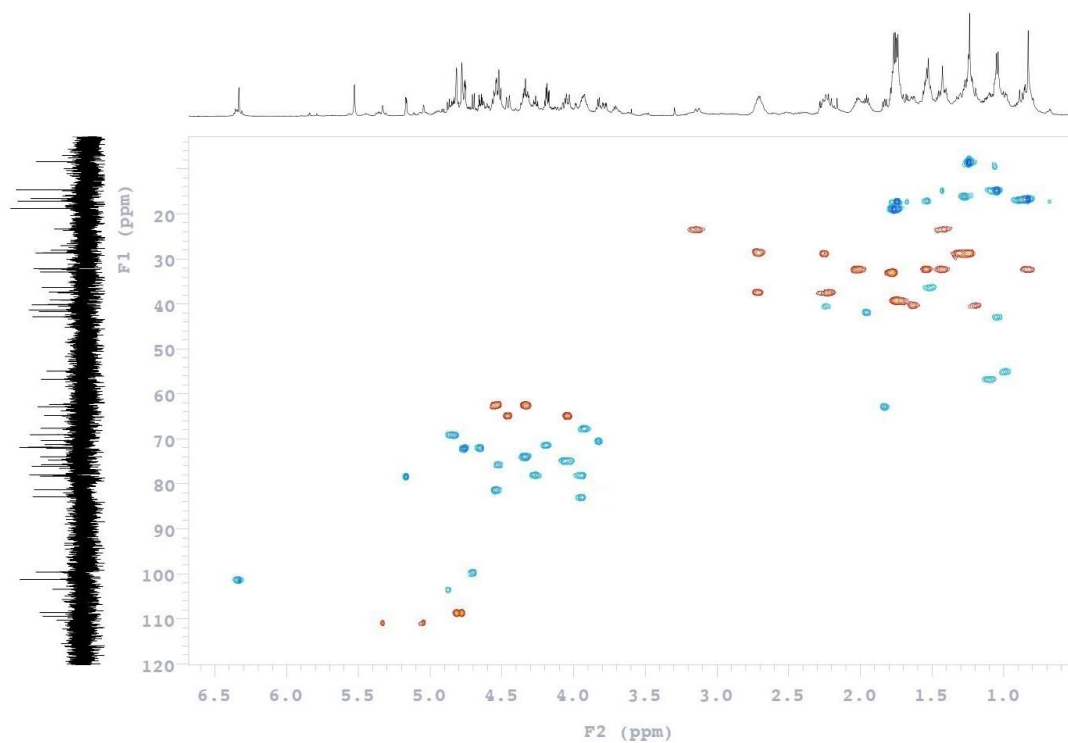


Figure 11. HSQC spectrum of compound 2 in  $C_5H_5N$  at 600 MHz

