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Isolation and Transformation of Tefrosin From The Seed of *Tephrosia Vogelii* With Selectfluor™

Ana Yulvia*[a], Elvira Hermawati[b], Ade Danova[b], Ika Oktavianawati[a], Muhammad Reza[a]

Abstract: In this study, tefrosin (1), a known phenolic compound, was successfully isolated and identified from the seed extract of *Tephrosia vogelii*. The structure of this compound was determined based on 1D and 2D NMR spectroscopy. Furthermore, the isolated compound was transformed using 0.5 equivalent of selectfluor™ in acetonitrile solvent at 100 °C for 3 hours. The reaction product, namely dehydrotephrosine (2), is new reaction product from selectfluor™ reagent as a catalyst in tertiary alcohol dehydration in aromatic group. This finding highlights the effectiveness of selectfluor™ as a catalyst in dehydration reactions, demonstrating its potential to introduce new chemical properties to compounds. The study underscores the versatility of selectfluor™ and its ability to facilitate the generation of valuable derivatives from phenolic compounds. These results provide insights into the reactivity of tefrosin and offer a new approach for chemical transformations involving phenolic substrates.

Keywords: Tefrosin, *tephrosia vogelii*, isolation, transformation, selectfluor™.

INTRODUCTION

The plant *Tephrosia vogelii* (*T. vogelii*) Hook. f. (Fabaceae) is widely grown in the tropics and subtropics [1], especially in Africa and India [2]. Based on ethnopharmacological investigation, several species are used as folk medicine for treatment of several diseases. For example, the roots of *T. vogelii* was used to eliminate fungal infections, treat tuberculosis, and treat typhoid fever [3, 4]. The leaves of *T. vogelii* was used as a poison for fish, an abortion agent, menstruation inducer, and a laxative [2, 5-8]. Previous phytochemical studies on the *T. vogelii* resulted in the isolation of various types of secondary metabolites derived from flavonoids and isoflavonoids including flavones, flavanones, chalcones, isoflavones, and rotenoids [1].

Several reports on the genus *Tephrosia* have demonstrated the presence of the oxygen functional groups that are common in aromatic compounds are found in the form of methylated. Furthermore, generally these compounds also exist as isoprenyl derivatives, either in the free state or cyclized with the -OH group to form a pyran group such as tefrosin which have been isolated from the leaves and roots of *T. vogelii* [9-11]. As an oxygenated aromatic derivative, it can be assumed that the aromatic ring in secondary metabolites of genus *Tephrosia* acts as a nucleophile.

Nowadays, much effort has been devoted to the synthesis and structural modification of various flavonoids to alter their biological properties. Selectfluor™ or 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octanebis[tetrafluoroborate] is an electrophilic fluorination reagent that is reported to play an important role in the fluorination of drug candidate compounds [12]. Replacing hydrogen with fluorine in organic compounds can create significant changes in the biological activity and physical properties of the original compound [13]. Based on literature studies, selectfluorTM is a 'pseudopositive' or 'electrophilic' fluorine source that can be used in electrophilic substitution reactions, either in aromatic rings or positive hydrogen in aliphatic structures [14].

In addition, selectfluor™ also has other chemical properties that is playing a role in non-fluorination reactions including oxidation of benzylic alcohol, oxidation of tertiary carbon centers, mediating reactions between aldehydes and imines to produce homoallylic alcohols and amines, and removing several groups of protecting groups [15, 16]. It has been reported in previous studies that alkylated aromatic rings can undergo iodination reactions with excellent results when reacted in acetonitrile using iodine and selectfluor™ [17]. Inspired by the facts above, it is certainly very interesting to further examine other plant parts of *T. vogelii* and study the chemical properties of flavonoid and isoflavonoid derivative compounds isolated from the seeds of *T. vogelii* using the selectfluor™ reagent.

