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STUDY OF RING-OPEN FRAGMENTATION IN TWO ROSANE-TYPE DITERPENOID LACTONES BY ESI-MS^n (QUADRUPOLE TIME-OF-FLIGHT AND ION TRAP)**

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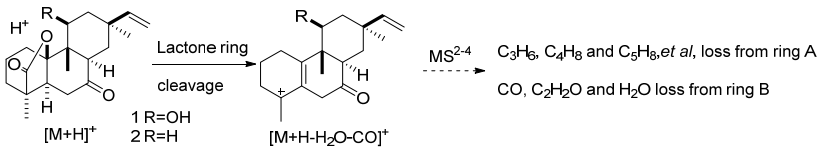
Ring-open fragmentation of two rosane-type lactones of 11*β*-hydroxyrosenonolactone (**1**) and rosenonolactone (**2**) was studied by ESI-MS^n technology. Experiments showed that both of them fragmented in lactone ring, followed by losing alkyl moieties and C=O related moieties from the skeleton rings. Mostly, an identical H_2O loss from C=O group in ring B was proposed by a hypothetical mechanism of [1,5] H-20 rearrangement, C8-C9 bond break and dehydration. All the possible product ions were established according to the accurate HR- ESI-MS^2 measurements.



INTRODUCTION

Rosane and *ent*-rosane diterpenes (RDs and *ent*-RDs) contain an identical backbone skeleton, except for the main difference of the orientation on methyl group at C-9 (Figure 1). The stereochemical structures of them primarily indicate that *β*-orientation of Me-9 is for RDs, and α-orientation of Me-9 is for *ent*-RDs.[1] The notable structural feature is tricyclic skeleton, containing three six-membered rings. Naturally, they have highly oxygenated substituents, such as hydroxyl, methoxyl, ketone and ester groups. These chemical and structural characteristics have attracted a lot of interest of chemists and pharmacologists in biological occurrence and bioactivities.[2-5]

RDs and *ent*-RDs distribute mainly in both plant and fungal sources. The phytochemical investigation demonstrated that they were the important secondary metabolites, distributing in genus *Euphorbia* of Euphorbiaceae family, and indicating important biological functions, such as anti-inflammatory, anti-tuberculosis effects, and anti-cancer activities, *et al*.[1-5] In recent years, many researches indicated that RDs were found in the fungal secondary metabolites, namely engleromycenol, engleromycenolic acid B, rosoloactone, rosenonolactone, and so on.[6-9] Among these compounds, some were tricyclic, the others were tetracyclic increasing one lactone ring located at C-4 and C-10. For instance, rosoloactone displayed significant anticancer activity, and had been developed as an effective therapeutic agent for treating cancer cell lines of human cervical cancer Hela cells and human ovarian cancer cells.[10-11] RDs are structurally and biologically interesting fungal metabolites, and may be produced by sequential microbial transformation.

Basing on further chemical, pharmaceutical and biological application, two RDs lactones of 11*β*-

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hydroxyrosenonolactone (**1**) and rosenonolactone (**2**) were selected for structural investigation by multi-stage electrospray ionization mass spectrometry (ESI-MS^n, n=2–4). Present study focused on the characteristic fragmentation and diagnostic ions of six-membered rings and lactone ring of them, and the accurate ion formulas were depended on high resolution tandem ESI technology (HR-ESI-MS/MS).[12]

RESULTS AND DISCUSSION

The MS and MS^n were exanimated in automatic mode of HCT spectrophotometer, and their precursor ions of protonated molecules $[M+H]^+$ were acquired to record MS^{2-4} spectra. For subsequent multi-stage MS studies in MS^3 and MS^4 spectroscopy (Table 1), the abundant or key ions in MS^{2-3} spectra were reasonably introduced as mother ions for collision-induced dissociation (CID) experiments to generate product ions, which were the important messages for proposing their fragmentation pathways. It should be noted that the alternative pathways in proposing fragmentation patterns can reasonably interpret the product ions by comparing and analyzing their MS^{3-4} data, and by the accurate ion formula from HR-ESI-MS^2 data (Table 2 and 3).

