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Isolation And Structural Elucidation Of Two New Compound Munduleaxanthone And Jipaflavonone From Mundulea Antanossarum Baill. (Syn. Mundulea Anceps Var. Mangokyensis R.Vig. P.P.A, Leguminosae) Originated From Madagascar

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Abstract – Mundulea antanossarum (Leguminosae), known by its vernacular name "Malaignarety", is a species of plant endemic to the southern part of Madagascar. It is a very important plant for society in this region, due to its therapeutic virtues in traditional Malagasy medicine.
Phytochemical studies carried out on the pith of this plant isolated two pure products, and their chemical structures were elucidated using mass spectrometry (TOF-SM-EIS), ultra-violet visible (HPLC-UV-DAD), infra-red (IR) and nuclear magnetic resonance spectroscopy (1D and 2D NMR).
The results of our studies carried out on this plant led to the discovery of two new molecules, Munduleaxanthone and Jipaflavonone, included in the flavonone chemical family with a reputation for biological activity.

Keywords – Munduleaxanthone, Jipaflavonone, Mundulea Antanossarum, Phytochemistry, NMR (1D and 2D) and TOF-SM-EIS.

I. INTRODUCTION

Madagascar abounds in an extraordinary wealth of flora with a very high rate of endemicity [1, 2, 3,4], of the twelve thousand species recorded, 85% are endemic to Madagascar [5-7]. This large island is isolated in the middle of the Indian Ocean and has all the characteristics of a continent [8]. It is world-renowned for its exceptional biodiversity and high rate of endemicity [9, 10]. This variety and endemicity of Malagasy biodiversity is due to the variation of climates in Madagascar from more arid regions to regions saturated with humidity [11]. Malagasy flora is therefore of particular scientific interest due to its diversity, age and great richness. As such, this biodiversity hot spot is a prime area for research into new molecules of biopharmaceutical interest [12, 13].

For this reason, many national and/or foreign scientific researchers are trying to unlock the secrets of Madagascar's plants through traditional medicine and empirical plant use [14], to discover their secrets, in an attempt to find one or more new molecules of biopharmaceutical interest.

This situation prompted us to carry out phytochemical studies on the plant known by the vernacular name "Malaignarety" (Malagasy name, Mahafaly dialect) and scientifically called Mundulea antanossarum (Leguminosae).

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It is an endemic plant of southern Madagascar and is highly recognized in this region and important to the local population for its therapeutic virtues.

The aim of this study is to investigate and isolate the new molecule(s) present in the pith of this plant, using fractionation techniques with a chromatography column (silica gel, sephadex).

II. MATERIALS AND METHODS

2.1 General

Silica gel 60 and 100, and TLC precoated plates were purchased from Merck, Darmstadt, Germany, Analytical HPLC was performed on a Waters system (Millenium 32 workstation, 2690 separation module, 996 photodiode array detector) equipped with HiChrom Lips 100-5- 250D column (4.6 x 250nm: LiChrospher Phase 5 µm, Si 100). A Perkin-Elmer 241 polarimeter was used for measurement of optical rotation.

The 1D and 2D vasoconstrictive, hypertensive, and cardiac stimulant action and can act as an allergenic substance. Salsola species have antioxidant and anti-inflammatory properties (Ahlam & Fatma).

Alkaloid experiments were performed at 600 MHz and 400MHz for 1 H and 13Crespectively, on a Bruker Avance 600MHz instrument equipped with an Ultra Shield Plus magnet and triplet resonance cryoprobe with gradient unit. Sample temperatures were stabilized at 298 K. The deuteriomethyl13C signal and the residual 1 H signal of the solvent (DMSO-D6) were used as secondary references (δ39.6and δ2.49 from tetramethylsilane, respectively). The exact molecular mass (HRMS) of molecular ion $[M-H]^+$ and fragment F^+ were determined with a Micromass QTof-2 mass spectrometer equipped with an electrospray ion source and a Micromass QTof-2JOEL mass spectrometer equipped with a Direct Analysis in Real Time (DART) atmospheric pressure ion source.

2.2 Plant material

The plant pith samples were collected in Tranoroa village, district of Ampanihy (Southwest of Madagascar) on April 2019. The plant sample was identified using taxonomic keys and by comparison with reference specimens available at the Department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo. Voucher specimens with assigned sample number PIJ-01 was deposited at the herbarium of the Laboratory of Applied Chemistry, Layflaylle Street, University of Toliara, Madagascar.

2.3 Extraction

The plant of stem bark (16kg of Mundulea antanossarum) was kept at room temperature (25°C to 30°C) for air drying (two week). The air-dried powdered stem bark of Mundulea antanossarum (Leguminosae) (12kg) was extracted by repeated with a mixture of water-ethanol (20/80) at room temperature. After filtering the mixture, aqueous ethanol filtrates were pooled dried over Na_2CO_3 and evaporated to dryness under reduced pressure using a rotary evaporator to yield crud extract.

The crude extract were suspended in water and sequentially partitioned with n-hexane, dichloromethane, ethyl acetate, n-butanol to yield the corresponding extract fractions. The different extracts were evaporated to dryness on an evaporator apparatus to yield the different crud extract. All extracts were stored at 4°C.