METHODS

General experimental prosedures

1D and 2D NMR spectra were measured in chloroform-d with a spectrometer of Agilent DD2 system operating at 500 (^{1}H), 125 (^{13}C), and 470 (^{19}F) MHz. Vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were conducted on Merck silica gel 60 GF_{254} art. 7731 and 7749, respectively. Thin layer chromatography (TLC) analysis was done using precoated silica gel plates (Merck Kielselgel 60 GF_{254}, 0.25 mm thickness). Spots on TLC were detected by UV irradiation, and sprayed by 1.5% $Ce(SO_4)_2$ in H_2SO_4, which was followed by heating. Solvents (MeOH, acetone, EtOAc, and n-hexane) for extraction, fractionation, and purifcation were of technical grades, which were distilled before used. $CHCl_3$ used in the purifcation was a pro analysis grade. The isolated compounds were transformed using 0.5 equivalents of selectfluor™ in acetonitrile at 100 °C for 3 hours.

Plant materials

In January 2021, the seed samples of *Tephrosia vogelii* were collected from Bandung, located in the West Java Province of Indonesia, and transported to the BSCA Building within

[a] A. Yulvia, I. Oktavianawati, M. Reza
Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Jember
[b] E. Hermawati, A. Danova
Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung
*e-mail: anayulvia@unej.ac.id

Faculty of Mathematics and Natural Sciences at the Institut Teknologi Bandung.

Extraction and isolation

The air-dried and powdered seeds of *Tephrosia vogelii* (2.0 kg) were maserated with acetone for 3x24 hours at room temperature. After the acetone extract was partitioned with n-hexane then removal of the solvent under reduce pressure, the acetone extract (20.7 g) was obtained. The acetone extract was then chromatographed with a vacuum liquid chromatography (VLC) (Merck silica gel 7731, 20 g) using gradient elution with n-hexane-EtOAc (9:1 to 1:1, 10% stepwise EtOAc addition, followed by EtOAc and MeOH, each 2 × 200 mL) to yield five fractions (F1-F5). The fraction F3 (1809 mg) was further fractionated with a vacuum liquid chromatography (VLC) with a gradient solvent system (eluent: n-hexane-EtOAc = 19:1) then purified with a radial chromatography (eluent: n-hexane-EtOAc = 39:1) to yield compound **1** (1880 mg), which were determined using 1D and 2D NMR methods.

Transformation with selectfluor™

The isolated compound **1** (200 mg) from the seeds of *T. vogelii* was reacted with 0.5 equivalents of selectfluor™ in acetonitrile (CH_3CN) for 3 hours at at 100 °C. Analysis of the reaction products formed was carried out based on a comparison of the TLC pattern between the isolated compound and the reaction product. Based on the TLC analysis of the reaction showed that the reaction product is formed, further separation and purification are carried out. Separation and purification of the reaction product using a radial chromatography (eluent: n-hexane-EtOAc = 19:1) to yield compound **2** (56 mg), which were determined using 1D and 2D NMR methods and compared with the starting material, compound **1,** to analyze the structural changes that occurred after the transformation with Selectfluor™

RESULT AND DISCUSSION

Compound **1** was obtained as a white solid. The ^{13}C-NMR spectrum of compound **1** (**Table 1**) shows the presence of 23 carbon signals consisting of two carbon signals in the methyl shift δ_C 28.3 and 28.52 ppm; two carbon signals originating from the methoxy group δ_C 55.8 and 56.3 ppm; four carbon signals from alkoxy groups (-O-CH_2; –O-CH; and -O-Cq), δ_C 63.8; 67.4; 76.2; and 78.0 ppm, respectively; nine carbon signals from sp^2 alkene carbon (δ_C 101.1; 108.6; 109.1; 109.4; 111.1; 111.9; 115.4; 128.6; and 128.8 ppm); five carbon signals from the oxyaryl group δ_C 143.9; 148.4; 151.1; 156.6; and 160.8 ppm; and one signal from the conjugated carbonyl group δ_C 191.4 ppm. From the ^{13}C-NMR signal it can be seen that compound **1** has an isoprene group which is characterized by the presence of two methyl groups (δ_C 28.3 and 28.5 ppm). The presence of an alkoxy group (–O-CH_2) in compound **1** is a characteristic of rotenoid compounds. The absence of one -CH carbon signal and the addition of one alkoxy carbon signal indicates that compound 4 has one –OH substituent as evidenced by the presence of a wide singlet signal at δ_H 4.47 ppm. Additionally, the ^{1}H-NMR spectrum (**Table 1**) shows that most of the signals are in the aliphatic shift δ_H < 6 ppm and the aromatic shift δ_H 6-8 ppm. The presence of many signals in the aromatic shift indicates that compound **1** is an aromatic compound. Other evidence that confirms that compound **1** is a rotenoid group is the presence of three double doublet (dd) proton signals at δ_H 4.57 ppm (J= 2.4; 1.2 Hz); 4.63 ppm (J= 12.1; 2.5 Hz); and 4.50 ppm (J= 12.1; 1.2 Hz), two shielding carbon signals at δ_C 76.2 and 63.8 ppm, one ketone conjugated carbon signal at δ_C 191.4 ppm. Thus, it can be concluded that compound **1** has a structure that matches with tefrosin (**Figure 1**) which has been reported in previous studies.

