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DIAGNOSTIC PRODUCT IONS AND FRAGMENTATION PATTERNS FOR THE CYCLOMYRSINANE-TYPE DITERPENOIDS BY HIGHER-ENERGY COLLISIONAL DISSOCIATION MASS SPECTROMETRY

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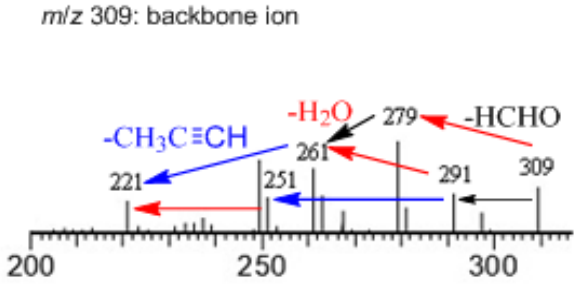
Seven cyclomyrsinane-type diterpenoids (cyclo-MDs) were studied on the diagnostic product ions and fragmentation patterns by mass spectrometry techniques with ESI source. The method consists of two individual mass spectrometric experiments, including high-resolution higher-energy collisional dissociation tandem (MS/MS) experiments and collision induce dissociation multi-stage (MS^n) experiments, the latter of which enable identification of the losing sequence of acyloxy groups. In positive-ion mode, the candidate compounds produced $[M+NH_4]^+$ and $[M+Na]^+$ pseudo-molecular ions. The fragmentation patterns were proposed and demonstrated that penta-esters of cyclo-MDs underwent the elimination of five acyloxy groups to give ion *m/z* 309, followed by the loss of a molecular water to achieve ion *m/z* 291. While to those hexa-esters of cyclo-MDs, they were found to departure directly all six acyloxy groups to get ion *m/z* 291; hypothetically, ion 309 of them was proposed by the cleavage of five acyloxy groups and one CH_2=CO molecule from 10-position acetoxyl group. All compounds shared common fragments of *m/z* 309, 291, 279, 251, 249 and 221, which were considered as diagnostic product ions, and exhibited the structural skeleton, four-membered ring and oxymethylene bridge of cyclo-MDs.



INTRODUCTION

Cyclomyrsinane-type diterpenoids (Cyclo-MDs) contained a 5-6-7-4 tetracyclic skeleton, owing a typical oxymethylene bridge between C-6 and C-13. The carbon skeleton was oxidated with functionalities at many positions, the most representative positions were at C-3, C-5, C-7, C-8, C-10. C-13, C-14, C-15, and C-17, and sometimes it will be uncommonly oxidated at C-2 position with a hydroxy or its related substituent. The functional positions were partially or fully esterified with various acyloxy groups of aliphatic and/or aromatic acids.[1] Because of the acyloxy groups linking to its carbon core, these diterpenoids showed significant biological activities. For example, Zolfaghari *et al.* regarded them as taxonomic marker to evaluated the anti-cancer activities of EJ-138 and Jurkat T cell lines.[1]Some candidate compounds from *Euphorbia prolifera* could reduce the triglyceride level by over 50% at a low concentration in antiadipogenic activity, furthermore, derivatives with a free hydroxyl group at C-8 after structural modification were beneficial to the activities.[2] Some of selected Cyclo-MDs compounds from *E. falcata* possessed blocking activity on G protein-activated inwardly rectifying potassium ion (GIRK) channels.[3]

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	1	2	3	4	5	6	7
R_1	Pr	Ac	Pr	Bu	Pr	Ac	Pr
R_2	Bz	Bz	Bz	Bz	Bz	Bz	*i*Bu
R_3	Bz	Bz	Ac	Ac	Ac	Ac	Ac
R_4	OH	OH	OH	Ac	Ac	Ac	Ac

Fig. 1 – Candidate chemical structures of Cyclo-MDs **1-7**.