2.4 Isolation

Fourteen grams (14g) of the ethyl acetate crude extract was first subjected to fractionation using silica gel column chromatography eluting whit a mixture of hexane/ethyl acetate/methanol (2/7/1), the column was in isocratic regime and at the end, it resulted into seven fractions. The fraction was selected for the following steps. The fraction F4 was checked for its purity by analytical TLC, and the zones were detected both with a UV lamp at 254 nm and 365 nm and by spraying with sulfuric vanillin acid, followed by heating at 120 °C during 1-5 min. The fraction F4 was resubmitted to silica gel column chromatography. The elution was done using cyclohexane and a gradient of ethyl acetate, which resulted into five fractions and 180 mg of F43, was subjected to further purification using a silica gel column chromatography, with hexane and a gradient of ethyl acetate for elution. The latter provided two pure compounds. The purity of Munduleaxanthone and Jipaflavonone was then detected by analytical HLPC with the mixture of chloroform and methanol 1:1 (v/v) as mobile phase, and the chromatography was performed with

isocratic regime during 25 min. The eluted compound was detected based on its UV absorption in the wavelength range from 190 nm to 315 nm.

III. RESULTS

The stem bark powder of Mundulea antanossarum (Leguminosae) collected from Tranoroa village, district of Ampanihy (Southwest of Madagascar), was extracted with a mixture of water-ethanol (20/80). The crude extract was suspended in water and was partitioned successively with different organic solvents of croissant polarity (Hexane, methylene chloride, ethyl acetate and n-butanol) in order to yield with the corresponding soluble extract. Repeated silica gel column chromatography of the ethyl acetate-soluble extract led to the isolation of Munduleaxanthone and Jipaflavonone, in pure forms as proved by HPLC analysis.

3.1. Determination of pure product structures

3.1.1. Ultraviolet-visible mass spectrometry analysis of compound-1

The result of product-1 using Q-TOF mass spectrometry equipped with chromatographic separation and a 6.97eV electron-sprite ionization source (EIS) operating in positive mode shows a parent peak corresponding to the molecular ion of m/z = 413.62045 $[M+H]^+$. This molecular ion has enabled us to hypothesize that the gross formula of product-1 corresponds to $C_{22}H_{20}O_8$.

The UV-visible spectrum of this molecule recorded by HPLC-UV-DAD shows a maximum at 273nm and 204nm. This maximum absorption band shows the presence of dihydroxyflavonone in this molecule. The IR spectrum proves the presence of this dihydroxyflavonone skeleton in product-1, as their λmax at 1619 Cm^{-1} and 3475 Cm^{-1} show that ketone groups and hydroxyl functions are present in the chemical structure of product-1.

From the literature written by Fatiany Pierre Ruphin et al, 2015 (Journal of Pharmacognosy and Phytochemistry, 2015; 3(6): 155-160) mentioned that this maximum absorption band is similar to that of Elieaflavonone. However, the crude formula of product-2 differs from that of Elieaflavonone by two carbons, two hydrogens and three oxygens, corresponding to the molecular ion of m/z = 74, which explains the presence of a hydroxyl function and an ethanoate compared to Elieaflavonone, In addition, the number of unsaturations and rings (NIC) of product-2 shows that their chemical structure must comprise thirteen (13) unsaturations according to our calculation given by NIC = X-Y/2+Z/2-1 ($N_XH_YN_ZO_T$). Structural analysis of Elieaflavonone, then, led us to a second hypothesis about the chemical structure of product-1, since Elieaflavonone must be substituted by a hydroxyl function and an ethanoate.

3.1.2. One-dimensional proton and carbon-13 NMR analyses of product-1

a. One-dimensional proton NMR spectrum (1D - ^{1}H NMR)

The general appearance of the 1D proton spectrum gives an idea of the protons present in product-1, enabling us to verify the hypothesis of the chemical structure of compound-1 on the above Q-TOF-EIS-SM analysis result. The proton displacements of product-1 emerge in the spectral band between 1.50 and 12.34ppm. This spectral band a reveals the four specific proton features including:

- Protons emerge a δH between 1 and 4 attributed to alkyl protons,

- Protons emerging in the spectral band between 4ppm and 6ppm are attributed to alkene protons,

- protons emerging between 6ppm and 7.50ppm are protons from benzene rings, and protons emerging between 11 and 12.40ppm are labile protons with acidic (OH) properties.

Interpretation of the proton 1D-NMR spectrum revealed:

- The presence of a very long singlet peak at δH 1.50; the area of integration of this peak attributed to six protons and these protons signifying the presence of two methyl groups linked in a ring indicating the characteristics of 2,2-dimethyl-chromane,

- Two doublet signals emerge at 3.74ppm and 3.81ppm respectively, unambiguously attributed to methylene (-CH_2-) protons from different chemical environments,

- Two signals in doublet form emerge at δH 5.95 and δH 6.88 respectively are from two alkene protons attributed to dimethyl-pyran protons.

Next, analysis of this 1D proton spectrum identified the presence of four aromatic ring protons with different characteristics:

- Two doublet signals at δH 7.16 and δH 7.24 respectively are attributed to benzene protons with axially symmetrical features,

- The two singlet signals at δH 6.21 and δH 5.69 respectively are attributed to the benzene protons of rings A and B.

b. Interpretation of carbon-13 1D-NMR spectra (^{13}C-NMR)

Carbon 13 NMR is the same as ^{1}H, but differs in natural abundances. The ^{13}C-NMR spectrum of product-1 is performed in Broad Band (BB) recording mode, and the signals of observed carbons appear as vertical lines. In fact, the BB spectrum of product-1 shows that it comprises twenty-two (22) carbons, including:

Ten quaternary carbons are identified:

- The two signals emerging at 176.2ppm and 196.9ppm are unambiguously attributed to carbonyl carbons of the acid function and the pyranozyl carbonyl group respectively,

- The peaks of the carbons that emerge at 101.7ppm, 102.6ppm, 133.6ppm, 139.9ppm, 160.2ppm, 161.5ppm and 163.3ppm respectively are attributed to the ethylenic quaternary carbons of the benzene rings, and then the signal emerges at 77. 18ppm is attributed to the quaternary carbon of the chroman ring and the carbon peaks emerge respectively at 104.7ppm a quaternary and at100.7ppm a CH are all attributed to pyranozyl group carbons.