Compound **2**, the dehydration products of compound **1**, there is a change in the aromatic proton signal to the more deshielding area, namely at the C-5 position (δ_H 8.04 ppm) and C-6' (δ_H 8.45 ppm). This is due to the anisotropy effect of alkenes formed at C2-C3. Based on the ^{13}C-NMR signal it can be seen that compound **2** has two sp^2 carbons (δ_C 110.6 and 156.2 ppm) which previously appeared in compound **2** as an alkoxy carbon signal. This indicates that compound **2** experienced the addition of one alkene group. The appearance of the addition of the alkene group was confirmed by the ^{1}H-NMR spectrum which was characterized by the disappearance of one double doublet (dd) proton signal and one widened singlet signal at δ_H 4.46 ppm as well as the presence of a singlet proton signal for oxymethine at δ_H 5.02 ppm. This is supported by the HMBC data (**Figure 2**), which shows a correlation between the hydrogen at position 2a and the carbons at C-2, C-3, and C-2'. This compound was identified as the dehydration derivative of compound **1**, dehydrotephrosine **(2)**, resulting from the loss of the hydroxyl group at the tertiary alcohol center and the formation of a double bond. These results demonstrate that selectfluor™ can act as an effective catalyst for the dehydration of tertiary alcohols in aromatic compounds.

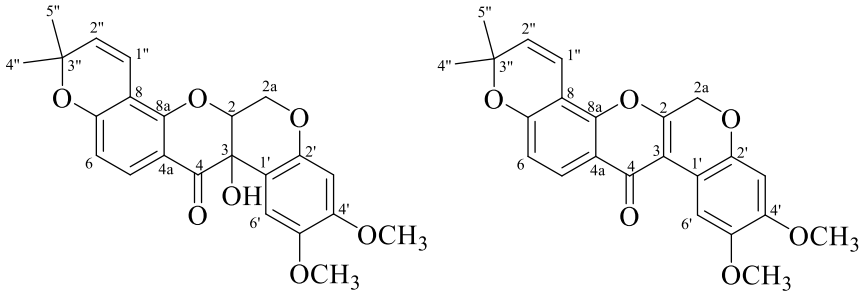

Figure 1. Structure of tefrosin **(1)** and dehydrotephrosine **(2)**

Table 1. ^{1}H-NMR[a,c] and ^{13}C-NMR[b,c] data of tefrosin **(1)** and dehydrotephrosine **(2)**

Position	Tefrosin **(1)**		Dehydrotephrosine **(2)**	
	δ_H (*mult, J* Hz)	δ_C (ppm)	δ_H (*mult, J* Hz)	δ_C (ppm)
2	4.57 (dd, 2.4 & 1.2)	76.2	-	156.2
3	-	-	-	110.6
3-OH	4.47 (s br)	67.4	-	-
4	-	191.4	-	174.4
4a	-	111.1	-	111.8
5	7.73 (d, 8.9)	128.6	8.04 (*d*, 10.0)	126.5
6	6.47 (d, 8.9)	111.9	6.87 (*d*, 10.0)	115.4
7	-	160.8	-	157.2
8	-	109.1	-	109.1
8a	-	156.6	-	151.1
2a	4.63 (dd, 12.1 & 2.5) 4.50 (dd, 12.1 & 1.2)	63.8	5.02 (*s*)	64.9
1’	-	108.6	-	118.5
2’	-	148.4	-	146.3
3’	6.48 (s)	101.1	6.55 (*s*)	100.4
4’	-	151.1	-	149.0
5’	-	143.9	-	144.1
6’	6.57 (s)	109.4	8.45 (*s*)	110.0
1’’	6.60 (d, 10.1)	115.4	6.77 (*d*, 10.0)	114.7
2’’	5.56 (d, 10.1)	128.8	5.73 (*d*, 10.0)	130.6
3’’	-	78.0	-	77.8
4’’	1.45 (s)	28.5	1.26 (*s*)	29.7
5’’	1.39 (s)	28.3	1.50 (*s*)	28.1
4’-OCH_3	3.82 (s)	56.3	3.87 (*s*)	55.9
5’-OCH_3	3.73 (s)	55.8	3.96 (*s*)	56.3