Higher-energy collisional dissociation (HCD), and Collision-induced dissociation (CID) of mass spectrometry can generate structure specific fragmentation to propose the fragmentation pattern of candidate compounds in biopharmaceutical and organic chemistry areas, which could help for rapid screening and identification.[4-6] In methodology, F. Griaud developed an approach by middle-down mass spectrometry technique to identify novel diagnostic ions in chimeric spectra.[7] The tandem mass spectrometry (MS^n) with CID or HCD techniques greatly benefit the rapid structural identification and elucidation for known and unknown compounds in crude plant extracts by specific fragmentation features and diversity of fragmentation pathways. The fragmentation patterns of clerodane-type diterpenes from *Casearia* species were studied by tandem mass spectrometry in space and time (quadrupole time-of-flight and ion trap) analyses, ten compounds were used for investigation to provide the fragmentation pathways of sequential neutral losses of ester groups from clerodane skeleton.[8] Eight cannabinoids were evaluated the fragmentation profiles by tandem mass spectrometry by adequate collision energies. Their fragmentation profiles can help to classify different samples, and their fragmentation patterns can be applied as a preliminary tool in the analysis of unknown samples.[9]

In this work, we focused on the diagnostic product ions and the fragmentation patterns of losing carboxylic acids, but not on the detailed analysis of fragmentation behavior of seven reference compounds, namely Proliferin D (**1**), Euphorbiaproliferin E (**2**), Euphorprolitherin D (**3**), Euphordraculoin P (**4**), *Euphorbia* substance SPr5 (**5**), Euphordraculoin O (**6**) and *Euphorbia* substance SPr4 (**7**), respectively (Figure 1). These compounds were classified as penta-esters of cyclo-MDs (**1-3**) and hexa-esters of cyclo-MDs (**4-7**).[10]

RESULTS AND DISCUSSION

High-resolution ESI-MS spectra were recorded with a Q Exactive Focus instrument, the main data present in the full mass spectra were summarized in Table 1 and 2. The published data of losing sequence are supported by combined use of high resolution ESI-HCD-MS/MS, as well as extensive ESI-CID-MS^n (n=2-4) techniques (Table S1, Figures S15-17), but sometimes it would be disputable. In the positive mode of HR-ESI-MS, all compounds were readily observed the pseudo-molecular ions of $[M+NH_4]^+$ and $[M+Na]^+$ in the single stage spectra, most of which were base peaks or highly abundant peaks. As mentioned further, the expected $[M+NH_4]^+$ and $[M+Na]^+$ ion were selected and attempted to undertake for HCD experimental analysis. The HCD-MS/MS analysis of $[M+NH_4]^+$ ion afforded a base peak of 77Da loss from its pseudo-molecular ions, suggesting that the neutral loss of NH_3 and AcOH was simultaneous. After then the immonium ions spontaneously exchanged to protonated ions that will give identical fragmentation routes to those of the protonated pseudo-molecules.[11]

Acyloxy and hydroxyl groups elimination of penta-esters of cyclo-MDs

Penta-esters of cyclo-MDs (**1-3**) possessed a single free hydroxyl group at 15-position. Mostly their HR-ESI-MS/MS spectra exhibited the difference between two prominent peaks were 60, 74, 88 or 122 Da, which were respectively corresponded to the molecular weight of acetic acid, propionic acid, butyric acid (or isobutyric acid) and benzoic acid. Correspondingly, the multi-stage CID experiments of low resolution for **2** demonstrated the information that the high abundant peaks in the product ion spectra exhibited the elimination of carboxylic acids from the pseudo-molecular $[M+Na]^+$ ion (Figure S15).

Owing to steric interactions, the AcO- moiety at 5-position with α-orientation was easy to eliminate by expellant with its neighbor acyloxy groups of 3-position and $-CH_2O-$ moiety, and gave rise the most abundant product ion PB of $[M+Na-60]^+$ or $[M+NH_4-17-60]^+$, which demonstrated two possible

pathways to fragment its side substituent groups. The former is effective elimination of two molecular acids from 8- and 10-positions to expand conjugated structure with C=O group, generating ions of PC and PD, followed by losing 3-position acid to produce ion PE. The latter is firstly caused by losing the 3-position acid to form the conjugated structure with C4-C5 double bond, and followed by departure of two acids from 8- and 10-positions to yield ion PE. The last acyloxy group at 14-position without *β*-H preferred to departure with cleavage of sodium carboxylate or carboxylic acid from its precursor ion PE to the product ion PF, which dehydrated to get ion PG. Both ions of PF and PG demonstrated the carbon backbone of Cyclo-MDs (Scheme 1).

In most case, loss of -CH_2O- moiety occurs in minor abundance but it's significant fragmentation process, which agrees perfectly with those precursor structures containing a C4-C5 double bond (ions PB-PE) or a cation at 14-position (ion PF) that could induce the cleavage of -CH_2O- segment from oxymethylene bridge. Similarly, a neutral loss of 42Da could be interpreted by elimination of ketene from acetoxyl groups with hydrogen shift and formed a free hydroxyl group.