Again, in the BB spectrum, five very characteristic carbon signals are observed, at 97.2ppm, 116.2ppm, 127.5ppm, 128.3ppm and 129.9ppm respectively, attributed to the CH carbons of the benzene rings. In addition, two carbons emerge at the same time at 127.5ppm and 129.9ppm.

This explains the presence of a di-substituted benzene ring in the para position in this molecule, even though it has an axis of symmetry.

Two outgoing carbon signals at δC 28.2 and δC 40.17 are attributed to the methyl group (CH_3) and methylene (CH_2) respectively, and the peak at δC 28.2 is very intense, signifying the presence of two methyl groups.

3.1.3. Two-dimensional NMR analysis of product-1

a. COSY spectrum (Correlation Spectroscopy)

It is used to identify protons linked by scalar coupling. These are ^{1}H-^{1}H correlations in a square. Indeed, we observe the following correlations:

The proton exiting at δH 7.24 as a doublet is coupled with the proton exiting δH 7.16 as a doublet too,

The proton exits at 5.98ppm as a doublet correlates with the proton exits at 6.88ppm as a doublet,

The proton exits at δH 5.69 with weak correlations with labile protons in the form of humps that were poorly resolved, exiting at 4.14ppm and 3.72ppm respectively.

b. HSQC spectrum (Heteronuclear Single Quantum Correlation)

This shows the correlations between protons and the carbons that carry them. The spots indicate the connectivity between the carbon and the proton. It can be used to deduct the number of protonated carbons and the corresponding proton allocation to each carbon, so that non-equivalent geminate protons can be easily identified. The results of HSQC analysis are presented in Table 1.

Table.1: HSQC (^{13}C - ^{1}H) and COSY (^{1}H - ^{1}H) correlation of the product-1 molecule.

δ (1H) ppm and their characteristics	Multiplicité	δ (13C) ppm	COSY
1.50 (methyl proton)	s	28.2	-
3.27(OH)	s	104.7	-
3.71 (methylene proton not equivalent)	d	40.17	3.84
3.84 (methylene proton not equivalent)	d		3.71
4.14 (OH)	s	100.7	-
5.69 (dihydroxyflavonone proton)	m	100.7	3.27 et 4.14
5.98 benzene proton	d	128.3	6.88
6.21 benzene proton	s	97.2	-
6.88 benzene proton	d	116.1	5.98
12.12 (OH phenol)	s	160.2	-
12.34 (OH acid)	s	176.2	-

c. Hetero - Molecular Band Correlation (HMBC) spectrum of compound-1

The HMBC spectrum of compound-1 identified correlations between protons and long-range carbons, generally at α and β, and substantiated the connectivity of the molecule. Indeed, we identify correlations concerning alkene protons that were attributed to dimethyl -pyran protons namely from:

The proton exiting at 6.88ppm carried by the carbon at δC 116.10 correlates with the three quaternary carbons exiting at 77.18ppm, 160.2 ppm and 163.3ppm respectively. According to COSY, the proton at δH 6.88 is coupled with the proton at δH 5.98, carried by the carbon at δC 128.3, yet this proton shows a task of correlations with the carbons exiting at 102.6ppm a quaternary and the methyls at 28.20ppm.

Then the proton exits at 6.21ppm as a singlet attributed to the alkene proton of the benzene ring correlates with the carbons exiting at 101.7ppm, 102.6 and at 1161.5ppm. In addition, the hydroxyl proton (OH) exiting at 12.15ppm, in the Para position to this proton, correlates weakly with the carbons exiting at δC 102.6 and δC 101.7, and the two methyls exiting at 1.50ppm correlate with the carbon at 77.18ppm.

Taken together, the above correlations provide the first sequence (fig.1) of this product-1 molecule.

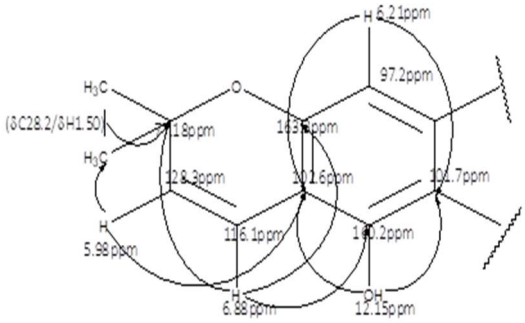


Figure 1: The molecule's first sequence

The second sequence of the molecular structure of product-1 was identified from the proton exits at δH 5.69. This proton correlates with the carbons exiting at 101.7ppm and 139.9ppm respectively, which are attributed to the quaternary alkene carbons of the benzene ring, and the carbon exiting at 196.9ppm attributed to carbonyl; then the hydroxyl proton exits at 4.14ppm carried by the carbon at δC 100.7 correlates with the carbons exiting at 196.9ppm and 104.7ppm carrying the proton (OH) exits at δH3.27.