[a] Data were recorded at 500 MHz
[b] Data were recorded at 125 MHz
[c] Data were measured in chloroform-d solvent

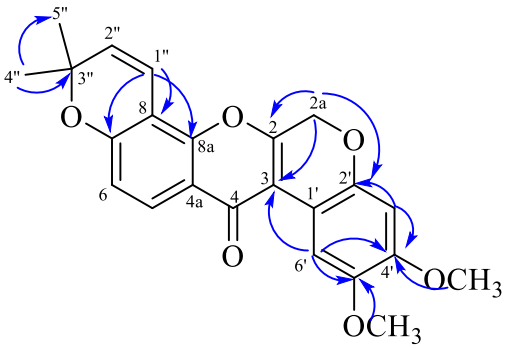

Figure 2. HMBC Correlations of dehydrotephrosine **(2)**

CONCLUSION

This study successfully isolated tefrosin from *Tephrosia vogelii* seeds and identified its structure using 1D and 2D NMR spectroscopy. Tefrosin **(1)** was then transformed into a new compound, dehydrotephrosine **(2)**, using selectfluor™ as a catalyst, demonstrating its effectiveness in dehydrating tertiary alcohols. The research highlights the ability of selectfluor™ to create novel derivatives from phenolic compounds, offering new insights into the chemical reactivity of tefrosin **(1)** and presenting potential approaches for transforming phenolic compounds in organic synthesis. These findings underscore the versatility of selectfluor™ as a catalyst and its significance in expanding the scope of chemical transformations for various applications, including drug development and structural modifications of aromatic compounds.

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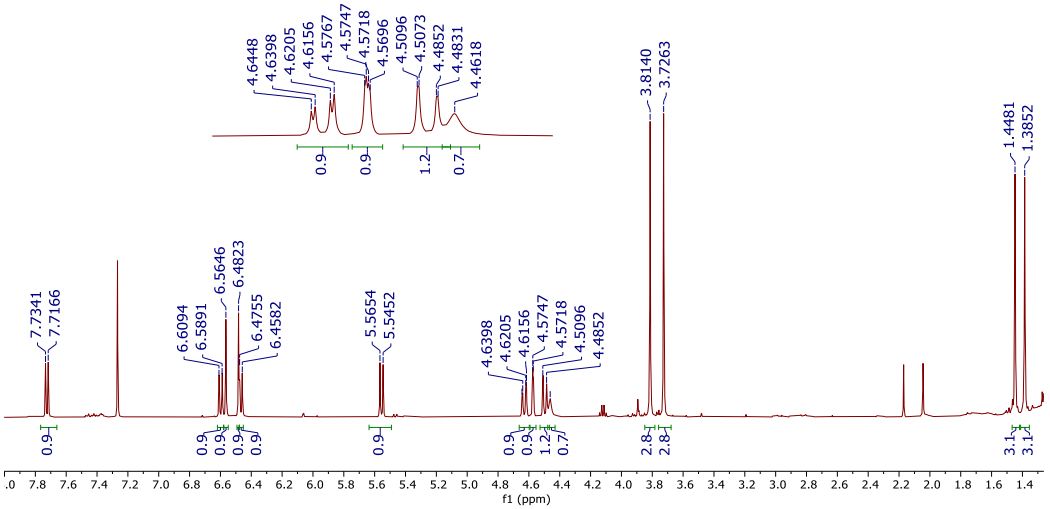
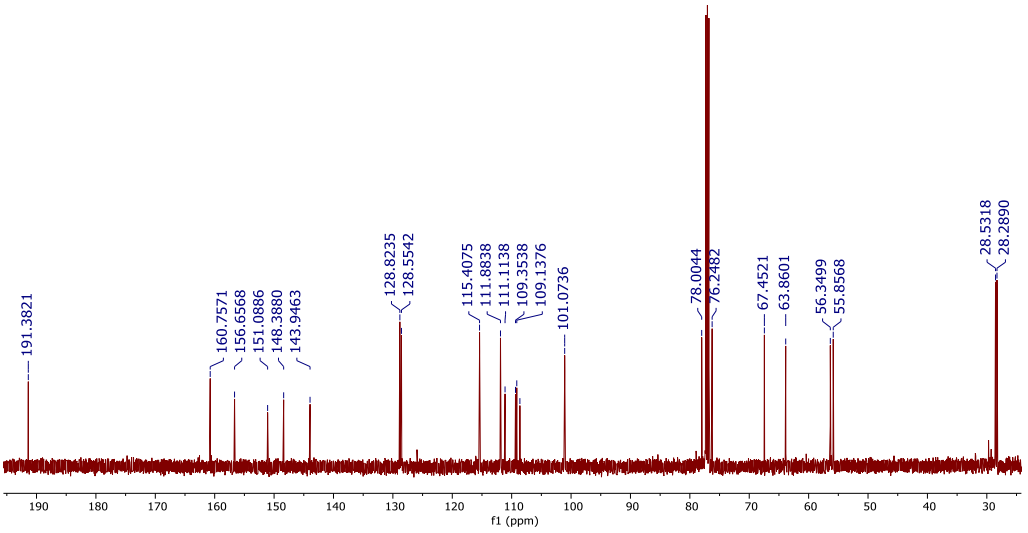
APPENDICES