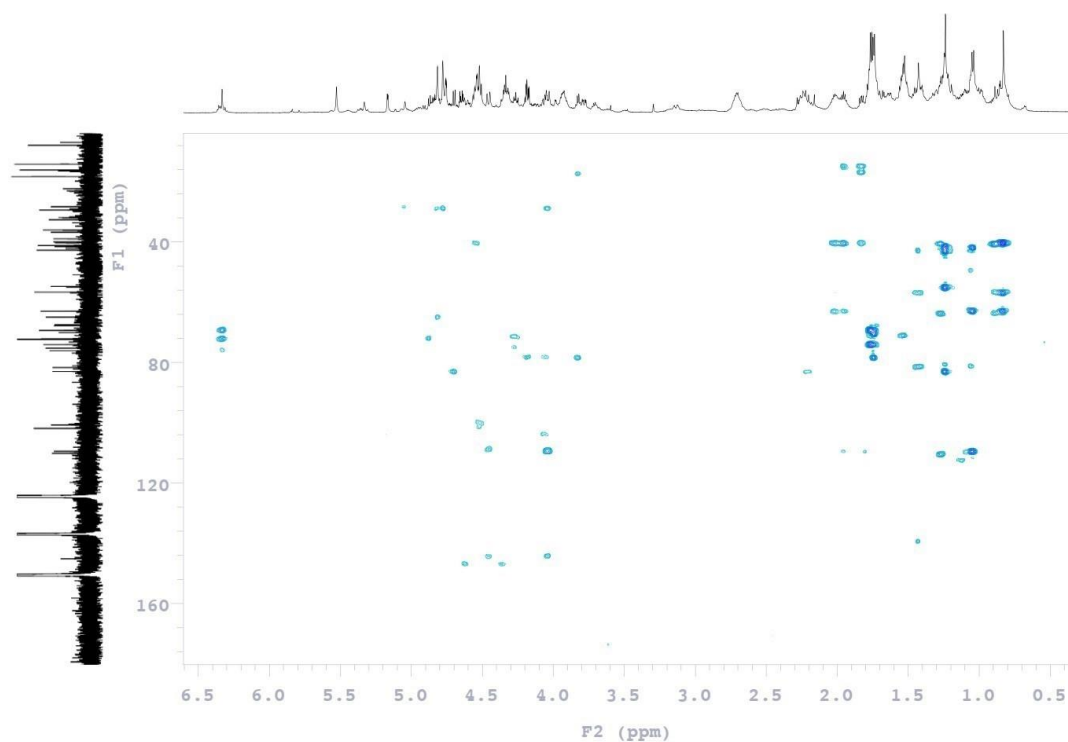
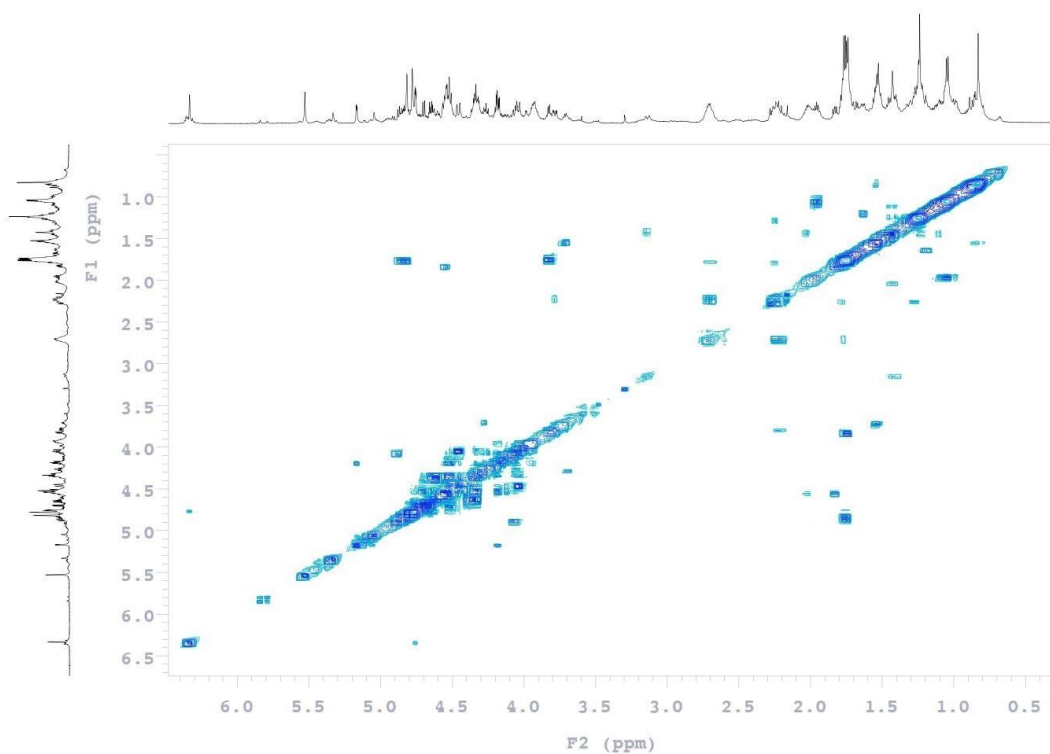
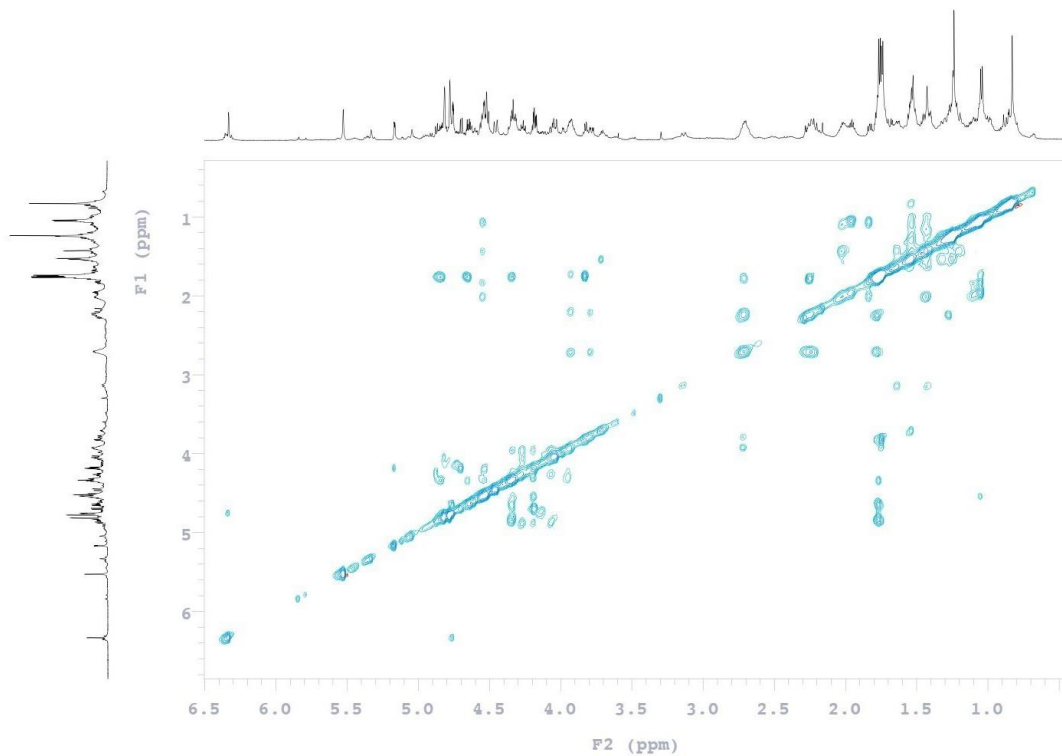


