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LC–HRESI–MS/MS Profiling of Flavonoids from *Chlorophora regia* (Moraceae)

James Oppong Kyekyeku[1*], Reimmel Kwame Adosraku[1] and Samuel Asare–Nkansah[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JOK and RKA designed the study and wrote the protocol. Author SAN managed the analyses of the study and interpretation of the data. Author JOK wrote the first and the final draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study was undertaken to characterize flavonoids from the stem bark of *Chlorophora regia* based on their HRESI–MS/MS fragmentation pattern in positive mode.

Study Design: Isolation and identification of flavonoids from the methanol–chloroform extract of the stem bark and the HRESI–MS/MS characterization of the flavonoids.

Place and Duration of Study: Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Ghana, Technische Universität Dortmund, Germany, between July 2014 and October 2016.

Methodology: Six flavonoids were isolated and purified using various chromatographic techniques. Their structures were elucidated by extensive analyses of their spectroscopic data (UV, 1D and 2D NMR, MS). Tandem mass spectroscopy was further employed to characterize the isolated flavonoids.

**Corresponding author: E-mail: jamakegh@yahoo.com;*

Results: Three flavonols including 3,5,7,4'–Tetrahydroxy–2'–methoxyflavonol, quercetin, kaempferol and three flavanones, 5,7,4'–Trihydroxy–2'–methoxyflavanone, 5,7,3',5'–Tetrahydroxyflavanone, naringenin were isolated. The MS fragmentation patterns of the flavonoids, in positive mode, were proposed. Retro Diels–Alder (RDA) fragmentation of the dihydropyran ring (ring C) of the chromane substructure of the flavonoids led to characteristic fragments that were used to identify the major flavonoid subgroup of the isolated compounds. Furthermore, the substitution pattern of the benzo (ring A) and phenyl (ring B) residues of the flavonoid nucleus was obtained through the RDA fragmentation.

Conclusion: The RDA fragments of the flavonoids obtained from the HRESI–MS/MS spectrum, could be employed in the identification and the determination of the substitution pattern of flavonoids in medicinal plants without the necessity of isolating them.

Keywords: Flavonoids; Chlorophora regia; HRESI–MS/MS; Moraceae; Retro Diels–Alder.

1. INTRODUCTION

Flavonoids are polyphenolic compounds with diverse biochemical and pharmacological effects and are distributed widely in plants [1–4]. Flavonoids are potent free radical scavengers due to the diphenylpropane (C6–C3–C6) skeleton in their structures. This three carbon link together with hydroxyl substitution on the aromatic rings make flavonoids good hydrogen and electron donors [5–8]. Based on the substitutions on ring (C) of the diphenylpropane skeleton, flavonoids are classified into six sub–groups; flavonols, flavan–3–ols, isoflavones, flavanones, flavones and anthocyanins [9,10].

HRESI–MS/MS analyses of flavonoids have been reported to show similar fragments for compounds in a particular sub–group classification. The fragmentation fission of flavonoids follows the Retro Diels–Alder (RDA) cleavage of the C–ring bonds. The fragmentation pattern, therefore, could be employed as a qualitative tool in the identification of an unknown flavonoid aglycone [6,11]. In our previous work on the chemical composition of the important Ghanaian medicinal plant, *Chlorophora regia* A. Chev, we reported the isolation of six flavonoids [12] including three flavonols, 3,5,7,4'–Tetrahydroxy–2'–methoxyflavonol (**1**) [12,13], quercetin (**2**) [14], kaempferol (**3**) [15] and three flavanones, 5,7,4'–Trihydroxy–2'–methoxyflavanone (**4**) [16], 5,7,3',5'–Tetrahydroxyflavanone (**5**) [17], naringenin (**6**) [18], (Fig. 1). Herein we report the HRESI–MS/MS fragmentation profile of the isolated flavonoids from *C. regia* to support earlier reports of the use of MS/MS fragments to identify flavonoids and as prove of the concept [6,11]. The identification of the isolated flavonoids was established by analyses of their HRESI–MS, 1D and 2D NMR data and correlating the observed spectra with those of reported literature.