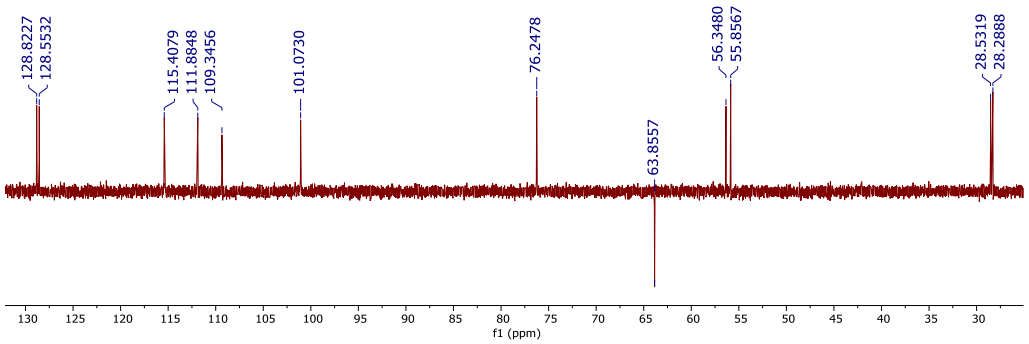
^{1}H-NMR Spectrum of Tefrosin **(1)** (500 MHz, $CDCl_3$)



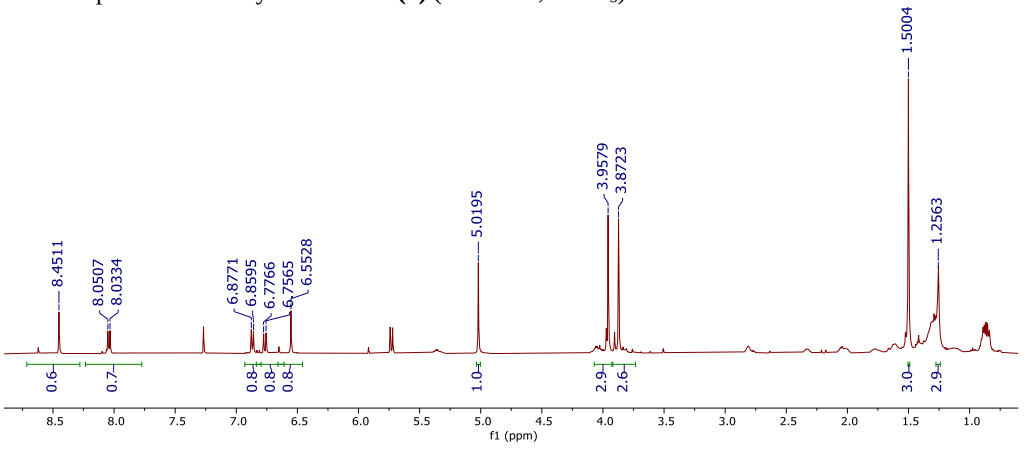
^{13}C-NMR Spectrum of Tefrosin **(1)** (125 MHz, $CDCl_3$)