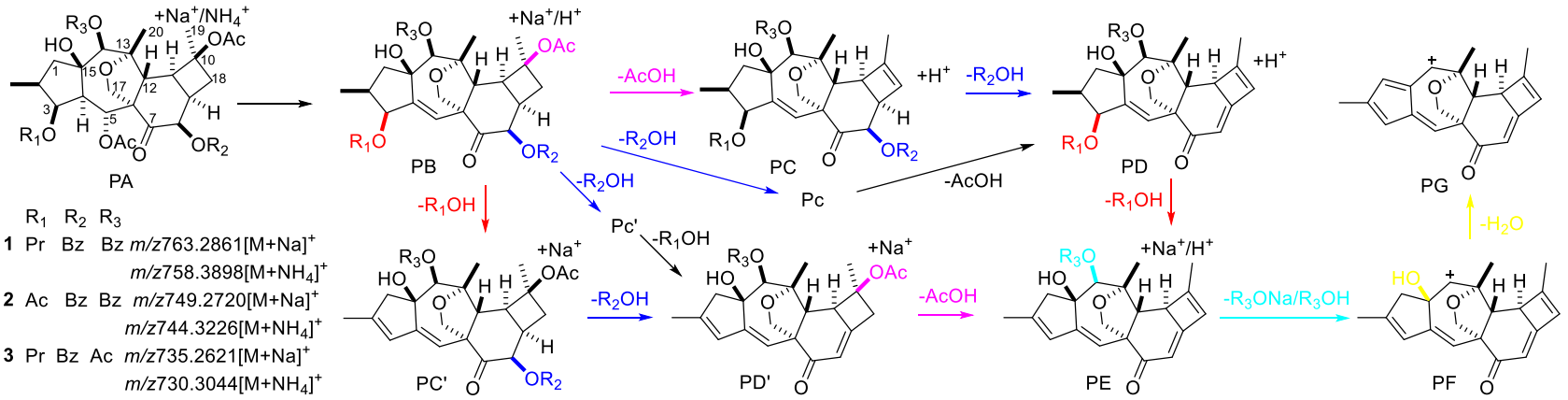
Acyloxy groups elimination of hexa-esters of cyclo-MDs

In this part the proposed fragmentation pathways were briefly analyzed for the elimination of its acyloxy groups, depending on HR-MS/MS and partial LR-MS^n (n=2-4, as shown to compound **4** and **7** in Table S1 and Figure S16-17) techniques (Scheme 2). As mentioned above, hexa-esters of cyclo-MDs demonstrated the expelling of neighboring constituents to acyloxy group at 5-position, giving rise to the base peak with 60 Da segment loss. Similarly, both of the acyloxy groups at 14- and 15-position are with β-orientation, their steric interaction led to the elimination of AcOH from 15-position to produce ion HB. The proposed fragmentation pathways of them were summarized in scheme 2, where the product ion HD was generated from the precursor ion HB through two possible pathways. One way was the first loss of acyloxy group by 1,4-elimination reaction from 14-position to get ion HC, and cleavage of sodium carboxylate or carboxylic acid from 3-position to obtain ion HD; Otherwise, different sequential departure of sodium carboxylate or carboxylic acid was observed on the other way (HB→Hc→HD). Furthermore, the carbon backbone ion HG of *m/z* 291 was obtained by successively losing acids from 8- and 10-positions. Moreover, ion HF of *m/z* 309, a hypothetical parent ion of *m/z* 291 by dehydrating reaction, was interpretated according to ketene cleavage by H rearrangement (HD→HE) and consecutive acid elimination of R_2OH.