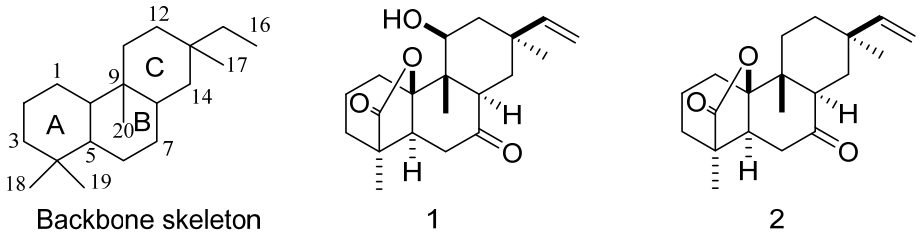


Fig. 1 – Backbone skeleton of Rosane-type diterpene, 11β-hydroxyrosenonolactone (**1**) and rosenonolactone (**2**).

Table 1

ESI-MS^n (n=2 to 4) data (150-350 Da) of 11β-hydroxyrosenonolactone (**1**) and Rosenonolactone (**2**)

Comp.	MS^n	Ion for CID	Fragment ions: *m/z* (%base peak)
1	MS^2	333 $[M+H]^+$	315(39.6),297(27.9),287(43.0),269(100),251(39.6),241(18.2),227(20.3),209(13.9), 195(6.3)
	MS^3	315	297(67.6),287(7.1),269(66.7),241(8.2),227(20.3),213(8.4),195(5.9),171(9.2),163(100)
		297	279(10.5),269(45.3),251(100),223(11.3),209(25.7),195(15.0),163(17.7)
		287	269(89.9),251(21.5),241(60.3),227(100),185(36.0)
		269	251(33.4),241(58.5),227(100),213(39.2),185(23.8),171(40.2)
		251	236(41.0),223(26.0),209(100),195(90.9),181(15.8)
		227	209(6.4),199(22.9),185(261),171(100),157(16.9)
2	MS^2	317 $[M+H]^+$	299(69.1),271(100),261(18.4),253(89.6),243(35.0),229(13.8),215(21.8),197(23.1), 163(19.8),153(47.6)
	MS^3	299	271(22.7),253(100),243(19.9),225(25.7),197(32.2),183(9.7),171(9.7),155(14.6)
		271	253(100),243(36.0),229(24.4),225(24.9),215(51.9),211(23.2),197(28.2),189(16.3), 183(15.6),163(37.1)
		253	238(1.9),225(13.9),211(49.2),197(100),183(26.0),171(44.7),169(26.4),157(21.0), 155(18.7)
		243	215(7.8),197(100),187(8.4),173(8.1),159(9.5)
	MS^4	197	182(100),169(70.4),155(70.9)

Table 2

HR-ESI-MS^2 data (150-350 Da) of precursor and product ions of 11β-hydroxyrosenonolactone (**1**)

Proposed formula	Observed *m/z*	Calculated *m/z*	Δ (mmu)	RDB
$C_{20}H_{29}O_4$	333.2052	333.2060	-0.87	6.5
$C_{20}H_{27}O_3$	315.1947	315.1955	-0.77	7.5
$C_{20}H_{25}O_2$	297.1842	297.1849	-0.74	8.5
$C_{19}H_{27}O_2$	287.1998	287.2006	-0.76	6.5
$C_{19}H_{25}O$	269.1895	269.1900	-0.51	7.5
$C_{19}H_{23}$	251.1788	251.1794	-0.58	8.5
$C_{18}H_{25}$	241.1945	241.1951	-0.53	6.5
$C_{18}H_{20}$	236.1555	236.1560	-0.45	9.0

Table 2 (continued)

$C_{16}H_{19}O$	227.1426	227.1430	-0.45	7.5
$C_{17}H_{19}$	223.1478	223.1481	-0.38	8.5
$C_{15}H_{17}O$	213.1269	213.1274	-0.45	7.5
$C_{16}H_{17}$	209.1321	209.1325	-0.37	8.5
$C_{14}H_{17}O$	201.1272	201.1274	-0.21	6.5
$C_{15}H_{15}$	195.1168	195.1168	-0.01	8.5
$C_{14}H_{17}$	185.1325	185.1325	0	6.5
$C_{14}H_{13}$	181.1012	181.1012	0.06	8.5
$C_{13}H_{15}$	171.1164	171.1168	-0.42	6.5
$C_{10}H_{11}O_2$	163.0750	163.0754	-0.32	5.5