Taken together, the above correlations give the second sequence of the product-1 molecule shown in figure.2.

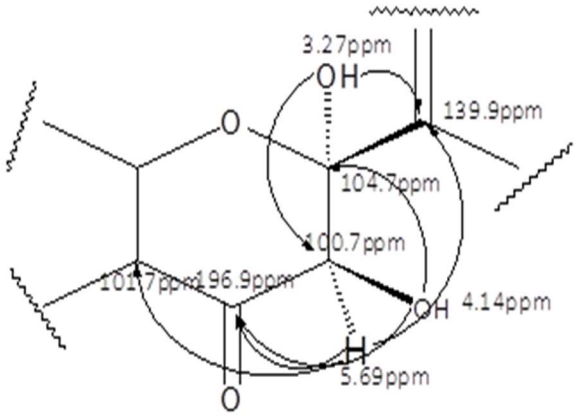


Figure 2: The second sequence of the molecule

The third fragmentation of the product.1 structure is determined from the correlation of two alkene protons of the benzene ring, which has an axis of symmetry of:

The proton exiting at 7.24ppm as a doublet carried by the carbon at δC 127.5ppm correlates with the carbons exiting at 133.6ppm and 104.7ppm. According to COSY, this proton is coupled with the 7.16ppm outgoing proton carried by the carbon at δC129.9ppm.

This doublet outgoing proton correlates with the quaternary carbon outgoing at 139.9ppm and also the methylene carbon at 40.17ppm carrying two non-equivalent geminal protons outgoing at 3.71ppm and 3.84ppm respectively. These two non-equivalent protons correlate with the benzene quaternary carbon at 133.6ppm and the carboxylic acid carbonyl at 176.2ppm.

Together, these correlations provide the final sequence for this molecule.

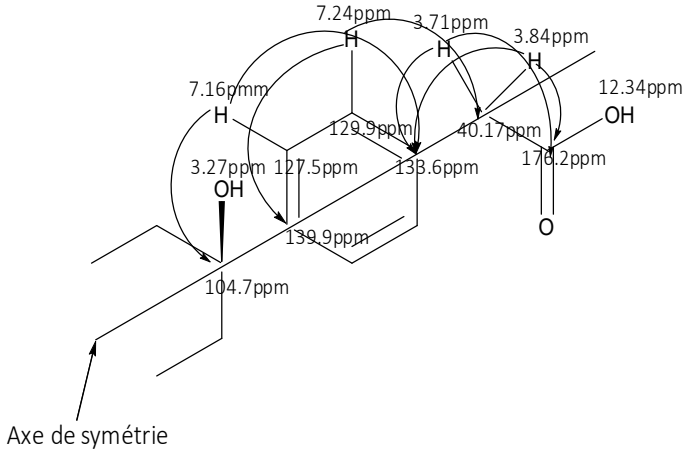
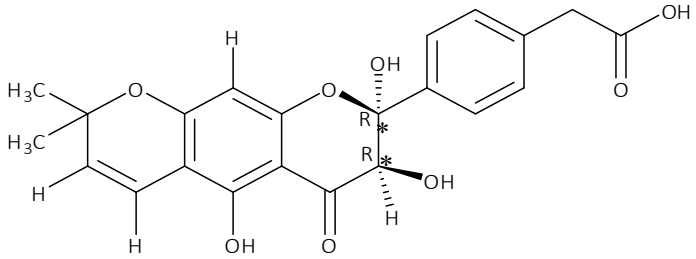


Figure 3: The sequence of the product-1 molecule

Putting together the three sequences of correlations obtained from the HMBC spectrum interpretations above, we obtain the complete structure of the molecule of the second pure compound (fig.4).



Molecular formula: C22H20O8

Molecular mass: 412

Molecular ion at m/z= 413.65045 $[M+H]^+$

Name: Jipaflavonone

Figure.4: Complete structure of product-1

Table.4: ^{1}H and ^{13}C chemical shift, the correlation ^{1}H-^{1}H COSY and important HMBC correlation of Jipaflavonone

Carbon N°	Types	1D NMR experiments ($_{1}H$ and ^{13}C)		2D NMR experiments	
		δH	δC	COSY	HMBC
2	COH	3.27(s)	104.7		C-1' et C-3
3	HCOH	4.14(s)			C-2 et C-4
		5.69 (s)	100.7		C-1' et C-10
4	CO (carbonyl)		196.6		
5	COH (phenol)	12.15(s)	160.2		C-6 et C-10
6	Cq		102.6		
7	Cq		163.2		
8	CH (benzenic)	6.21 (s)	97.2		C-6, C-9 et C-10
9	Cq		163.4		
10	Cq		101.7		
1'	Cq		139.9		
2'	CH (benzenic)	7.16(d)	127.5	H-3'	C-2, C-4' et C-6'
3'	CH (benzenic)	7.24(d)	129.9	H-2'	C-1', C-4'α et C-5'
4'	Cq		133.6		
4'α	CH_2 (gemini proton not equivalent)	3.71(d)		H-4'α_a	C-4'β et C-4'
		3.84(d)	40.1	H-4'α_b	C-4'β et C-4'
4'β	C-OH (acid)	12.34 (s)	176.2		
5'	CH (benzenic)	7.24(d)	129.9	H-6'	C-1', C-3'et C-4'α

6'	CH (benzenic)	7.16(d)	127.5	H-5'	C-2, C-2' et C-4'
2"	C-O(chroman core)		77.18		C-4'β
3"	CH (alkene)	5.98(d)	128.3	H-4"	C-5" et C-6
4"	CH (alkene)	6.88(d)	116.1	H-3"	C-2", C-5 et C-7
5"	2xCH_3	1.50(s)	28.2		C-2"

3.1.4. One-dimensional proton and carbon-13 NMR analysis of product-1

a. One-dimensional proton NMR spectrum (1D - 1H NMR)

Interpretation of 1D 1H NMR spectra is based on proton chemical shifts. Proton characteristics can be determined from these chemical shifts.