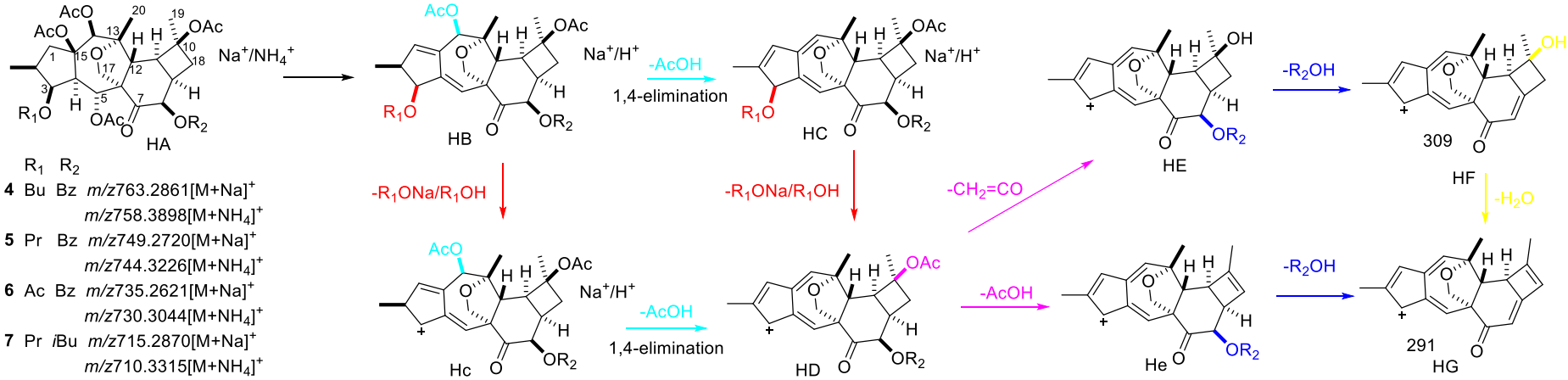
Analysis the common fragmentation ions of cyclo-MDs

Penta-esters of cyclo-MDs generated the ion *m/z* 309 (PF) by eliminating five carboxylic acids following one H_2O loss from OH-15 to yield ion *m/z* 291 (PG), differently hexa-esters were hypothesized to produce a free OH group (HE) according to the cleavage of ketene from 10-position acetoxyl group by H rearrangement mechanism. A pair of ions PF and HF with identical formula indicated different chemical structures corresponding to the variable position of hydroxyl groups, what was depended on the deduction of fragmentation patterns. The other pair of PG and HG was considered as identical chemical structure with conjugated system. Both of *m/z* 309 and *m/z* 291 demonstrated the cyclo-MDs carbon backbone, their daughter ions of *m/z* 279, 261, 251, 249 and 221, showed the characteristic information to its 9,11-cyclized four-membered ring and oxymethylene bridge structure. For instance, the pair-ions of *m/z* 291 and 251, *m/z* 261 and 221 were proposed to the cleavage of ring D by losing $CH_3C{\equiv}CH$ molecule. Furthermore, the transitions of *m/z* 309→279 and *m/z* 291→261 could be suggested to the loss of HCHO from oxymethylene bridge. All these ions were considered as diagnostic product ions, being routinely utilized in structural depth profiling of cyclo-MDs, including four-membered ring and oxymethylene bridge.

The mass data in table S2 pointed out that ions of *m/z* 251, 249 and 221 with losing identical $CH_3C{\equiv}CH$ segments had a higher probability of mass error value (>30ppm), but the masses of neutral $CH_3C{\equiv}CH$ loss were confidently deduced from mass differences between parent ions and product ions, such as *m/z* 291→251 and *m/z* 261→221. The elemental formulae of small neutral molecules were easily determined because the number of candidate formulae was limited.



Scheme 1 – Proposed fragmentation pathways of penta-esters of cyclo-MDs.



Scheme 2 – Proposed fragmentation pathways of hexa-esters of cyclo-MDs.

Table 1

Corresponding ions data for elemental composition and accurate masses of penta-esters of cyclo-MDs (**1**-**3**) measured by HR-ESI-HCD-MS/MS