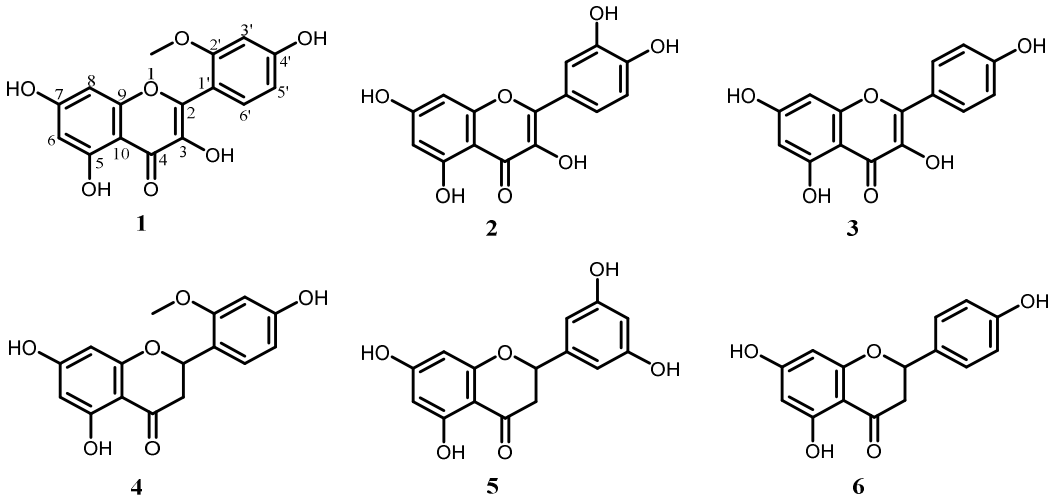


Fig. 1. Chemical structures of compounds 1–6

2. MATERIALS AND METHODS

2.1 Plant Material

The stem bark of *Chlorophora regia* was collected in the month of June 2014, from Asakraka forest (6°37'48,39"N0°41'6,87"W) in the Eastern Region of Ghana. The sample was authenticated by Mr. Cliford Asare at Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. A voucher specimen (KNUST/HM/CR1/2014/R002) was deposited at the herbarium.

2.2 Extraction and Isolation

The extraction and isolation of the compounds were as previously described [12]. The stem bark was air-dried, powdered (2 Kg) and extracted with a mixture of methanol–chloroform (80:20) at room temperature by cold maceration. The obtained crude extract was concentrated by using the rotary evaporator to yield a brownish residue (140 g). The concentrated residue was partitioned successively in cyclohexane, dichloromethane and methanol. The methanolic fraction (110 g) was fractionated by silica gel column chromatography using variable compositions of cyclohexane–ethyl acetate and ethyl acetate–methanol to obtain fourteen major fractions following HR–MS and TLC monitoring. The subfractions were subjected to various separation techniques including sephadex LH–20 column chromatography, silica gel column chromatography and *semi-preparative* HPLC to yield compounds **1**–**6**.

2.3 General Experimental Procedure

NMR spectra were recorded on a Bruker DRX–500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) using deuterated methanol (CD_3OD) and Dimethylsulfoxide (CD_3SOCD_3) as solvents. Chemical shifts (δ) were quoted in parts per million (ppm) using tetramethylsilane (TMS) as internal standard.

Semi-preparative HPLC was carried out on a Gynkotek pump equipped with a Dionex DG-1210 degasser, a Dionex Gina 50 auto-sampler, a Dionex UVD 340S detector, and a Phenomenex Gemini C18 column (10 x 250 mm, 10µm particle size) using a Chromeleon software system. Column chromatography was performed on Silica gel 60 (70-230 mesh; AppliChem, GmBH, Darmstadt, Germany) and Sephadex LH–20 (25-100µm, Amersham Biosciences). TLC was carried out on pre-coated silica gel 60 plates (0.25 mm; Merck, Darmstadt, Germany) and the developed spots were visualized under UV light and further by spraying with H_2SO_4–EtOH (1:9, v/v). All the solvents were of analytical grade.