The signals from protons emerging at 12.34ppm, 11.85ppm, 11.43ppm and 10.44pmm respectively can be attributed to phenol or acid protons. However, the general chemical proton shift table shows that acidic mobile protons emerge between 10 and 13ppm, while these proton shift values are included in this spectral band.

These four protons are therefore attributed to phenol and acid protons.

Next, five proton signals are identified: three singlet signals emerge at δH 6.74, δH 6.87 and δH 6.97 respectively, and two doublet signals emerge at 7.29ppm and 7.33ppm respectively. These are all attributed to the alkenes of the benzene ring. In addition, signals emerge at δH 6.33ppm as a doublet and at δH 5.89 as a doublet from linear alkenes, respectively. The general chemical shift table justifies these assignments, as protons from linear alkenes emerge between 4.68ppm and 6.45ppm.

Finally, the signals emerging at 3.64ppm and 3.71ppm respectively, all in doublet form, are attributed to non-equivalent geminated methylene (-CH2-) protons. In addition, the proton at δH 1.06 ppm in doublet form attributed to the proton of the methyl group.

b. Interpretation of carbon 13 1D-NMR spectra (13C-NMR)

13C NMR recorded in BB mode identified twenty-six carbon peaks. In addition, the DEPT (Distortion less Enhancement by Polarization Transfer) spectrum revealed the presence of one methylene carbon and ten methyne and methyl carbons. Whereas, according to DEPT, compound-2 has at least thirteen quaternary carbons. Interpretation of the 13C spectrum revealed the carbon signals with their attributions. Indeed:

Eight highly characteristic carbon signals are observed:

Four signals at 128.9ppm, 130.2ppm, 133.6ppm and 136.7ppm respectively are attributed to the alkene carbons of the di-substituted benzene ring, which has an axis of symmetry passing through the quaternary carbons at δC 133.6 and δC136.7,

Two carbon signals emerge at δC 176.2 and δC 179.9 attributed to carbonyl groups, one attributed to a carbonyl group of an acid function, as according to 1H NMR, there is an acid proton emerges at 12.34ppm,

A very long signal emerges at 22.6 ppm, attributed to methyl groups,

Two carbon signals emerge at 34.2ppm and 40.8ppm, attributed to a methyne group and a methylene group respectively.

Next, we observe a series of carbon signals characteristic of a xanthone nucleus, namely: 102.9ppm, 107.3ppm, 109.5ppm, 113.4ppm, 121.4ppm, 124.9ppm, 132.0ppm, 138.2ppm, 148.5ppm, 155.0ppm, 156.2ppm and 159.4ppm. Two carbon signals emerge at δC 125.9 and δC 142.4, attributed to linear alkene carbons bonded to an aromatic ring with NIC equal to four, and their double bond conjugates with the unsaturation of the aromatic ring.

3.1.5. Two-dimensional NMR analysis of the 2-product

a. Correlation Spectroscopy (COSY) spectrum

Interpretation of the COSY spectrum of product-2 enabled us to identify the proton-proton correlation and multiplicity order. The results of these interpretations are shown in Table 3.

HSQC spectrum

Interpretation of the HSQC spectrum enabled us to draw up sufficiently visible correspondences between the prevailing proton-carbon chemical shifts, and to define the degree of carbon substitution, as shown in Table.3.

Table.3: HSQC (13C - 1H) and COSY (1H - 1H) correlation of the product-2 molecule.

δH (ppm)	δC (ppm)	multiplicity	COSY	Degree of substitution
1.06	22.6	d	2.52	-CH_3
2.52	34.2	m-d	1.06 et 5.89	(CH) methyne
3.64	40.8	d	3.71	-CH2- not equivalent
3.71		d	3.64	
5.89	142.4	d-d	2.52 et 6.33	(-CH=) linear alkenes
6.33	125.9	d	6.33	
6.74	102.9	s	-	Proton of a xanthone-type nucleus
6.87	121.7	s	-	
6.97	109.5	s	-	
7.29	130.2	d	7.33	Alkenic proton of di-substituted benzene ring
7.33	128.9	d	7.29	
10.44	138.2	poorly resolved	-	Phenol
11.43	159.4	Recife	-	
11.85	155.0	poorly resolved	-	
12.34	176.2	bump	-	Proton acid carboxylic

b. Heteronuclear Multiple Bond Correlation (HMBC) spectrum of product-2

Interpretation of the HMBC spectrum allows us to determine the correlation between carbons and protons, and to provide information on the sequence of my molecule.

First, we identify the correlations concerning the benzene protons of the xanthone ring. Indeed:

The proton exits at 6.74ppm carried by the carbon at δC 102.9 correlates with the carbons exiting at 113.4ppm, 124.9ppm and 156.2ppm respectively. Next, the singlet proton signal exits a at δH 6.87 carried by carbon 121.7ppm correlates with the carbon exiting at δC 159.4 carrying a hydroxyl proton at 11.43ppm and the quaternary carbon at δC 156.2. However, this proton does not correlate with the carbon at δC 102.9 carrying a proton at 6.74ppm. Both protons are therefore in the par position in the benzene ring.