Comp.	Ion name	adduct: NH_4^+			adduct: Na^+		
		elemental composition	measured/calculated (*m/z*)	intensity(%)/ RDB/Err. (ppm)	elemental composition	measured/calculated (*m/z*)	RA(%)/RDB/ Err. (ppm)
1	PA	$C_{41}H_{50}O_{13}N$	764.2900/764.3277	12.9/17.5/-49.3	$C_{41}H_{46}O_{13}Na$	769.2756/769.2831	100/18.5/-9.7
	PB	$C_{39}H_{43}O_{11}$	687.2739/687.2800	100/18.5/-8.9	$C_{39}H_{42}O_{11}Na$	709.2551/709.2619	89.5/18.5/-9.7
	PC	$C_{37}H_{39}O_9$	627.2534/627.2589	62/18.5/-8.7	-	-	-
	Pc	$C_{32}H_{37}O_9$	565.2372/565.2432	36.0/14.5/-10.7	-	-	-
	PC’	-	-	-	$C_{36}H_{36}O_9Na$	635.2194/635.2252	23.5/18.5/-9.1
	Pc’	-	-	-	$C_{32}H_{36}O_9Na$	587.2198/587.2252	37.9/14.5/-9.1
	PD	$C_{30}H_{33}O_7$	505.1973/505.2221	25.8/14.5/-49.0	-	-	-
	PD’	-	-	-	$C_{29}H_{30}O_7Na$	513.1840/513.1884	19.2/14.5/-8.5
	PE	$C_{27}H_{27}O_5$	431.1816/431.1853	80.5/14.5/-8.6	$C_{27}H_{26}O_5Na$	453.1633/453.1672	6.1/14.5/-8.7
	PF	$C_{20}H_{21}O_3$	309.1459/309.1485	73.6/10.5/-8.6	$C_{20}H_{21}O_3$	309.1460/309.1485	0.7/10.5/-8.2
	PG	$C_{20}H_{19}O_2$	291.1356/291.1380	26.3/11.5/-8.2	$C_{20}H_{19}O_2$	291.1354/291.1380	0.6/11.5/-8.8
2	PA	$C_{40}H_{48}O_{13}N$	750.2687/750.3120	12.2/17.5/-57.7	$C_{40}H_{44}O_{13}Na$	755.2596/755.2674	88.5/18.5/-10.3
	PB	$C_{38}H_{41}O_{11}$	673.2584/673.2643	100/18.5/-8.9	$C_{38}H_{40}O_{11}Na$	695.2393/695.2463	100/18.5/-10.0
	PC	$C_{36}H_{37}O_9$	613.2379/613.2432	27.2/18.5/-8.6	-	-	-
	Pc	$C_{31}H_{35}O_9$	551.2230/551.2276	15.0/14.5/-8.3	-	-	-
	PC’	-	-	-	$C_{36}H_{36}O_9Na$	635.2191/635.2252	33.3/18.5/-9.6
	Pc’	-	-	-	$C_{31}H_{34}O_9Na$	573.2041/573.2095	37.8/14.5/-9.4
	PD	$C_{29}H_{31}O_7$	491.2024/491.2064	25.0/14.5/-8.2	-	-	-
	PD’	-	-	-	$C_{29}H_{30}O_7Na$	513.1839/513.1884	28.1/14.5/-8.7
	PE	$C_{27}H_{27}O_5$	431.1817/431.1853	19.5/14.5/-8.3	$C_{27}H_{26}O_5Na$	453.1631/453.1672	6.8/14.5/-9.2
	PF	$C_{20}H_{21}O_3$	309.1459/309.1485	11.2/10.5/-8.5	$C_{20}H_{21}O_3$	309.1459/309.1485	0.8/10.5/-8.5
	PG	$C_{20}H_{19}O_2$	291.1354/291.1380	0.04/11.5/-8.7	$C_{20}H_{19}O_2$	291.1352/291.1380	0.7/11.5/-9.3
3	PA	$C_{36}H_{48}O_{13}N$	702.3057/702.3210	8.8/13.5/-9.0	$C_{36}H_{44}O_{13}Na$	707.2604/707.2674	84.2/14.5/-9.9
	PB	$C_{34}H_{41}O_{11}$	625.2584/625.2643	100/14.5/-9.6	$C_{34}H_{40}O_{11}Na$	647.2401/647.2463	100/14.5/-9.5
	PC	$C_{32}H_{37}O_9$	565.2382/565.2432	12.6/14.5/-8.9	-	-	-
	Pc	$C_{27}H_{35}O_9$	503.1987/503.2276	1.6/10.5/-57.4	-	-	-
	PC’	-	-	-	$C_{31}H_{34}O_9Na$	573.2042/573.2095	36.8/14.5/-9.2
	Pc’	-	-	-	$C_{27}H_{34}O_9Na$	525.2049/525.2095	40.0/10.5/-8.8
	PD	$C_{28}H_{27}O_5$	443.1808/443.1853	3.3/15.5/10.2	-	-	-
	PD’	-	-	-	$C_{24}H_{28}O_7Na$	451.1687/451.1727	24.0/10.5/-8.9
	PE	$C_{22}H_{25}O_5$	369.1664/369.1697	1.8/10.5/-8.9	$C_{22}H_{24}O_5Na$	391.1480/391.1516	6.6/10.5/-9.3
	PF	$C_{20}H_{21}O_3$	309.1458/309.1485	2.2/10.5/-8.8	$C_{20}H_{21}O_3$	309.1458/309.1485	2.1/10.5/-8.8
	PG	$C_{20}H_{19}O_2$	291.1352/291.1380	0.5/11.5/-9.4	$C_{20}H_{19}O_2$	291.1354/291.1380	1.3/11.5/-8.7

