RETRO-DIELS-ALDER FRAGMENTATION OF COUMARONOCHROMONES AND COMPLETE ASSIGNMENTS OF ^{13}C NMR DATA FOR LUPINALBIN A AND AYAMENIN B

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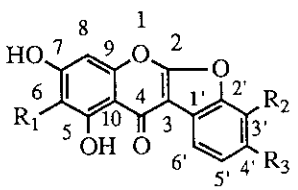
Abstract - A new retro-Diels-Alder fragmentation pathway for coumaronochromones was found in ei ms, and complete assignments of ^{13}C nmr data for simple coumaronochromones (lupinalbin A and ayamenin B) were presented.

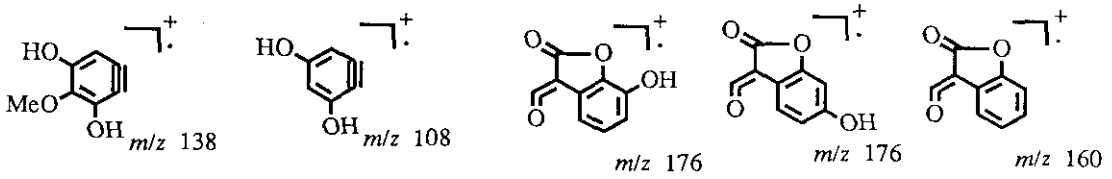
In 1966, Falshaw *et al.* [1] reported the isolation and structure elucidation of lisetin, the first derivative of coumaronochromone with the basic structure of 11*H* -benzofuro [2,3-*b*] [1]-benzopyran-11-one as a natural product. We also found fifteen coumaronochromones from Leguminosae [2-6] and Iridaceae. [7] Although more than nineteen coumaronochoromones are known,[2-10] there has been no study on the mass fragmentation pattern for them. Here we present a new retro-Diels-Alder (RDA) fragmentation pathway for coumaronochromones in ei ms spectroscopy. Moreover, there has been no investigation on complete assignments of ^{13}C nmr data for any coumaronochromone. Yang *et al.* [11] tried to assign the ^{13}C nmr data for lupinalbin A (**1**) isolated from *Lotus creticus*, however their assignments were quite tentative. In the present study, ^{13}C nmr spectra of lupinalbin A (**1**) and ayamenin B (**2**) were unambiguously assigned by application of 2D nmr techniques (CH-COSY, COLOC and HMBC). The isoflavone numbering system was used in this study.[1]



1: R_1, R_2 = H, R_3 = OH
2: R_1 = OMe, R_2, R_3 = H
3: R_1, R_2, R_3 = H
4: R_1, R_3 = H, R_2 = OH
5: R_1 = OMe, R_2 = H, R_3 = OH
6: R_1 = OMe, R_2 = OH, R_3 = H

Table 1. RDA fragments (rel. int., %) derived from A and B rings of coumaronochromones

	fragment from A ring		fragments from B ring	
compd *m/z*	138	108	176	160
ayamenin A (**2**)	-			19
ayamenin B (**3**)		10		16
ayamenin D (**5**)	-		22	
ayamenin C (**4**)		7	8	
compound **6**	-		21	
lupinalbin A (**1**)		6	11	



Retro-Diels-Alder fragmentation of coumaronochromone

Isoflavone exhibits RDA fission in ei ms [12] as shown in Figure 1, but the presence of an additional furan ring in coumaronochromones never permits them to be cleaved in the same manner as isoflavones. We have isolated six simple coumaronochromones, lupinalbin A (**1**),[2] ayamenins A-D (**2-5**), and 5,7,3'-trihydroxy-6-methoxycoumaronochromone (**6**) from *Iris pseudacorus* ,[7] and found that their ei ms spectra were quite simple. The precise analyses of their mass spectra allowed us to find coumaronochromone-type RDA fission (Figure 1). As shown in Table 1, all coumaronochromones with a monohydroxylated B ring exhibited a fragment ion at *m/z* 176, whilst those unsubstituted in the ring, *m/z* 160. These fragments must be derived from the B ring by RDA-type fission characteristic of coumaronochromones. Compounds containing no methoxyl group on the A ring showed a fragment ion at *m/z* 108 which was attributable to fragment from the A ring. However, compounds with a methoxy at C-6 did not show the corresponding benzyne fragment at *m/z* 138, probably because the methoxyl group would be cleaved much faster than the D ring to yield RDA fragments as