2.3.1 HPLC–HRMS^n

The HPLC–HRMS^n experiments were carried out on an LTQ–Orbitrap spectrometer (Thermo Fisher, USA) equipped with a HESI-II source. The spectrometer was equipped with an Agilent 1200 HPLC system (Santa Clara, USA) including pump, PDA detector, column oven (30°C), and auto-sampler (injection volume 5µL for Fullscan, 7 µL for MS^n). MS^2 experiments were measured by CID (collision-induced decay, 35eV) mode. HPLC analyses were performed on a Luna C18 (2) column (50 x 3 mm, 3 µm particle size) from Phenomenex (Torrance, USA) using a mobile phase system of water (+ 0.1% formic acid) (A) and methanol (B) gradient (flow rate 350 µL/min). The gradient parameters were set as follows: linear gradient from 95% A to 100% B over 14 min, 100% B isocratic for 4 min, the system returned to initial conditions within 0.5 min of 95% A and was equilibrated for 4.5 min.

2.4 Spectral Analysis

3,5,7,4'–Tetrahydroxy–2'–methoxyflavonol (**1**): Yellow amorphous solid; LC–UV [MeOH–H_2O (0.1% HCOOH)]; λ_{max} 250 and 350 nm; ^{1}H NMR (500 MHz in DMSO–d_6) and ^{13}C NMR (125 MHz, DMSO–d_6) see Table 1. Positive HRESI–MS: *m/z* 317.0657 $[M + H]^+$ (calcd. for $C_{16}H_{13}O_7$, 317.0656), *m/z* 339.0478 $[M + Na]^+$ (cald. for $C_{16}H_{12}O_7Na$, 339.0475).

Quercetin (**2**): Yellow needles; LC–UV [MeOH–H_2O (0.1% HCOOH)] λ_{max} 254 and 370 nm; ^{1}H NMR (500 MHz in CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 1. Positive HRESI–MS: *m/z* 303.0501 $[M + H]^+$ (calcd. for $C_{15}H_{11}O_7$, 303.0499).

Kaempferol (**3**): Yellow amorphous solid; LC–UV [MeOH–H_2O (0.1% HCOOH)]; λ_{max} 265 and 366 nm.^{1}H NMR (500 MHz in CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 1. Positive HRESI–MS: *m/z* 287.0550 $[M + H]^+$ (calcd. for $C_{15}H_{11}O_6$, 287.0550), *m/z* 309.0369 $[M + Na]^+$ (cald. for $C_{15}H_{10}O_6Na$, 309.0370).

5,7,4'–Trihydroxy–2'–methoxyflavanone (**4**): Yellow needles; LC–UV [MeOH–H_2O (0.1%

HCOOH)]; λ_{max} 225 and 288 nm;^{1}H NMR (500 MHz in CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 2. Positive HRESI–MS: *m/z* 303.0864 $[M + H]^+$ (calcd. for $C_{16}H_{15}O_6$, 303.0863), *m/z* 325.0680 $[M + Na]^+$ (cald. for $C_{16}H_{14}O_6Na$, 325.0683).

5,7,3',5'–Tetrahydroxyflavonone (**5**): Colourless amorphous solid; LC–UV [MeOH–H_2O (0.1% HCOOH)]; λ_{max} 228 and 288 nm; ^{1}H NMR (500 MHz in CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 2. Positive HRESI–MS: *m/z* 289.0708 $[M + H]^+$ (calcd. for $C_{15}H_{13}O_6$, 289.0707).