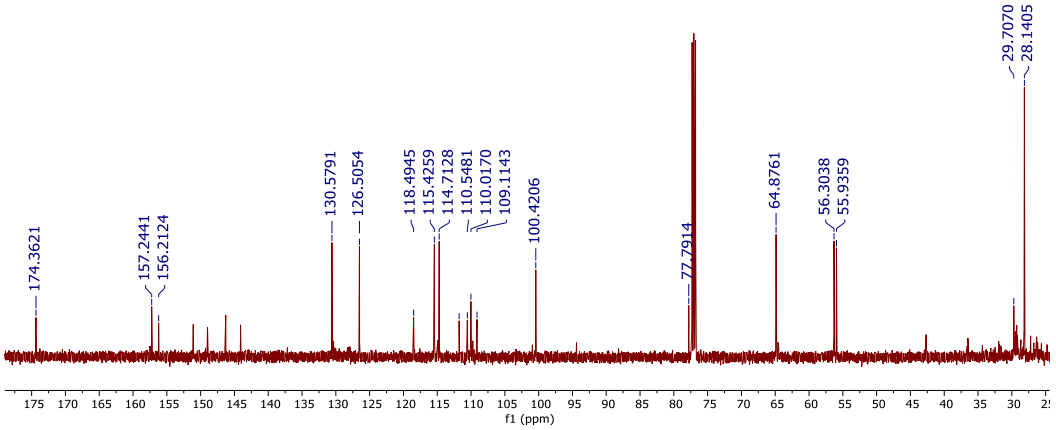
DEPT-135 Spectrum of Tefrosin **(1)**



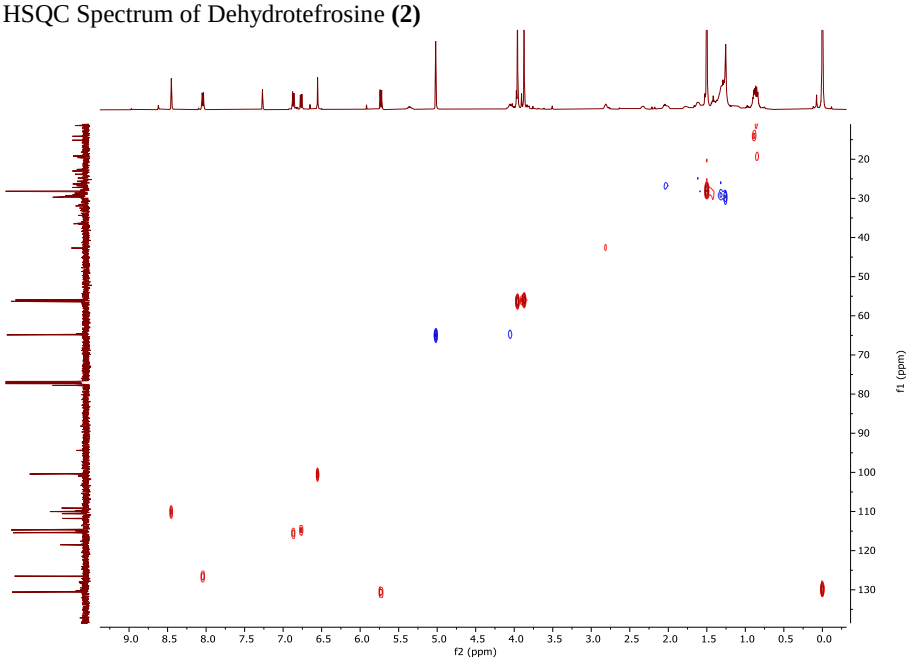
^{1}H-NMR Spectrum of Dehydrotefrosine **(2)** (500 MHz, $CDCl_3$)



^{13}C-NMR Spectrum of Dehydrotefrosine **(2)** (125 MHz, $CDCl_3$)



HSQC Spectrum of Dehydrotefrosine **(2)**



HMBC Spectrum of Dehydrotefrosine **(2)**