Figure 12. HMBC spectrum of compound 2 in  $C_5H_5N$  at 600 MHz

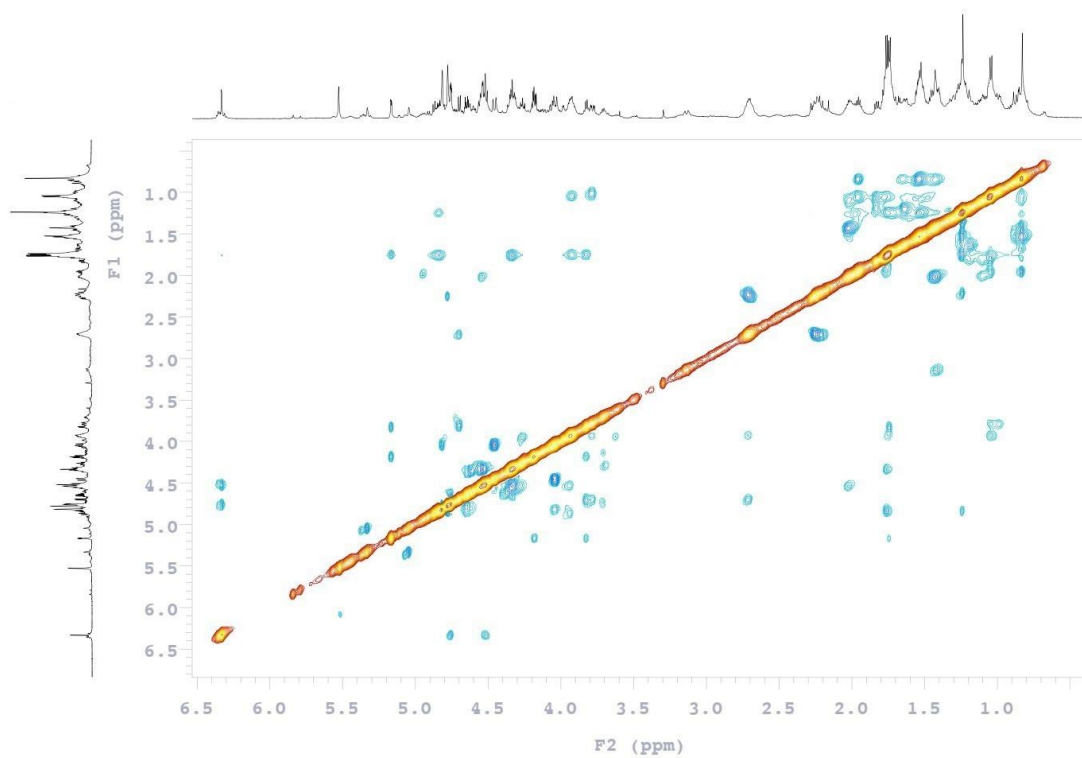


**Figure 13.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 2 in  $\text{C}_5\text{H}_5\text{N}$  at 600 MHz

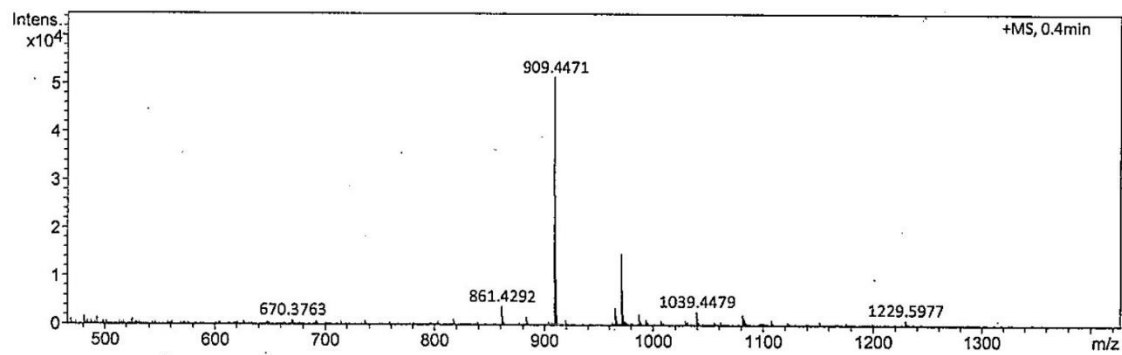


**Figure 14.** TOCSY spectrum of compound 2 in  $\text{C}_5\text{H}_5\text{N}$  at 600 MHz





**Figure 15.** ROESY spectrum of compound 2 in  $C_5H_5N$  at 600 MHz



**Figure 16.** Mass spectrum (HR-ESI-MS in positive-ion mode) of compound 2