Table 3

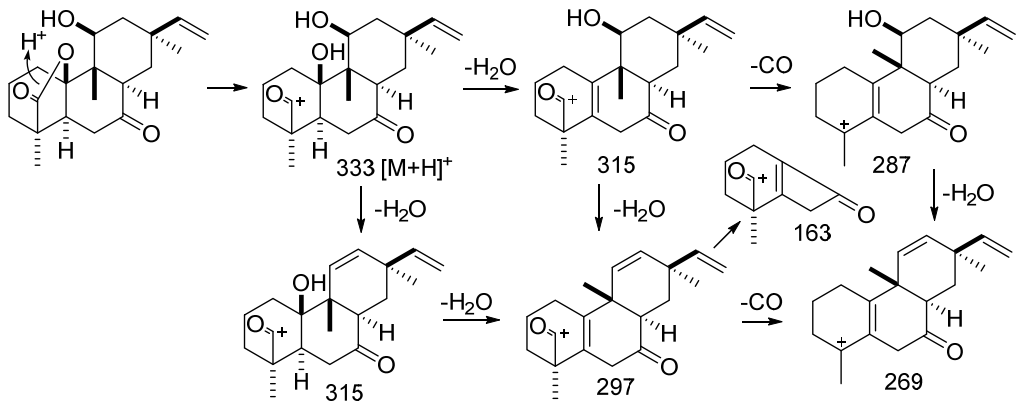
HR-ESI-MS^2 data (150-350 Da) of precursor and product ions of rosenonolactone (**2**)

Proposed formula	Observed *m/z*	Calculated *m/z*	Δ (mmu)	RDB
$C_{20}H_{29}O_3$	317.2105	317.2111	-0.64	6.5
$C_{19}H_{26}O_3$	302.1870	302.1876	-0.61	7.0
$C_{20}H_{27}O_2$	299.1998	299.2006	-0.76	7.5
$C_{19}H_{27}O$	271.2047	271.2056	-0.90	6.5
$C_{16}H_{21}O_3$	261.1478	261.1485	-0.72	6.5
$C_{19}H_{25}$	253.1945	253.1951	-0.61	7.5
$C_{16}H_{19}O_2$	243.1375	243.1380	-0.45	7.5
$C_{18}H_{22}$	238.1711	238.1716	-0.46	8.0
$C_{17}H_{25}$	229.1946	229.1951	-0.47	5.5
$C_{17}H_{21}$	225.1633	225.1638	-0.48	7.5
$C_{15}H_{19}O$	215.1426	215.1430	-0.42	6.5
$C_{16}H_{19}$	211.1477	211.1481	-0.44	7.5
$C_{15}H_{17}$	197.1323	197.1325	-0.14	7.5
$C_{13}H_{17}O$	189.1273	189.1274	-0.09	5.5
$C_{14}H_{19}$	187.1480	187.1481	-0.14	5.5
$C_{14}H_{17}$	185.1325	185.1325	0.06	6.5
$C_{14}H_{15}$	183.1169	183.1168	0.02	7.5
$C_{14}H_{14}$	182.1092	182.1090	0.22	8.0
$C_{13}H_{17}$	173.1323	173.1325	-0.16	5.5
$C_{13}H_{15}$	171.1165	171.1168	-0.31	6.5
$C_{13}H_{13}$	169.1008	169.1012	-0.41	7.5
$C_{11}H_{15}O$	163.1114	163.1117	-0.38	4.5
$C_{12}H_{17}$	161.1321	161.1325	-0.37	4.5
$C_{12}H_{15}$	159.1165	159.1168	-0.34	5.5
$C_{12}H_{13}$	157.1009	157.1012	-0.24	6.5
$C_{12}H_{11}$	155.0852	155.0855	0.29	7.5