The alkene proton exits at 6.33ppm carried by the carbon at δC 125.9 correlates with the carbons exiting at 34.2ppm, 121.7ppm and 159.4ppm respectively. However, the carbon exiting at 121.7ppm is the proton carrier at δH 6.87, so both protons are in the meta position. Furthermore, the carbon exiting at δC 34.2 is the carrier of the proton exiting at 2.52ppm and this proton has been coupled with the alkene proton at 5.89pp but not with the proton at 6.33ppm. So the proton at δH 2.52 is in the β position relative to the proton at δH 6.33.

Finally, the proton exiting at 5.89ppm correlates with the carbons exiting at δC 124.9 and δC22.6 respectively. All these correlations resulted in the first sequence of the product-2 molecule (fig.5).

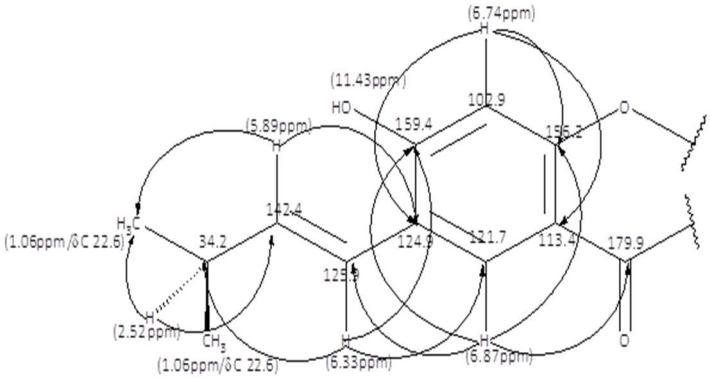


Figure.5: first sequence of the product-2 structure

The second sequence of the molecular structure of product-2 was identified from the proton which exits at 6.87ppm. This proton correlates with carbons exiting at 107.3ppm, 138.2ppm, 136.7ppm respectively.

The phenol proton exits at 11.85ppm with the carbons exiting at δC 107.3, at δC 109.5 and a weak correlation with the carbonyl at 179.9ppm. However, the favorable correlation for the phenol hydroxyl proton is in the ortho position, while the carbonyl group carbon is in the Meta position.

What's more, the hydroxyl proton at δH 10.44 correlates with the outgoing carbons at δC 132.0 and δC 148.5. Taken together, these correlations enabled us to find the second sequence in the structure of product-2 (fig.6).

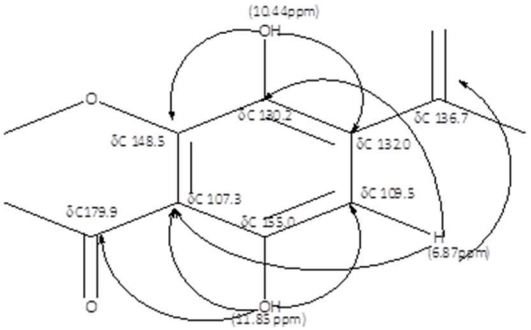


Figure 6: Second sequence of the compound-2 molecule

The final sequence of the product-2 structure is observed from the correlation of the alkene protons of the di-substituted benzene ring emerge at 7.29ppm and 7.33ppm respectively, which exhibits axial symmetry. Indeed:

The doublet proton signal at 7.29ppm correlates with carbons 136.7 ppm and 40.8ppm. However, the carbon at δC 40.8 is the carrier of two non-equivalent geminate protons, and these non-equivalent protons correlate with the quaternary carbon at δC 133.6 and the acid carbonyl at 176.2ppm. The doublet proton signal at δH 7.33 correlates with the carbons at δC 133.6 and δC 132.0. All these correlations, complemented by symmetry, have enabled us to elucidate the third and final sequence of the product-2 structure (fig.7).

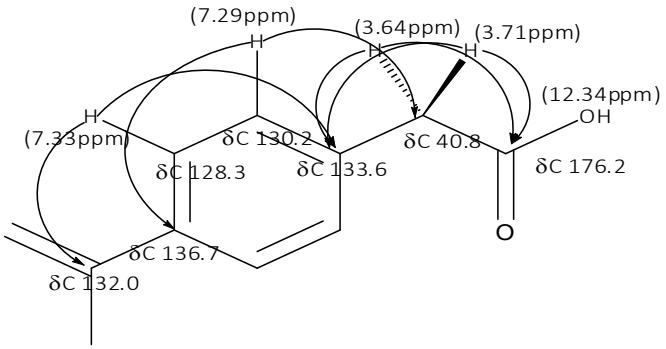
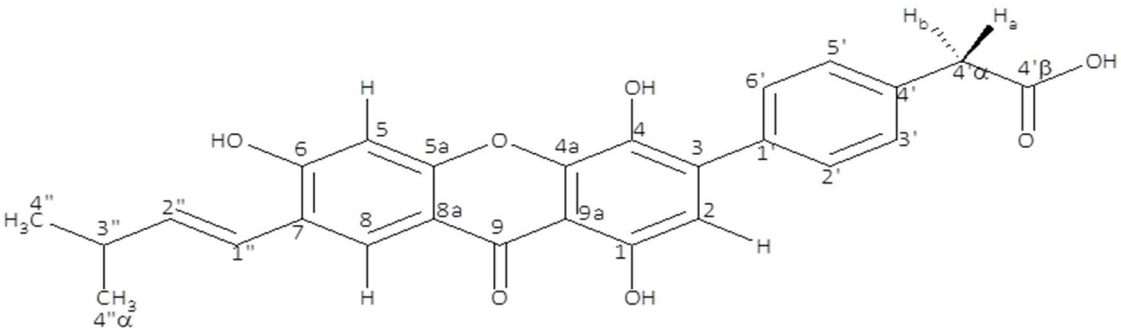


Figure.7: The third sequence of the product-2 structure.