Naringenin, 5,7,4'–Trihydroxyflavanone (**6**): Yellow amorphous solid; LC–UV [MeOH–H_2O (0.1% HCOOH)]; λ_{max} 214 and 289 nm; ^{1}H NMR (500 MHz in CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data see Table 1. Positive HRESI–MS: *m/z* 273.0754 $[M + H]^+$ (calcd. for $C_{15}H_{13}O_5$, 273.0758), *m/z* 295.0571 $[M + Na]^+$ (cald. for $C_{15}H_{12}O_5Na$, 295.0577).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as yellow amorphous solid. The molecular formula was assigned as $C_{16}H_{12}O_7$ based on the pseudo–molecular ion peak at *m/z* 317.0657 $[M + H]^+$ (calcd. 317.0656 for $C_{16}H_{13}O_7$) in the HRESI–MS spectrum, in positive mode. The ^{1}H NMR spectrum (Table 1) showed the presence of methoxy protons at δ_H 3.70 (3H, s, 2'–MeO), three aromatic protons attached to ring B at δ_H 6.50 (1H, d, *J* = 2.0 Hz, H–3'), δ_H 6.45 (1H, dd, *J* = 8.5, 2.0 Hz, H–5'), δ_H 7.23 (1H, d, *J* = 8.5 Hz, H–6') and two aromatic protons attached to ring A at δ_H 6.17 (1H, d, *J* = 2Hz, H–6), δ_H 6.27 (1H, d, *J* = 2Hz, H–8). The position of the methoxy moiety was confirmed through HMBC correlation between the methoxy protons and δ_C 158.9 (C–2'). Based on detailed analysis of the 1D and 2D NMR and comparison with reported data, the structure of **1** was elucidated as 3,5,7,4'–Tetrahydroxy–2'–methoxyflavonol [12,13]. The HRESI–MS/MS fragmentation of **1** follows the fragmentation pattern typical of flavonol aglycones [6,11]. The main fragment was observed at *m/z* 261 $[M + H–2CO]^+$ which corresponds to a loss of two molecules of carbon monoxide. Subsequent losses of CH_3OH and CO was observed at *m/z* 257.04 $[M + H–CH_3OH–CO]^+$. Retro Diels–Alder (RDA) cleavage of the C–ring resulted in further fragments which were described based on the nomenclature of Ma et al. [19] (Table 3). Retrocyclization cleavage of the protonated molecule of **1** produced RDA fragments $^{0,2}A^+$ at *m/z* 165, $^{0,2}B^+$ at *m/z* 151, $[^{0,2}B^+–CH_3]$ at *m/z* 139, $^{1,3}A^+$ at *m/z* 153 and $^{1,3}B^+$ at *m/z* 163 (Scheme 1).

Compound **2** was obtained as yellow needles with a molecular formula of $C_{15}H_{10}O_7$, determined by HRESI–MS showing a quasi–molecular ion peak at *m/z* 303.0501 $[M + H]^+$ (calcd. 303.0499 for $C_{15}H_{11}O_7$). The ^{1}H NMR spectrum (Table 1) indicated the presence of two aromatic protons attached to ring A at δ_H 6.33 (1H, d, *J* = 2.0 Hz, H–8), δ_H 6.12 (1H, d, *J* = 2.0 Hz, H–6) and three aromatic protons attached to ring B at δ_H 7.67 (1H, d, *J* = 2.0 Hz, H–2'), δ_H 6.83 (1H, d, *J* = 8.5 Hz, H–5'), δ_H 7.57 (1H, d, *J* = 2.0, 8.5 Hz H–6'). The absence of higher upfield proton signals revealed there were no protons attached to ring C. The structure of **2** was concluded to be quercetin after analysis of the ^{1}H and ^{13}C NMR, and comparing same to reported data [14]. HRESI–MS/MS fragmentation profile of **2**, expectedly, showed RDA fragments similar to that of **1** which are generally typical of flavonol aglycones. Dehydration of the protonated molecular ion gave a peak at *m/z* 285. Loss of water followed by a loss of carbon monoxide gave the principal fragment at *m/z* 257 $[M + H–H_2O–CO]^+$. A further loss of one molecule of carbon monoxide was observed at *m/z* 229 $[M + H–CH_3OH–2CO]^+$. RDA fragments observed following fission of the C–ring were $^{0,2}A^+$ at *m/z* 165, $^{1,3}A^+$ at *m/z* 153, $^{0,2}B^+$ at *m/z* 137 and $^{1,3}B^+$ at *m/z* 149.

Compound **3** was obtained as yellow amorphous solid with a molecular formula of $C_{15}H_{10}O_6$, determined by HRESI–MS showing a peak at *m/z* 287.0550 $[M + H]^+$ (calcd. 287.0550 for $C_{15}H_{11}O_6$). In the ^{1}H NMR spectrum, two aromatic protons assigned to ring A at δ_H 6.33 (1H, br s, H–8), δ_H 6.12 (1H, d, *J* = 1.5 Hz, H–6) and two sets of equivalent aromatic protons assigned to ring B at δ_H6.85 (2H, d, *J* = 9.0 Hz, H–3'/5'), δ_H8.02 (2H, d, *J* = 8.5 Hz, H–2'/6') were observed. The structure of **3** was elucidated as kaempferol based on analysis of the ^{1}H and ^{13}C NMR data [15]. The HRESI–MS/MS fragmentation of **3** produced fragments similar to that of **1** and **2** (Table 3).