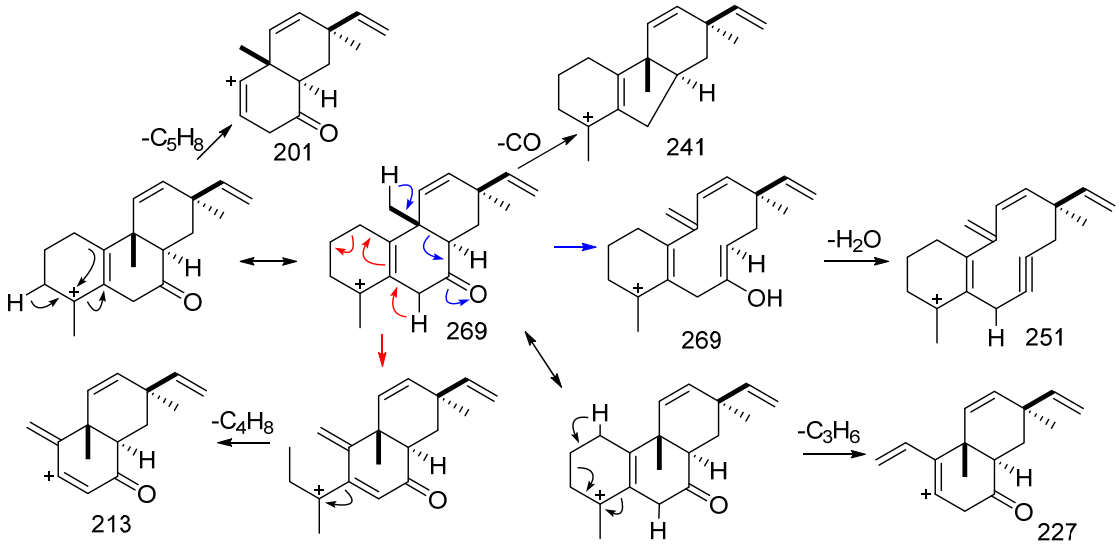
Fragmentation of 11β-hydroxyrosenonolactone (**1**)

The spectra of 11β-hydroxyrosenonolactone indicated continuous elimination of three H_2O molecules (*m/z* 333-315-297 and *m/z* 269-251) from those three oxygenated groups of ester, hydroxyl and C=O groups, respectively. For the ESI-CID-MS^n analysis, **1** fragmented originally by the lactone ring cleavage. Subsequent elimination of two H_2O molecules from precursor $[M+H]^+$ ion (*m/z* 333) led to generate ion *m/z* 297, followed by CO loss to produce a more stable allyl-type tertiary carbonium ion *m/z* 269 at C-3 position, which was showed as key intermediate ion with high abundance (Table 1). The experimental data indicated that the key ion *m/z* 269 could be yielded from another pathway of *m/z* 333-315-287-269 corresponding to the consecutive loss of H_2O, CO and H_2O (Scheme 1).

Carbocyclic ring-open fragmentation of **1** mostly happened in its ring A and B from the key ion *m/z* 269. By analyzing the ions formula difference of *m/z* 269 to 227, 213 and 201, it was suggested that the alkyl loss of C_3H_6, C_4H_8 and C_5H_8 should be fragmented from ring A induced by C-3 tertiary ion. In CID experiment of *m/z* 269, the product ions of *m/z* 251 and 241 were proposed by losing H_2O and CO from ring B, both of which were from the third oxygen source of C=O at C-7 position. H_2O elimination was suggested from C=O group by a possible hydrogen [1,5] transfer mechanism from H-20, and followed by C8-C9 bond break to form a ten-membered cycloalkyne ion *m/z* 251 (Scheme 2).



Scheme 1 – Lactone ring cleavage was proposed by H_2O and CO loss of 11β-hydroxyrosenonolactone (**1**).



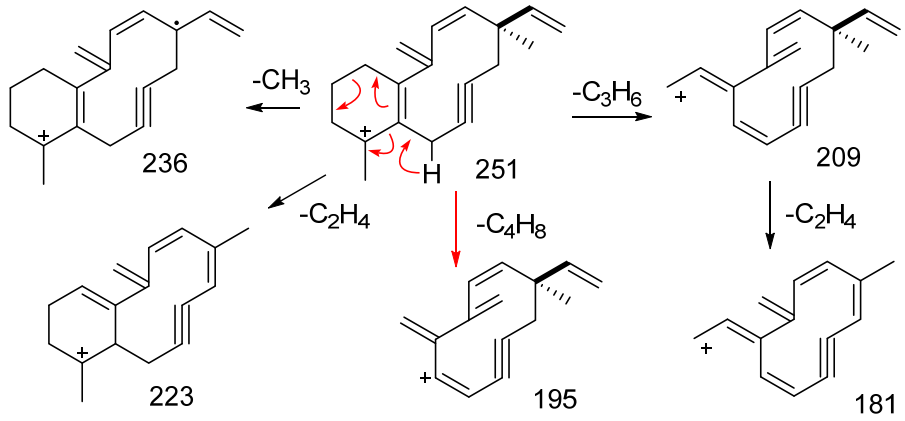
Scheme 2 – Alkyl moieties loss from ring A, CO and H_2O loss from ring B of 11β-hydroxyrosenonolactone. Blue arrow: H_2O loss from C=O group of ring B (*m/z* 269 to 251); red arrow: C_4H_8 loss from ring A (*m/z* 269 to 213).