Table 2. Hr ei ms analysis for RDA fragments of ayamenins A, B and lupinalbin A

Fragments	ayamenin A (**2**)	ayamenin B (**3**)	lupinalbin A (**1**)
B ring *m/z*	160.0143	160.0162	176.0113
molecular formula	$C_9H_4O_3$	$C_9H_4O_3$	$C_9H_4O_4$
calcd	160.0160	160.0160	176.0109
A ring *m/z*		108.0197	108.0209
molecular formula		$C_6H_4O_2$	$C_6H_4O_2$
calcd		108.0221	108.0221

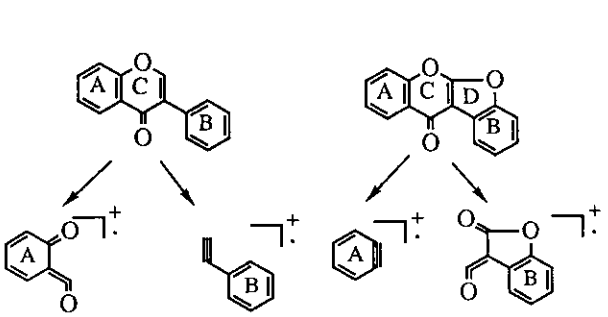
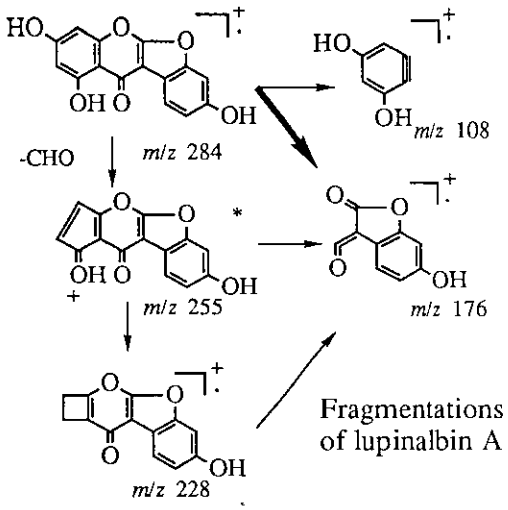
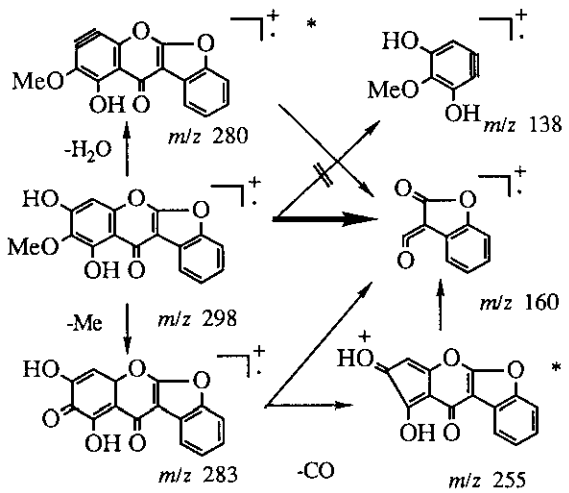


Figure 1. Retro-Diels-Alder fragmentation of isoflavones (left) and coumaronochromones (right)