Compound **4** was obtained as yellow needles. The molecular formula was assigned as $C_{16}H_{14}O_6$ based on the quasi–molecular ion peak at *m/z* 303.0864 $[M + H]^+$ (calcd. 303.0863 for $C_{16}H_{15}O_6$) in the HRESI–MS spectrum. The presence of methoxy protons at δ_H 3.83 (3H, s, 2'–MeO) were observed in the ^{1}H NMR spectrum (Table 2). The spectrum further showed the

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compounds 1, 2 and 3

Position	1[a]		2[b]		3[b]	
	δH multi. (*J* in Hz)	δC	δH multi. (*J* in Hz)	δC	δH multi. (*J* in Hz)	δC
2		148.6		149.1		148.4
3		136.9		137.5		137.4
4		176.9		177.6		177.7
5		160.9		162.8		162.8
6	6.17, d (2.0)	98.3	6.12, d (2.0)	99.6	6.12, d (1.5)	99.6
7		163.8		165.9		165.9
8	6.27, d (2.0)	93.7	6.33, d (2.0)	94.7	6.33, br s	94.8
9		157.1		158.5		158.6
10		103.8		104.8		104.9
1'		110.7		124.5		124.0
2'		158.9	7.67, d (2.0)	116.3	8.02, d (8.5)	131.0
3'	6.50, d (2.5)	99.7		146.5	6.85, d (9.0)	116.6
4'		160.8		148.3		160.8
5'	6.45, dd (8.5, 2.0)	107.4	6.83, d (8.5)	116.5	6.85, d (9.0)	116.6
6'	7.23, d (8.5)	132.2	7.57, dd (8.5, 2.0)	122.0	8.02, d (8.5)	131.0
2' –MeO	3.70, s	55.9	-	-	-	-

[a]Measured in DMSO-d_6; [b]Measured in CD_3OD

presence of five aromatic protons, two were assigned to ring A at δ_H 5.81 (1H, d, *J* = 2.0 Hz, H–8), δ_H 5.83 (1H, d, *J* = 2.0 Hz, H–6) and three assigned to ring B at δ_H 6.39 (1H, d, *J* = 2.0 Hz, H–3'), δ_H 6.36 (1H, dd, *J* = 8.5, 2.0 Hz, H–5'), δ_H 7.23 (1H, d, *J* = 8.5 Hz, H–6'). The observation of two proton signals upfield which were absent in **1** and comparing the Double Bond Equivalent (DBE), indicated a loss of the usual double bond between δ_C 75.9 (C–2) and δ_C 43.5 (C–3). Thus three protons were successfully assigned to ring C at δ_H 5.54 (1H, dd, *J* = 13.0, 3.0 Hz, H–2), δ_H 2.95 (1H, dd, *J* = 17.0, 13.0 Hz, H–3a), δ_H 2.59 (1H, dd, *J* = 13.0, 3.0 Hz, H–3b). A further comparison of the ^{13}C NMR data (Table 2) of **4** to that of **1,** assisted in successfully assigning the position of the methoxy group to δ_C 54.9 (C–2'). The structure of **4** was determined to be 5,7,4'–Trihydroxy–2'–methoxyflavanone [16]. The fragmentation pattern of **4** in the HRESI–MS/MS was characteristic of flavanone aglycones [6,11]. A loss of a CH_2CO group $[M + H–CH_2CO]^+$ was observed at *m/z* 261. Retrocyclization cleavage of **4** produced the following RDA fragments $^{0,4}B^+$ at *m/z* 177, $^{1,3}A^+$ at *m/z* 153, $[M + H–B–ring]^+$ at *m/z* 179 (Table 3 and Scheme 2).