The intermediate ions *m/z* 251 and 227 were introduced for CID, which let the cleavage of ring A and B, respectively (Scheme 3 and 4). Scheme 4 showed that the positive charge in Ring B could potentially induced the fragmentation of C=O and CH_2=CO. MS^3 spectrum of *m/z* 227 presented the neural loss of 42 Da, proposed as the elimination of ketene molecule (CH_2=CO) by decarboxylation. Alternatively, a 28 Da loss, from *m/z* 199 to 171, was assigned to another decarboxylation of C=O. Otherwise, ring C bearing a double bond carried out the favorite elimination of side-chain groups of methyl and C_2H_4 to form the stable p-π or π-π conjugated system (*m/z* 236 and 223).

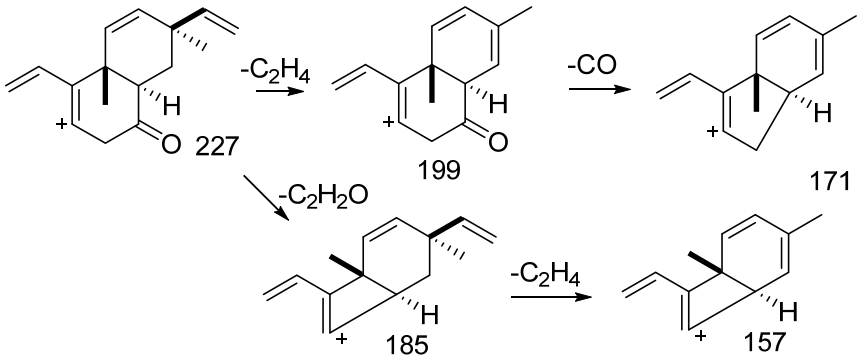
Fragmentation of Rosenonolactone (2)

Rosenonolactone, reduced product of 11β-hydroxyrosenonolactone, was used to support the hypothesis fragmentation pattern, and the results of ESI-MS^n indicated some similar preferential cleavage on ring-open and dehydration from C=O group. The sequence loss of 18 Da and 28 Da masses to give the product ions *m/z* 299 and 271, respectively, confirmed a two-step cleavage of lactone ring (Scheme 5). After that, CID spectrum of ion *m/z* 271 could produce ions of *m/z* 229 and 163, both of which were generated by ring B cleavage; meanwhile product ion *m/z* 253 was yielded by dehydration from C=O group of ring B initiated from the remote [1,5] H rearrangement, which was considered as key ion and introduce for next CID experiment (Scheme 6).