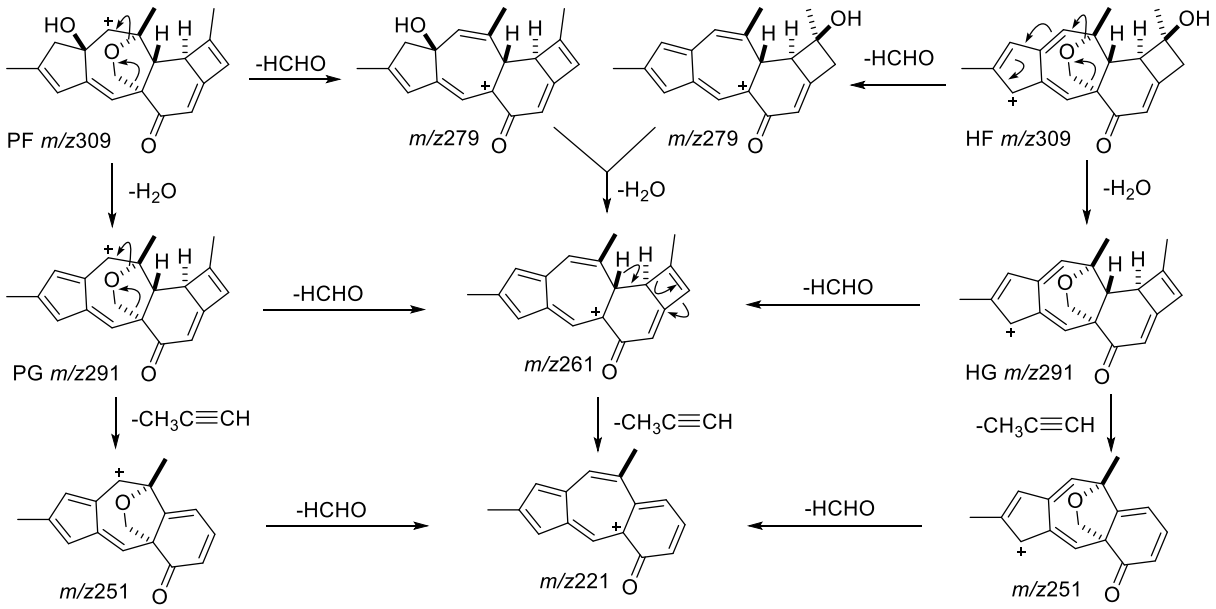
RA: Relative Abundance; RDB: Ring and Double Bond equivalent; “-”: not detected.

Table 2

Corresponding ions data for elemental composition and accurate masses of hexa-esters of cyclo-MDs (**4**-**7**) measured by HR-ESI-HCD-MS/MS