Compound **5** was isolated as colorless amorphous solid. The molecular formula was determined as $C_{15}H_{12}O_6$ based on the pseudo–molecular ion at *m/z* 289.0708 $[M + H]^+$ (calcd. 289.0707 $C_{15}H_{13}O_6$) in the HRESI–MS spectrum. The ^{1}H NMR spectrum (Table 2) indicated the presence of two aromatic protons attached to ring A at δ_H 5.82 (1H, d, *J* = 2.5 Hz, H–8), δ_H 5.84 (1H, d, *J* = 2.0 Hz, H–6) and three aromatic protons attached to ring B at δ_H 6.73 (1H, s, H–2'/6'), δ_H 6.86 (1H, s, H–4'). Two proton signals at δ_H 5.22 (1H, dd, *J* = 13.0, 3.0 Hz, H–2), δ_H 3.01 (1H, dd, *J* = 17.0, 12.5 Hz, H–3a), δ_H 2.64 (1H, dd, *J* = 17.0, 3.0 Hz, H–3b) were observed indicating the absence of the usual unsaturation in ring C characteristic of flavanones. The structure of **5** was confirmed to be 5,7,3',5'–Tetrahydroxyflavonone after comparing ^{1}H and ^{13}C NMR data (Table 2) to reported literature [17]. The HRESI–MS/MS profile of **5** followed a fragmentation pattern typical of flavanone aglycones [6,11]. A stepwise loss of two H_2O molecules gave peaks at *m/z* 271 $[M + H–H_2O]^+$ and *m/z* 253 $[M + H–2H_2O]^+$. CH_2CO group loss was observed at *m/z* 247 $[M + H–CH_2CO]^+$. Retro Diels–Alder fission of ring C resulted in the fragments $^{0,4}B^+$ at *m/z* 163, $^{1,3}A^+$ at *m/z* 153 and $[M + H–B–ring]^+$ at *m/z* 179 (Table 3 and Scheme 2).

Compound **6** was obtained as yellow amorphous solid with a molecular formula of $C_{15}H_{12}O_5$ based on the ion peak at *m/z* 273.0754 $[M + H]^+$ (calcd. 273.0758 for $C_{15}H_{13}O_5$). The ^{1}H NMR spectrum (Table 2) indicated the presence of two aromatic protons attached to ring A at δ_H 5.82 (1H, d, *J* = 2.0 Hz, H–8), δ_H 5.83 (1H, d, *J* = 2.5 Hz, H–6), four aromatic protons attached to ring B at δ_H 7.25 (2H, d, *J* = 8.5 Hz, H–2'/6'), δ_H 6.75 (2H, d, *J* = 8.5 Hz, H–3'/5') and three saturated protons attached to ring C at δ_H 5.27 (1H, dd, *J* = 13.0, 3.0 Hz, H–2), δ_H 3.04 (1H, dd, *J* = 17.0, 13.0 Hz, H–3a), δ_H 2.63 (1H, dd, *J* = 17.0, 3.0 Hz, H–3b).

The structure of **6** was elucidated as 5,7,4'–Trihydroxyflavanone (Naringenin) after comparing the ^{1}H and ^{13}C NMR data (Table 2) with reported literature [18]. The HRESI–MS/MS fragmentation information confirmed **6** as a flavanone type flavonoid. A loss of CH_2CO was observed at *m/z* 231 $[M + H–CH_2CO]^+$. Retrocyclization cleavage of ring C produced RDA fragments $^{0,4}B^+$ at *m/z* 147, $^{1,3}A^+$ at *m/z* 153 and $[M + H–B–ring]^+$ at *m/z* 179 of the protonated molecular ion (Table 3 and Scheme 2).