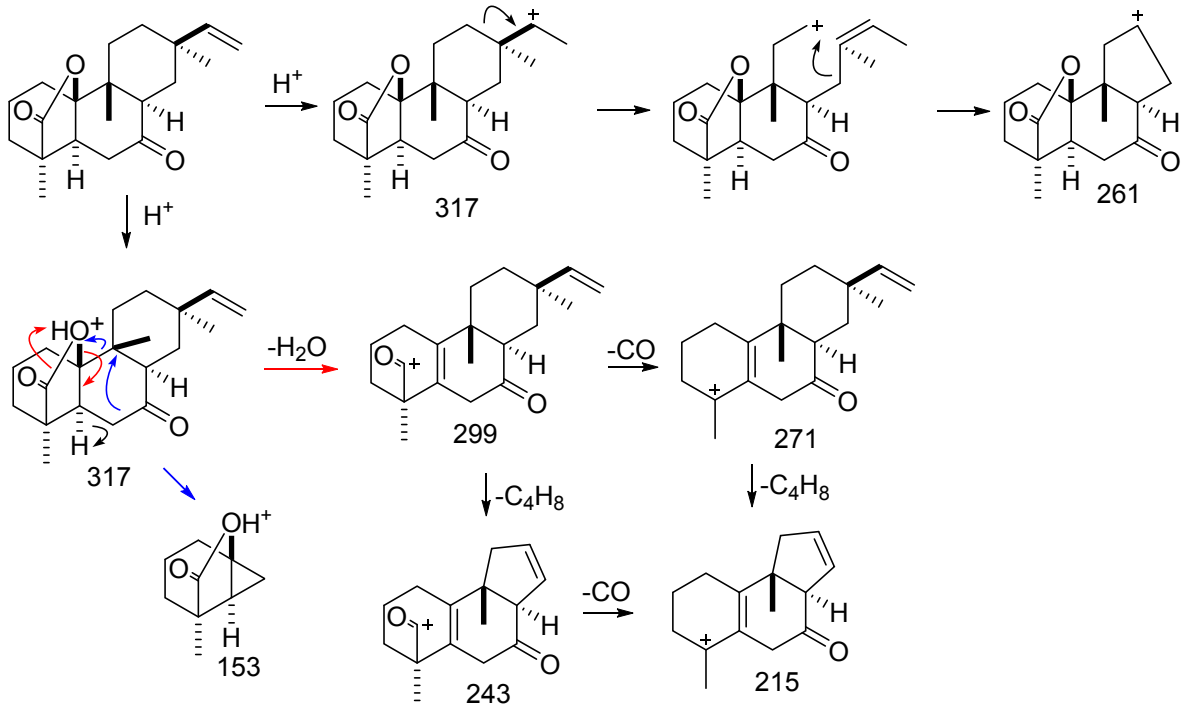
Ring A can cleavage by degrading serial neutral or radical moieties, such as C_2H_4, C_3H_6. In the MS^3 spectrum of *m/z* 253, the similar loss of C_4H_8 and C_6H_{10} produce ions of *m/z* 197 and 171, respectively (Scheme 7). The decarboxyl group from ring B was observed in MS^3 spectrum of ion *m/z* 215, which could produce the ions *m/z* 187 and 173 by losing CO and ketene moiety, respectively (Scheme 8).



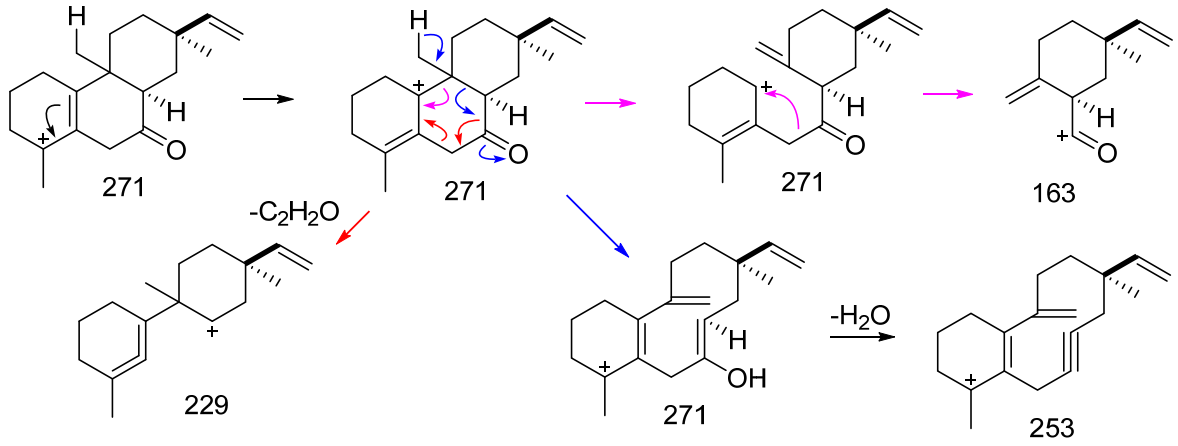
Scheme 3 – Alkyl moieties loss from product ion of m/z 251 of 11β-hydroxyrosenonolactone. Red arrow: C_4H_8 loss from ring A by [1,3] H-6 transfer (*m*/*z* 251 to 195).