Putting these three sequences together and considering the coupling constant 1J, we end up with the complete flat componse-2 structure (fig.8



Molecular formula: $C_{26}H_{22}O_7$

Molecular mass: 446

Molecular ion at m/z= 447.15085 $[M+H]^+$

Name: Munduleaxanthone

Figure.8: Complete structure of product-2

Table.4: 1H and 13C chemical shift, the correlation 1H-1H COSY and important HMBC correlation of Munduleaxanthone

N°	Types	1D- NMR (1H and 13C)		2D-NMR experiments	
		δH (^{1}H ppm)	δC(^{13}C ppm)	COSY	HMBC
1	C-OH phenol)	11.85	155.0		C-2, C-9a et faible avec C-9
2	CH benzene alkene	6.97	109.5		C-1', C-4 et C-9a
3	Cq		132.0		
4	C-OH(phenol)	10.44	138.2		C-3 and C-4a
4a	Cq		148.5		
5	CH benzene alkene	6.74	102.9		C-7 and C-8a
5a	Cq		156.2		
6	C-OH(phenol)	11.43	159.4		C-5 and C-7

7	Cq		124.9		
8	CH benzene alkene	6.87	121.7		C-5a, C-6, C-9 and C-1"
8a	Cq		113.4		
9	CO (carbonyl)		179.9		
9a	Cq		107.3	H-3'and H-5'	C-3 and C-4'
1'	Cq		136.7	H2' and H-6'	C-1' and C-4'α
2' et 6'	CH benzene alkene	7.29	130.2		
3' et 5'	CH benzene alkene	7.33	128.3	H-4'α_b	C-4' and C-4'β
4'	Cq		133.6	H-4'α_a	C-4' and C-4'β
4'α	-CH2- gemini proton not equivalent	3.64	40.8		
		3.71		H-2"	C-6, C-8 and C-3"
4'β	C-OH (acid)	12.34	176.2	H-2"and H-3"α	C-7 and C-4"
1"	CH linear alkene	6.33	125.9	H-2" and H-3"α	C-2" and C-4"
2"	CH linear alkene	5.89	142.2	H-3"	C-3"

IV. Discussion

Higher plants have the capacity to synthesize, through complex metabolic pathways at the end of the photosynthesis reaction, numerous compounds which they use for various adaptive functions they may undergo [19-23]. They therefore contain a wide variety of chemical molecules such as terpenoids, alkaloids, lipids, flavonoids and their derivatives, complex phenolic compounds and their derivatives, with different properties depending on their structure [15-18]. It is also now recognized that plants are an important source of new molecules [24, 25].

Ethnobotanical surveys carried out in the south-western region of Madagascar led to the selection of the plant known by its vernacular name "Malaignarety", scientifically called Mundulea antanossarum (Leguminosae) [26], and endemic to southern Madagascar. Phytochemical screening of the ethyl acetate extract from the stem bark of this plant shows that Mundulea antanossarum is rich in flavonoids, quinones, terpenoids, complex phenolic compounds and saponins. Bibliographical research on this plant shows that no in-depth phytochemical studies have been carried out.

Phytochemical studies carried out on the ethyl acetate extract of this plant isolated two products, attributed to Munduleaxanthone and Jipaflavonone respectively. The bibliographical studies carried out on this molecule show that it was not described in the literature, and the plant matrix studied is endemic to Madagascar. This justifies our results, as this plant has not been found elsewhere. These two isolated molecules have different basic structures and are attributed to Xanthone and flavonone respectively, and all included in the family of complex phenolic compounds. The presence of these molecules was justified by phytochemical screening tests, as this plant is rich in complex phenolic compounds, quinones, terpenoids and flavonoids.

Both pure products are soluble in a medium-polar organic solvent, which means that both molecules are medium-polar. Structurally, both molecules contain carbonyl groups. The presence of this function favors solubility in this medium-polar solvent, as ketones have a nucleophilic center, and the phenolic protons of these molecules take on acidic properties, as well as strong resonance due to the existence of hydrogen bonds, which explains why phenol is always acidic, and the negative charge on the oxygen is conjugated to the π electrons of the benzene ring.

Next, a study of the stereochemistry of these molecules shows that Jipaflavonone has two asymmetric carbons, and these asymmetric carbons have two configurations: either RR or SS. Polarimeter analysis of this molecule shows that it is deviated to

the right [α]= +53°, which explains why it has a dextrorotatory effect (D). This analytical result confirms that the stable configuration of this molecule is RR.