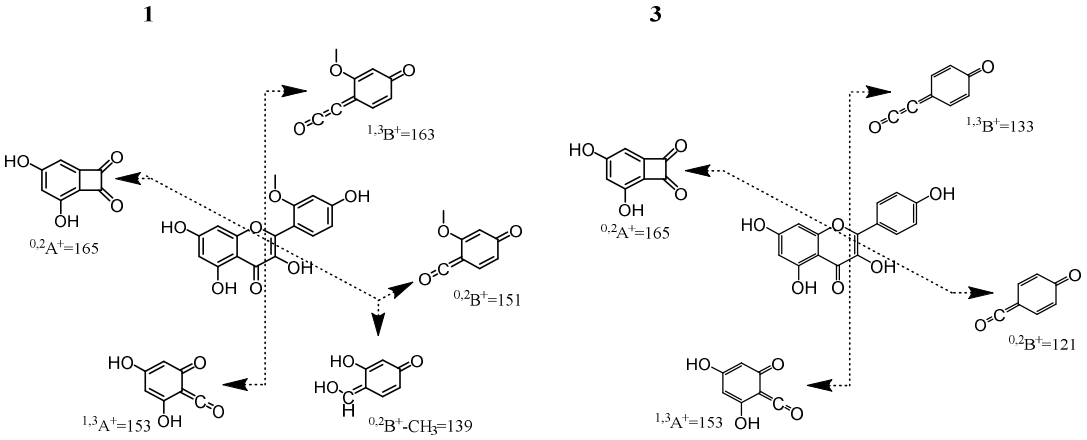
Table 2. ^{1}H and ^{13}C NMR data for compounds 4, 5 and 6 in CD_3OD

Position	4		5		6	
	δH[a]multi. (*J* in Hz)	δC[b]	δH[a] multi. (*J* in Hz)	δC[b]	δH[a] multi. (*J* in Hz)	δC[b]
2	5.54, dd (13.0, 3.0)	75.9	5.22, dd (13.0, 3.0)	80.8	5.27, dd (13.0, 3.0)	80.8
3	2.95, dd (17.0, 13.0) 2.59, dd (17.0, 3.0)	43.5	3.01, dd (17.0, 12.5) 2.64, dd (17.0, 3.0)	44.4	3.04, dd (17.0, 13.0) 2.63, dd (17.0, 3.0)	44.3
4		198.6		198.0		198.1
5		165.8		165.7		165.2
6	5.83, d (2.0)	97.3	5.84, d (2.0)	97.3	5.83, d (2.5)	97.4
7		168.6		168.7		168.7
8	5.81, d (2.0)	96.4	5.82, d (2.5)	96.5	5.82, d (2.0)	96.5
9		165.6		165.1		165.8
10		103.6		103.7		103.7
1'		119.6		119.5		131.4
2'		159.3	6.73, s	116.6	7.25, d (8.5)	129.3
3'	6.39, d (2.0)	100.2		147.2	6.75, d (8.5)	116.6
4'		160.5	6.86, s	132.1		159.3
5'	6.36, dd (8.5, 2.0)	108.5		146.8	6.75, d (8.5)	116.6
6'	7.23, d (8.5)	129.1	6.73, s	115.0	7.25, d (8.5)	129.3
2' –MeO	3.73, s	56.2	-	-	-	-