Fragmentations of ayamenin A

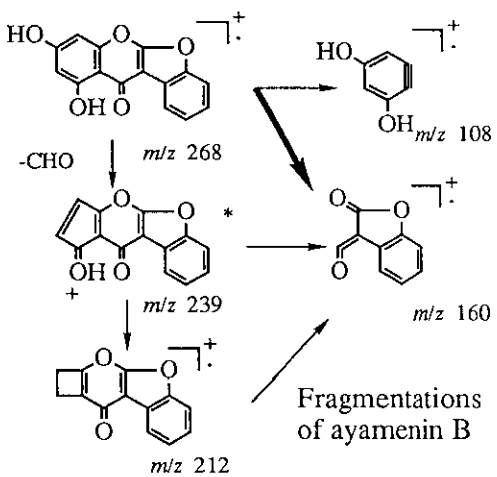


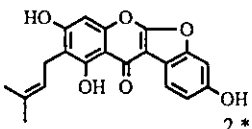
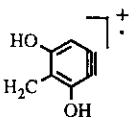
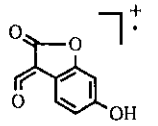
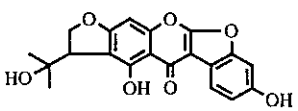
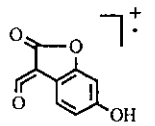
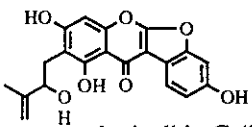
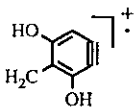
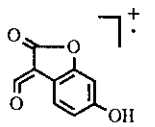
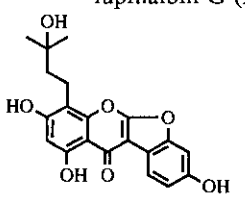
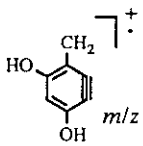
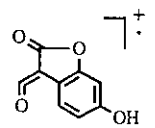
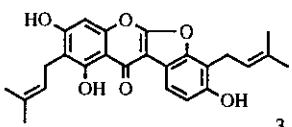
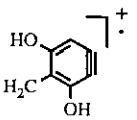
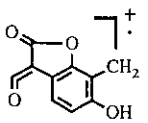
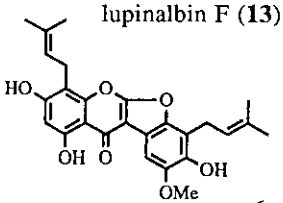
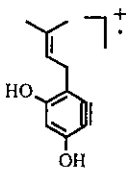
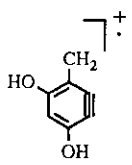
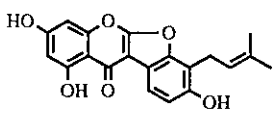
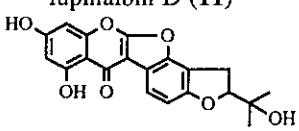
Figure 2. Fragmentations of simple coumaronochromones (* Several fragmentation patterns resulting in same *m/z* are also possible.)

shown in the fragmentation of ayamenin A (**2**) in Figure 2.

The results of high resolution ei ms (hr ei ms) of those fragments (*m/z* 176, 160 and 108) fully supported the presence of expected RDA fission of coumaronochromones (Table 2). Moreover ayamenins A (**2**) and B (**3**), and lupinalbin A (**1**) were subjected to ei ms ms analysis. Figure 2 shows their fragmentation patterns based on the coumaronochromone-type RDA fission. In ei ms ms of lupinalbin A , fragment ions both at *m/z* 176 (11 %) and 108 (6 %) were derived not from the deformylation fragment at *m/z* 255 (5 %), but from the molecular ion at *m/z* 284 (100 %). As for ayamenin B, fragment ions at *m/z* 160 (16 %) and