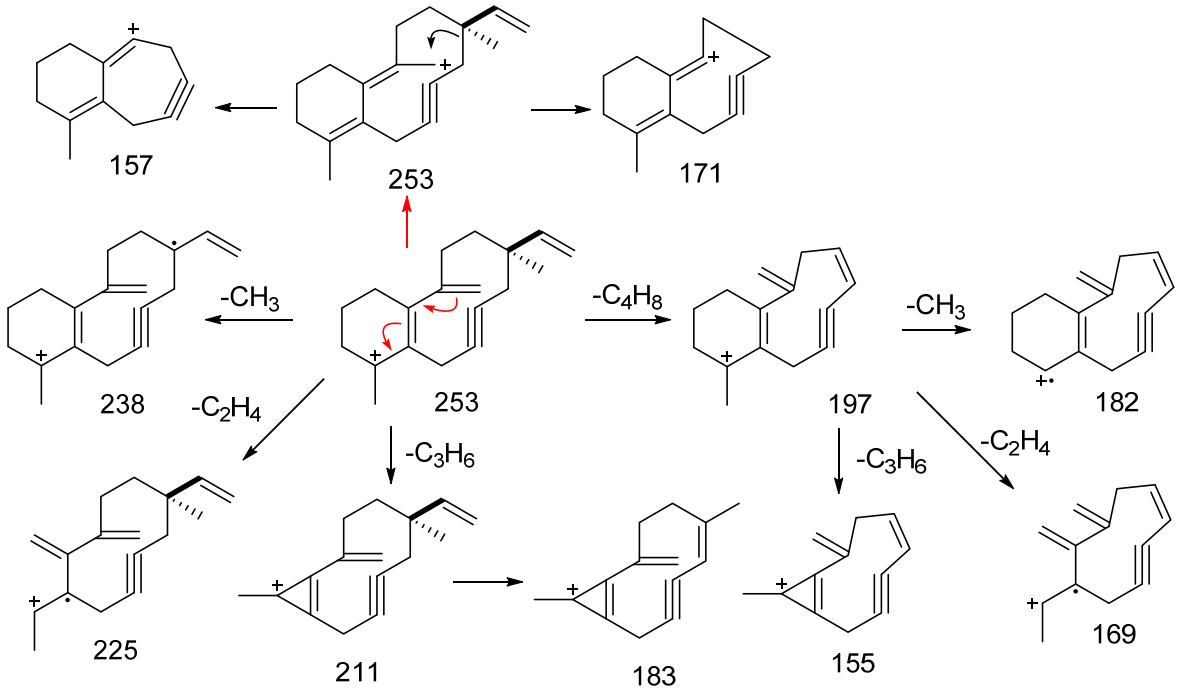
Scheme 4 – CO and C_2H_2O loss from ring B of 11β-hydroxyrosenonolactone.



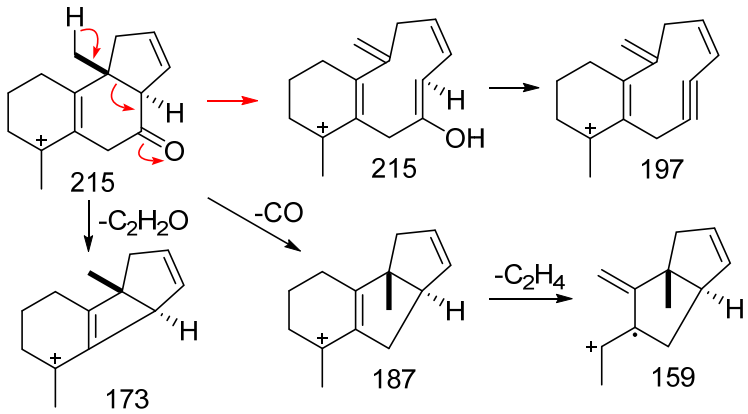
Scheme 5 – Lactone ring cleavage and H_2O loss from C=O group of ring B, with the alkyl moieties loss from ring C of Rosenonolactone. Blue arrow: ring B cleavage (*m*/*z* 317 to 153); red arrow: H_2O loss from lactone ring (*m*/*z* 317 to 299).



Scheme 6 – Ring B cleavage to lose CO, C_2H_2O and H_2O of Rosenonolactone. Blue arrow: H_2O loss from C=O group (*m/z* 271 to 253); red arrow: C_2H_2O loss (*m/z* 271 to 229); purple arrow: ring B cleavage (*m/z* 271 to 163).