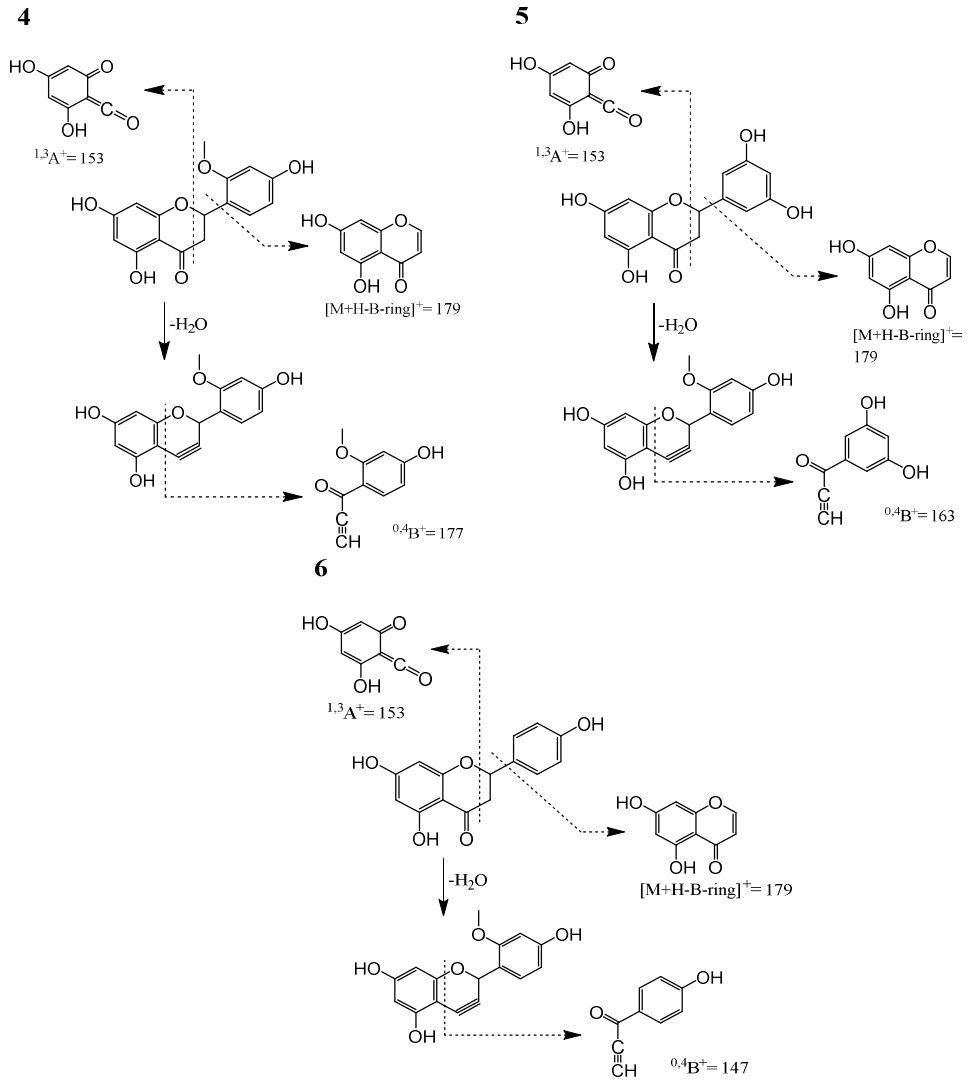
[a]Recorded at 500 MHz; [b]Recorded at 125 MHz

Table 3. Defining structural HRESI–MS/MS fragments of 1–6

Fragment	1	2	3	4	5	6
$[M + H–2CO]^+$	261(100)	247 (28)	231 (34)			
$[M + H–CO]^+$			259 (20)			
$[M + H–CH_3OH–H_2O]^+$	257(4)					
$[M + H–CH_2CO]^+$				261(8)	247 (4)	231 (6)
$[M + H–H_2O]^+$		285 (50)	269 (30)		271(20)	
$[M + H–H_2O–CO]^+$		257 (100)	241 (100)			
$[M + H–H_2O–2CO]^+$		229 (60)	213 (84)			
$[M + H–2H_2O]^+$					253 (8)	
RDA fragments						
$^{0,2}A^+$	165 (16)	165 (46)	165 (84)			
$^{0,2}B^+$	151 (5)	137 (12.5)	121 (22)			
$[^{0,2}B^+–CH_3]^+$	139 (18)					
$^{0,4}B^+$				177 (80)	163 (100)	147 (84)
$^{1,3}A^+$	153 (8)	153 (10)	153 (42)	153 (100)	153 (32)	153 (100)
$^{1,3}B^+$	163 (4)	149 (6)	133 (16)			
$[M + H–B–ring]^+$				179 (5)	179 (26)	179 (6)



Scheme 1. Retrocyclization cleavage of the C–ring of compounds 1 and 3



Scheme 2. Retrocyclization cleavage of the C-ring of compounds 4–6

4. CONCLUSION

This study provides further evidence of the potential use of Retro Diels–Alder (RDA) fragmentation of the dihydropyran ring (ring C) of the chromane substructure to identify bio-active flavonoids. The substitution pattern of the benzo (ring A) and phenyl (ring B) residues of the flavonoids could also be predicted based on the fragmentation pattern. This approach will eliminate the arduous task of isolating flavonoids from natural sources for the purposes of identification.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

SUPPLEMENTARY DATA

Supporting information to this article can be found online.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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