m/z 108 (10 %) were derived from the molecular ion at *m/z* 268 (100 %), and the former RDA fragment (*m/z* 160) was also formed as a daughter ion from the *m/z* at 239 ion. This fact shows that the RDA fission occurs not only on the molecular ion but also on the fragment ion at *m/z* 239. However, since the relative intensity of fragment ion at *m/z* 239 (4 %) is quite smaller than that of molecular ion (100 %), almost all of the RDA fragment of the B ring must be derived from the molecular ion. In the case of ayamenin A, the molecular and intense fragment ions [*m/z* 298 $[M]^+$ (91 %), 283 $[M-Me]^+$ (62 %), 280 $[M-H_2O]^+$(52 %) and 255 $[M-Me-CO]^+$(100 %)] afforded the fragment ion at *m/z* 160. In this case, the contribution of each fragment ion to the formation of the RDA fragment may be significant (Figure 2). Therefore, it seems that the RDA fission of coumaronochromones mainly occurs on the molecular ion, and some fragment ions still take part in the formation of RDA fragment ions. We examined if this RDA fission was common to complex coumaronochromones, lupinalbins B-G, lupilutin and 8-prenyllisetin (Table 3), and found that the coumaronochromones with a monohydroxylated B ring tend to yield the RDA fragment derived from the B ring (*m/z* 176). Complex coumaronochromones with highly substituted B ring (e. g., lupinalbins D and E, and 8-prenyllisetin) gave no RDA fragment, whereas only lupinalbin F showed a fragment ion at *m/z* 189 (6 %) which might be derived from the B ring via the coumaronochromone-type RDA fission. RDA fragment ions from the A ring of complex counaronochromones were hardly observed. In the ms spectra of lupinalbins B, F and G, and lupilutin, an RDA fragment peak at *m/z* 121 (Table 3) was observed (< 1 %, 4 %, 4 % and 2.4 %, respectively), but not in those of lupinalbins C and E. 8-Prenlyllisetin showed two peaks at *m/z* 176 (5.6 %) and 121 (19 %) (Table 3) which were assignable to the A ring fragments. However those fragments were not confirmed whether they were exactly derived from the A ring as a result of coumaronochromone-type RDA fission. So far as we examined, RDA fragments mentioned above were consistently detected in ms spectra of coumaronochromones with unsubstituted or monohydroxylated B ring. However analysis of mass fragmentation of complex coumaronochromones especially possessing highly substituted A/B rings, e. g., euchretins A - C,[8-9] are rather complicated and hardly informative to structure elucidation. But it is at least true from the present study that coumaronochromones do not exhibit the isoflavone-type RDA fission. Therefore, it is probably unreasonable to try to explain ms spectrum of coumaronochromone such as euchretin A[8] according to fragmentation based on the isoflavone-type RDA fission.

Table 3. The application of coumaronochromone-type RDA fission for some coumaronochromones

Coumaronochromones	fragments from A ring	fragments from B ring
 lupinalbin B (**7**)[2]*	 *m/z* 121 (< 1 %)	 *m/z* 176 (2.2 %)
 lupinalbin C (**8**)[2]*	**	 *m/z* 176 (2.5 %)
 lupinalbin G (**9**)[4]	 *m/z* 121 (4.3 %)	 *m/z* 176 (7.5 %)
 lupilutin (**10**)[5]	 *m/z* 121 (2.4 %)	 *m/z* 176 (10.0 %)
 lupinalbin F (**13**)[3]	 *m/z* 121 (4 %)	 *m/z* 189 (6 %)
 8-prenyllisetin (**14**)[6]	 *m/z* 176 (5.6 %)  *m/z* 121 (19 %)	**
 lupinalbin D (**11**)[2]*	**	**
 lupinalbin E (**12**)[2]*	**	**

* Data were obtained on JEOL JMS D 300. ** Fragments were not detected.

Complete assignments of ^{13}C nmr data for lupinalbin A (1) and Ayamenin B (3)

Table 4 shows the cross peaks found in CH-COSY and HMBC spectra of ayamenin B. The correlation between a carbon signal δ x and a proton H-y were described as δ x [H-y]. From CH-COSY spectrum, carbons at δ 100.8 [H-6], 95.8 [H-8], 112.3 [H-3'] and 122.0 [H-6'] were assigned to carbons at C-6, 8, 3' and 6' respectively. Another two carbons at δ 126.4 and 126.3 showed cross peaks with two protons distinguishable as a proton in higher field (H) and a proton in lower field (L) around 7.50 ppm respectively. They might be assigned to C-4' or C-5', and HMBC analysis was carried out to assign acurate chemical shift for them. Since HMBC spectra showed cross peaks between a higher field proton and two carbons (C-3' and C-6'), whilst the lower field proton showed cross peaks with C-3' and C-1', it conforms that the higher field proton is attached to C-4' (δ 126.4) and the lower field proton to C-5' (δ 126.3). The carbon signals at δ 163.9 [H-6 (δ 6.39) and C-5-OH (δ 12.92)], δ 164.5 [H-8], δ 156.3 [H-8], δ 104.5 [H-6, H-8 and C-5-OH] and δ 123.4 [H-3' and H-5'] were assigned to C-5, C-7, C-9, C-10 and C-1'respectively. Among the signals at δ 179.9, 166.3 and 98.2 gave no cross peaks in both CH-COSY and HMBC spectra, a signal at δ 179.9 was assignable to carbonyl carbon at C-4. Remaining signals δ 166.3 (oxygenated sp^2 carbon) and 98.2