Comp.	Ion name	adduct: NH_4^+			adduct: Na^+		
		elemental composition	measured/calculated (*m/z*)	Intensity (%)/ RDB/Err. (ppm)	elemental composition	measured/calculated (*m/z*)	RI (%)/RDB/ Err. (ppm)
4	HA	$C_{39}H_{52}O_{14}N$	758.3898/758.3382	0.8/14.5/68.0	$C_{39}H_{48}O_{14}Na$	763.2864/763.2936	27.4/15.5/-9.4
	HB	$C_{35}H_{41}O_{10}$	621.2638/621.2694	65.2/15.5/-9.1	$C_{35}H_{40}O_{10}Na$	643.2456/643.2514	30.0/15.5/-9.0
	HC	$C_{33}H_{37}O_8$	561.2434/561.2483	13.2/15.5/-8.7	$C_{33}H_{36}O_8Na$	583.2253/583.2302	6.3/15.5/-8.4
	Hc	$C_{31}H_{33}O_8$	533.2125/533.2170	10.7/15.5/-8.4	-	-	-
	HD	$C_{29}H_{29}O_6$	473.1919/473.1959	26.1/15.5/-8.4	$C_{29}H_{29}O_6$	473.1920/473.1959	5.0/15.5/-8.1
	HE	$C_{27}H_{27}O_5$	431.1814/431.1853	8.3/14.5/-9.0	$C_{27}H_{27}O_5$	431.1817/431.1829	1.5/11.5/-2.7
	He	$C_{27}H_{25}O_4$	413.1710/413.1747	2.9/15.5/-9.1	$C_{27}H_{25}O_4$	413.1710/413.1747	1.0/15.5/-9.0
	HF	$C_{20}H_{21}O_3$	309.1458/309.1485	5.4/10.5/-8.9	$C_{20}H_{21}O_3$	309.1460/309.1485	2.3/10.5/-8.2
	HG	$C_{20}H_{19}O_2$	291.1356/291.1380	3.6/11.5/-8.2	$C_{20}H_{19}O_2$	291.1357/291.1380	2.0/11.5/-7.9
5	HA	$C_{38}H_{50}O_{14}N$	744.3226/744.3226	13.0/14.5/0	$C_{38}H_{46}O_{14}Na$	749.2720/749.2780	14.3/15.5/-7.9
	HB	$C_{34}H_{39}O_{10}$	607.2493/607.2538	53.0/15.5/-7.4	$C_{34}H_{38}O_{10}Na$	629.2310/629.2357	46.5/15.5/-7.6
	HC	$C_{32}H_{35}O_8$	547.2286/547.2326	20.0/15.5/-7.3	$C_{32}H_{34}O_8Na$	569.2105/569.2146	13.7/15.5/-7.3
	Hc	$C_{31}H_{33}O_8$	533.2130/533.2170	9.2/15.5/-7.5	-	-	-
	HD	$C_{29}H_{29}O_6$	473.1924/473.1959	41.2/15.5/-7.2	$C_{29}H_{29}O_6$	473.1923/473.1959	5.9/15.5/-7.4
	HE	$C_{27}H_{27}O_5$	431.1822/431.1853	13.6/14.5/-7.3	$C_{27}H_{27}O_5$	431.1821/431.1853	1.5/15.5/-7.4
	He	$C_{27}H_{25}O_4$	413.1717/413.1747	5.2/15.5/-7.3	$C_{27}H_{25}O_4$	413.1718/413.1747	1.3/15.5/-7.2
	HF	$C_{20}H_{21}O_3$	309.1462/309.1485	11.9/10.5/-7.6	$C_{20}H_{21}O_3$	309.1461/309.1485	3.2/10.5/-7.7
	HG	$C_{20}H_{19}O_2$	291.1356/291.1380	7.4/11.5/-8.2	$C_{20}H_{19}O_2$	291.1356/291.1380	2.8/11.5/-8.2
6	HA	$C_{37}H_{48}O_{14}N$	730.3044/730.3069	20.8/14.5/-3.4	$C_{37}H_{44}O_{14}Na$	735.2621/735.2623	17.8/15.5/-0.3
	HB	$C_{33}H_{37}O_{10}$	593.2358/593.2381	100/15.5/-3.8	$C_{33}H_{36}O_{10}Na$	615.2201/615.2201	40.4/15.5/0
	HC	$C_{31}H_{33}O_8$	533.2152/533.2170	25.1/15.5/-3.3	$C_{31}H_{32}O_8Na$	555.1990/555.1989	9.7/15.5/0.2
	Hc	-	-	-	-	-	-
	HD	$C_{29}H_{29}O_6$	473.1944/473.1959	11.7/15.5/-3.0	$C_{29}H_{29}O_6$	473.1962/473.1959	4.0/15.5/0.8
	HE	$C_{27}H_{27}O_5$	431.1838/431.1853	3.5/14.5/-3.7	$C_{27}H_{27}O_5$	431.1857/431.1853	1.0/14.5/1.0
	He	$C_{27}H_{25}O_4$	413.1735/413.1747	1.0/15.5/-3.0	$C_{27}H_{25}O_4$	413.1750/413.1747	0.9/15.5/0.8
	HF	$C_{20}H_{21}O_3$	309.1474/309.1485	2.0/10.5/-3.7	$C_{20}H_{21}O_3$	309.1487/309.1485	2.0//10.5/7.1
	HG	$C_{20}H_{19}O_2$	291.1368/291.1380	1.4/11.5/-4.1	$C_{20}H_{19}O_2$	291.1383/291.1380	1.7/11.5/11.0
7	HA	$C_{35}H_{52}O_{14}N$	710.3315/710.3382	14.3/10.5/9.4	$C_{35}H_{48}O_{14}Na$	715.2872/715.2936	18.5/11.5/-8.9
	HB	$C_{31}H_{41}O_{10}$	573.2641/573.2694	100/11.5/-9.3	$C_{31}H_{40}O_{10}Na$	595.2462/595.2514	37.6/14.5/-8.7
	HC	$C_{29}H_{37}O_8$	513.2439/513.2483	10.2/11.5/-8.6	$C_{29}H_{36}O_8Na$	535.2258/535.2302	10.4/11.5/-8.2
	Hc	$C_{28}H_{35}O_8$	499.2283/499.2326	13.0/11.5/-8.8	-	-	-
	HD	$C_{26}H_{31}O_6$	439.2077/439.2115	12.8/11.5/-8.7	$C_{26}H_{31}O_6$	439.2079/439.2115	4.9/11.5/-8.3
	HE	$C_{24}H_{29}O_5$	397.1973/397.2010	3.1/10.5/-9.2	$C_{24}H_{29}O_5$	397.1977/397.2010	11.4/10.5/-8.2
	He	$C_{24}H_{27}O_4$	379.1869/379.1904	1.1/11.5/-9.3	$C_{24}H_{27}O_4$	379.1871/379.1880	1.0/11.5/-8.6
	HF	$C_{20}H_{21}O_3$	309.1459/309.1485	2.2/10.5/-8.6	$C_{20}H_{21}O_3$	309.1459/309.1485	3.9/10.5/-8.6
	HG	$C_{20}H_{19}O_2$	291.1354/291.1380	1.3/11.5/-8.6	$C_{20}H_{19}O_2$	291.1356/291.1380	2.5/11.5/-8.2