At the end of the Jipaflavonone mass spectrum, five highly identifiable fragmentations are observed, starting with the peak related to the molecular ion m/z = 413.65045 [M+H]+. Attributed respectively to molecular weights yield m/z = 381 [M+H] +, m/z = 353 [M+H] +, m/z = 203 [M+H] + and m/z = 177 [M+H] +. These fragmentations are due to different reaction mechanisms:

The first fragmentation, at m/z = 381 [M+H] +, corresponds to the loss of methanol (CH_3OH). Jipaflavonone structure does not include methanol, but hydroxyl and methyl groups are present on this molecule and the aromatic and benzene rings are always acidic, as the negative charge is in conjugation with the double bond of the benzene ring. The methanol departure is therefore favorable to the radical cleavage followed by a rapid concerted mechanism. The second fragment, at m/z = 353 [M+H] +, corresponds to the departure of ethyl acetate ($C_2H_3O_2$) and was obtained by the Mc Lafferty reaction mechanism, with the acetate group stabilized by the mesomeric effect. The last two fragmentations were obtained from the retro Diels-Alder reaction (fig.9).

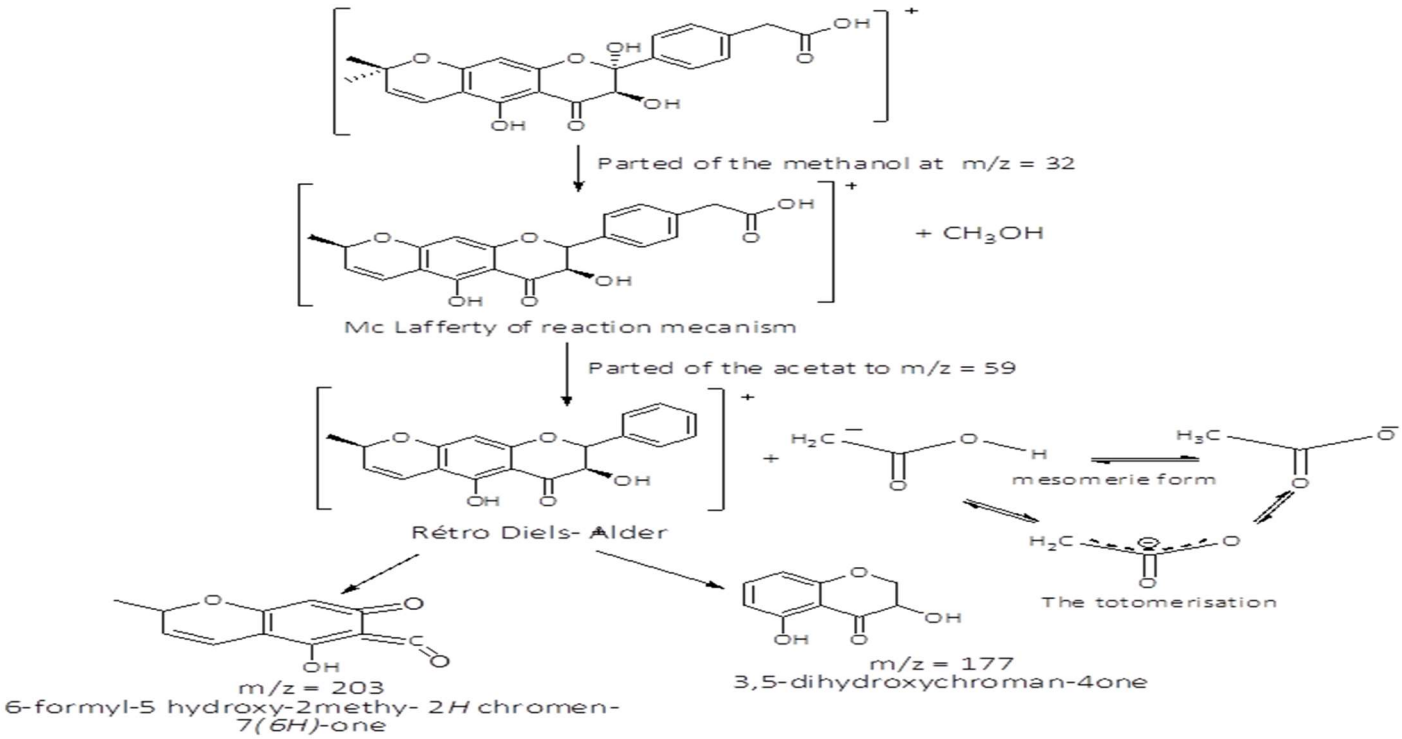


Figure 9. The fragments important for the structure elucidation of Jipaflavonone

V. CONCLUSION

The plant known by its vernacular name (Malagasy name, Mahafaly tribe), was collected in Tranoroa, Ampanihy district, southwestern Madagascar. It is very important to the local population in this region because of its therapeutic virtues. The aerial part of this plant is empirically used in infusion as an adjuvant to artemisinate to treat malaria. This therapeutic virtue has been proven by Fatiany PR et al, 2016 published in the journal Discovery Phytomedicine 2016; 3 (1): 1-6, but no in-depth phytochemical study has been carried out on this plant.

The phytochemical study carried out on the ethyl acetate extract of the stem bark of this plant, using various chromatographic techniques, isolated two new molecules named: Munduleaxanthone, Jipaflavonone. Their chemical structures have been identifiées grâce aux applications de méthodes des analyses spectroscopiques (RMN 1D et 2D, SM-HR-EIS en mode Q-TOF positif, HPLC-UV-DAD et IR).

Toutefois, l'espoir est encore grand car les plantes du Sud Malagasy ne cessent de nous fournir des nouvelles molécules et molécules qui présentent des intérêts biopharmaceutiques. Il est probable que dans un avenir proche, des molécules de grande valeur thérapeutique seront issues de la flore du de Madagascar.

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