Table 4. Cross peaks found in CH-COSY (☆) and HMBC (●) spectra of ayamenin B (**3**) (*acetone-d_6*)

Protons / Carbons		H-6 6.39	H-8 6.63	H-3' 7.7	H-4' 7.50 (H)*	H-5' 7.50 (L)*	H-6' 8.03	C-5-OH 12.92
C-2	166.3							
C-3	98.2							
C-4	179.9							
C-5	163.9	●						●
C-6	100.8	☆	●					●
C-7	164.5		●					
C-8	95.8	●	☆					
C-9	156.3		●					
C-10	104.5	●	●					●
C-1'	123.4			●		●		
C-2'	150.3						●	
C-3'	112.3			☆	●	●		
C-4'	126.4			●	☆		●	
C-5'	126.3			●		☆	●	
C-6'	122.0				●		☆	

* These two protons were distinguishable as a lower field proton (L) and a higher field proton (H).

(unoxygenated sp^2 carbon) were easily assigned to C-2 and C-3 respectively. As a result, all carbons were assigned as shown in Table 5.

By measuring the CH-COSY and COLOC spectra, the fifteen carbon signals for lupinalbin A (**1**) were also assigned in the same manner as described for ayamenin B (**3**). In the case of **1**, the chemical shifts for carbons in rings A and C were similar to those of ayamenin B (**3**). Because of the hydroxylation at C-4', the chemical shifts of the B ring carbons in **1** were different from those of **3**. The chemical shift of C-1'(δ 113.9) at the *para* position of C-4' was shifted to higher field about 10 ppm compared with that of C-1' in **3** (δ 123.4 ppm). The carbons at C-3' (δ 99.0) and C-5' (δ 114.0) which were *ortho* to the oxygenated C-4' also showed higher field shifts about 12 ppm (in ayamenin B : C-3', δ 112.3 and C-5', δ 126.4). But the *meta* carbons (C-2' and C-6') were not so affected by oxygenation at C-4'. Our assignments were shown in Table 6 associated with those of Yang *et al* .[11] On the basis of unequivocal assignments for ayamenin B (**3**) and lupinalbin A (**1**), the ^{13}C nmr data for ayamenin A (**2**) were similarly assigned as shown in Table 5. These two data (the RDA fission and ^{13}C nmr chemical shift values) will be useful to identify new coumaronochromones.

Table 5. ^{13}C Nmr data of lupinalbin A, ayamanin A and ayamanin B

	lupinalbin A (**1**)			ayamenin A (**2**)	ayamenin B (**3**)
carbon	(DMSO-d_6)	(DMSO-d_6)*	(acetone-d_6)	(acetone-d_6)	(acetone-d_6)
C-2	161.4	149.8 (C-2')	164.8	166.4	166.3
C-3	97.0	113.4 (C-1')	97.6	97.9	98.2
C-4	177.9	178.2	178.5	180.3	179.9
C-5	162.0	162.0	162.5	155.2	163.9
C-6	99.6	99.8	100.0	133.0	100.8
C-7	163.4	164.2	164.0	157.1	164.5
C-8	94.7	94.3	95.2	95.7	95.8
C-9	154.5	156.2 (C-4')	155.2	151.3	156.3
C-10	102.7	102.5	103.0	104.0	104.5
C-1'	113.1	96.9 (C-3)	113.9	122.3	123.4
C-2'	149.8	154.6 (C-9)	150.5	150.3	150.3
C-3'	98.6	98.7	99.0	112.3	112.3
C-4'	156.2	162.0 (C-2)	156.7	126.3	126.3
C-5'	113.6	113.7	114.0	126.5	126.4
C-6'	120.9	121.0	121.5	122.0	122.0
C-6-OMe			60.8		

* The assignment reported by Yang *et al* .[11]

EXPERIMENTAL

All compounds used in the present study were isolated from lupins and iris, and identified in our earlier papers (lupinalbin A,[2] ayamenins A - D[7] and 5,7,3'-trihydroxy-6-methoxycoumaronochromone[7]). Nmr spectra were obtained by a Bruker AM 500. Ei ms (direct inlet system, 70 eV ionization potential) and ei ms ms (70 eV, ionization potential and10 kV, acceleration potential) were determined by a JEOL JMS DX 300 or D 300, and JEOL JMS HX-100, respectively.

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