RA: Relative Abundance; RDB: Ring and Double Bond equivalent; "-": Not detected.



Scheme 3 – Proposed fragmentation pathways and relationship of diagnostic product ions.

EXPERIMENTAL

Reagents

Reference compounds (**1**-**7**) were provided from Dr. Li Wang, who performed the experiments to isolate and identify the chemical structures from *Euphorbia dracunculoides* Lam.[6] HPLC grade methanol was available from Tedia Company (USA), ammonia was purchased from Aladdin Reagent Company (China), and 0.45 μm mesh nylon filters from Jinteng Company (China). All samples without further purification were prepared by dissolving in methanol, alkalizing (a drop) by aqueous ammonia solution (0.1%), and then filtered onto 0.45 μm mesh nylon filters; a syringe pump was calibrated prior to feed the samples into ESI source by infusion at a continuous flow rate of 4 μL/min.

Analytical instrumentation and conditions

The Q Exactive Focus mass spectrometer (Thermo Scientific) was operated with an ESI interface in positive mode to record high resolution mass spectra. The data were processed using Thermo Scientific™ TraceFinder Software (version 4.1), which provides possible elemental molecular formulas, accurate masses and isotopic patterns. MS experiments occurred during by performing a fullscan without HCD followed by a full scan for MS/MS experiments with HCD, enabled collision energies were optimized for each individual compound of interest. High purity nitrogen was used as atomized gas and drying gas both with flow rate of 10 mL/min, the sheath gas temperature was set to 350°C. High purity argon was used as collision gas. The scan range was m/z 100-800 for all MS experiments.

Multi-stage mass (MS^n) spectra were recorded with a Bruker Esquire HCT spectrophotometer coupled with an ESI operating in positive ionization mode. The instrument was controlled by Esquire Control software, and automatic gain control target was set to record MS^n spectra. The mass spectrometric parameters were as follows: capillary temperature 143.5°C; the heater temperature 320°C; the positive spray voltage 4.20-4.50 kV. The sheath gas was high purity nitrogen, the collision gas was high purity argon. The scan range was *m/z* 100-800.

CONCLUSIONS

Among a growing number of traditional utilizations in Euphorbia species reported in the literatures, its macrocyclic structures and bioactivities have raised a special interest for researchers all around the world.[12]

In this study, the characteristic fragmentation patterns and diagnostic product ions of seven cyclo-MDs were systematically exploited for the first time by HR-ESI-MS/MS and assistant ESI-MS^n techniques. Their possible structures were investigated based on their elemental compositions, ring double-bond equivalents and accurate MS/MS characteristics for their reasonable cleavage. The survey about the fragmentation behavior of them showed that elimination of all acyloxy groups including free OH group could produce fragment ions of *m/z* 309 and 291 corresponding to diterpenoid carbon backbone. Due to fourth analysis, neutral losses of HCHO and $CH_3C{\equiv}CH$ were observed in reported diagnostic product ions, the latter $CH_3C{\equiv}CH$ loss could be used to discriminate from other type diterpenoids.

These results could be efficiently used to screen and identify these diterpenoids in plant extracts of Euphorbia species and to serve as a practical strategy to investigate cyclo-MDs.

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