Scheme 7 – Ring A and C cleavage to lose alkyl moieties from ion *m/z* 253 of Rosenonolactone.



Scheme 8 – CO, C_2H_2O and H_2O loss from ion *m/z* 215 of Rosenonolactone.

The similar fragmentation to **1** was also observed in the pathway of *m*/*z* 299-271-215 or *m*/*z* 299-243-215, from which product ion *m*/*z* 215 was obtained corresponding to neutral loss of C_4H_8 and CO, and C_4H_8 cleavage from ring C based on a controversial mechanism of hydrogen migration and ring-reducing degradation (Scheme 5). The obvious difference between **1** and **2** was that ring C of **2** could degrade to lose an alkyl C_4H_8 moiety. In the early fragmenting stage of **2**, a C_4H_8 moiety loss was assigned to the cleavage of ring C; and due to the alternative protonated site at vinyl group of C-13, the $[M+H]^+$ ion induced ring C opening followed by ring-reducing degradation to lose C_4H_8 moiety.

EXPERIMENTAL

Apparatus and analytical conditions

Multi-stage mass spectra were recorded with a Bruker Esquire HCT spectrophotometer equipped with an ESI source. The MS instrument was controlled by Esquire Control software, and operated in the positive mode with sheath gas of Nitrogen (99.9%) and collision gas of Helium (99.99%). The operating parameter of voltages was operated by automatic optimizing mode via instrument control software; Collision-induced dissociation (CID), 40V; scan range, 50–400 Da.

HR-ESI-MS/MS detection was carried out in Q Exactive Focus mass spectrometer (Thermo Scientific) in positive ionization mode, and data processing was performed using TraceFinder software version 4.1 (Thermo Scientific). Full mass $ddMS^2$ mode was used with MS full scan 70,000 FWHM, MS/MS full scan 15,000 FWHM. Other operating parameters were as follows: spray voltage, 4.4 kV; sheath gas pressure, 30.0 arb; aux gas pressure, 5.0 arb; capillary temperature, 250; heater temperature, 300; higher-energy collisional dissociation (HCD), 35 V; scan range, 150-350 Da.

Chemicals and samples

HPLC grade MeOH (≥99.8%) was purchased from Macron Fine Chemicals of China (Avantor), and formic acid was purchased from Aladdin Chemistry Co. Ltd, China. Compounds of 11β-hydroxyrosenonolactone and Rosenonolactone were kindly donated from Dr. Li Wang (Kunming Instituted of Botany, CAS, China). Samples were dissolved in methanol and acidized by formic acid with the approximate concentration of 0.5 mg/mL. Nitrogen (99.9%) and Helium (99.99%) were obtained from Jin-Hou Special Gas Co., Ltd. (Haikou, China).

CONCLUSIONS

The ESI multi-stage mass spectrometry by direct infusion, combining with HR-ESI-MS^2, analyzed the possible fragmentation pathway of two RDs lactones, and present study focused on the cleavage of their skeleton rings and lactone ring. Both of them always followed a typical fragmentation in MS^2 stage, and characterized by lactone ring cleavage to produce ion of $[M+H-H_2O-CO]^+$, which was define as the diagnostic ion for RDs lactone. And then, a characteristic water elimination pattern was assigned to ring-open and dehydration from C=O group at C-7 position by a possible mechanism of [1,5] H-20 rearrangement and C8-C9 bond break between ring B and C.

Dominant ring-open fragmentation was assigned to alkyl moieties loss from ring A, such as C_3H_6, C_4H_8 and C_5H_8, induced by tertiary allyl positive ion at C-4 position. In addition, the stability of conjugated system arising may explain the favorite loss of branched methyl or ethyl groups in ring C, instead of ring-open. Furthermore, ring C of 11β-hydroxyrosenonolactone contained a double bond formed by H_2O elimination, was highly stable and not found the cleavage in MS^n experiments, which suggested that it's more likely to form the p-π or π-π stable conjugated system by breaking the branch groups. On the contrarily, ring C of Rosenonolactone without unsaturated double bond could cleave to lose C_4H_8 moiety.

The present fragmentation study will provide valuable information for fast characterization and identification of RDs lactone from fungal fermentation broth, can provide reasonable understanding for ring-open fragmentation of them. To our knowledge, this methodology was a strategy for dereplication and identification, can be possibly applied to identify RDs, especially containing lactone ring.

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