# Supporting Materials

# Identification of the Chemical Constituents of an Anti-Arthritic Chinese Medicine Wen Luo Yin by Liquid Chromatography Coupled with Mass Spectrometry

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**Table 1. Characterization of the constituents of WLY by LC-MS/MS.**

| Peak no. | $t_R$ (min) | Identification | Formula | $[M+H]^+$ (m/z) | $[M-H]^-$ (m/z) | Fragment ions in positive ion mode | Structure class | Plant material |
|---|---|---|---|---|---|---|---|---|
| 1 | 2.4 | neoline | $C_{24}H_{39}NO_6$ | 438.4 | — | 420$[M+H-H_2O]^+$,388$[M+H-H_2O-CH_3OH]^+$ | alkaloid | RA |
| 2 | 3.6 | fuziline | $C_{24}H_{39}NO_7$ | 454.4 | — | 436$[M+H-H_2O]^+$,418$[M+H-2H_2O]^+$,404$[M+H-H_2O-CH_3OH]^+$, 386$[M+H-2H_2O-CH_3OH]^+$,372$[M+H-H_2O-2CH_3OH]^+$ | alkaloid | RA |
| 3 | 4.6 | talatizamine | $C_{24}H_{39}NO_5$ | 422.6 | — | 390$[M+H-CH_3OH]^+$,372$[M+H-CH_3OH-H_2O]^+$, 358 ($[M+H-2CH_3OH]^+$) | alkaloid | RA |
| 4 | 8.8 | 14-benzoyl-10-OH-mesaconine | $C_{31}H_{43}NO_{11}$ | 606.4 | — | 588($[M+H-H_2O]^+$),574($[M+H-CH_3OH]^+$), 556($[M+H-H_2O-CH_3OH]^+$),524($[M+H-H_2O-2CH_3OH]^+$) | alkaloid | RA |
| 5 | 16.2 | benzoylmesaconine | $C_{31}H_{43}NO_{10}$ | 590.6 | — | 572($[M+H-H_2O]^+$), 558($[M+H-CH_3OH]^+$), 540($[M+H-H_2O-CH_3OH]^+$),508($[M+H-H_2O-2CH_3OH]^+$) | alkaloid | RA |
| 6 | 18.7 | 14-benzoylaconine | $C_{32}H_{45}NO_{10}$ | 604.4 | — | 586($[M+H-H_2O]^+$),572($[M+H-CH_3OH]^+$), 554($[M+H-H_2O-CH_3OH]^+$),522($[M+H-H_2O-2CH_3OH]^+$) | alkaloid | RA |
| 7 | 21.4 | 14-benzoylhypaconine | $C_{31}H_{43}NO_9$ | 574.6 | — | 542($[M+H-CH_3OH]^+$), 510($[M+H-2CH_3OH]^+$),492($[M+H-H_2O-2CH_3OH]^+$) | alkaloid | RA |
| 8 | 24 | unknown | | 537.6 | — | 417,375 | | RA |
| 9 | 25.2 | 10-OH-mesaconitine | $C_{33}H_{45}NO_{12}$ | 648.4 | — | 588($[M+H-CH_3COOH]^+$),556($[M+H-CH_3COOH-CH_3OH]^+$), 528($[M+H-CH_3COOH-CH_3OH-CO]^+$) | alkaloid | RA |
| 10 | 25.2 | 14-benzoyldeoxyaconine | $C_{32}H_{45}NO_9$ | 588.2 | — | 556($[M+H-CH_3OH]^+$), 538($[M+H-H_2O-CH_3OH]^+$), 524($[M+H-2CH_3OH]^+$), 506($[M+H-H_2O-2CH_3OH]^+$) | alkaloid | RA |
| 11 | 30.6 | mesaconitine | $C_{33}H_{45}NO_{11}$ | 632.6 | — | 572($[M+H-CH_3COOH]^+$),540($[M+H-CH_3COOH-CH_3OH]^+$), 354($[M+H-CH_3COOH-3CH_3OH-C_6H_5COOH]^+$) | alkaloid | RA |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12 | 31.1 | 10-OH-aconitine | $C_{34}H_{47}NO_{12}$ | 662.4 | — | 602([M+H-$CH_3COOH$]$^+$),570([M+H-$CH_3COOH$-$CH_3OH$]$^+$), 384([M+H-$CH_3COOH$-3$CH_3OH$-$C_6H_5COOH$]$^+$) | alkaloid | RA |
| 13 | 33.2 | coumarin* | $C_9H_6O_2$ | 147.4 | — | 103([M+H-$CO_2$]$^+$),91([$C_7H_7$]$^+$),77([$C_6H_5$]$^+$),65([$C_5H_5$]$^+$) | phenylpropanoid | CR |
| 14 | 34.5 | hypaconitine* | $C_{33}H_{45}NO_{10}$ | 616.6 | — | 556([M+H-$CH_3COOH$]$^+$),524([M+H-$CH_3COOH$-$CH_3OH$]$^+$), 496([M+H-$CH_3COOH$-$CH_3OH$-CO]$^+$), 338([M+H-$CH_3COOH$-3$CH_3OH$-$C_6H_5COOH$]$^+$) | alkaloid | RA |
| 15 | 35.3 | aconitine* | $C_{34}H_{47}NO_{11}$ | 646.4 | — | 586([M+H-$CH_3COOH$]$^+$),554([M+H-$CH_3COOH$-$CH_3OH$]$^+$), 368([M+H-$CH_3COOH$-3$CH_3OH$-$C_6H_5COOH$]$^+$) | alkaloid | RA |
| 16 | 35.4 | 13-deoxyhypaconitine | $C_{33}H_{45}NO_9$ | 600.2 | — | 540([M+H-$CH_3COOH$]$^+$),508([M+H-$CH_3COOH$-$CH_3OH$]$^+$), 322([M+H-$CH_3COOH$-3$CH_3OH$-$C_6H_5COOH$]$^+$) | alkaloid | RA |
| 17 | 36.7 | 2-hydroxy cinnamaldehyde | $C_9H_8O_2$ | 149.4 | 147.2 | 131([M+H-$H_2O$]$^+$),121([M+H-CO]$^+$), 103 ([M+H-$H_2O$-CO]$^+$), 93([M+H-CO-$C_2H_4$]$^+$), 91([$C_7H_7$]$^+$), 77([$C_6H_5$]$^+$) | phenylpropanoid | CR |
| 18 | 40.1 | deoxyaconitine | $C_{34}H_{47}NO_{10}$ | 630.4 | — | 570([M+H-$CH_3COOH$]$^+$),538([M+H- $CH_3COOH$-$CH_3OH$]$^+$), 510([M+H-$CH_3COOH$-$CH_3OH$-CO]$^+$) | alkaloid | RA |
| 19 | 43.5 | cinnamic alcohol* | $C_9H_{10}O$ | 135.4 | 133.4 | 117 ([M+H-$H_2O$]$^+$), 91([$C_7H_7$]$^+$), | phenylpropanoid | CR |
| 20 | 49.3 | cinnamic acid* | $C_9H_8O_2$ | 149.4 | 147.2 | 131([M+H-$H_2O$]$^+$),103([M+H-$H_2O$-CO]$^+$),91([$C_7H_7$]$^+$),77([$C_6H_5$]$^+$) | phenylpropanoid | CR |
| 21 | 53.8 | cinnamic aldehyde* | $C_9H_8O$ | 133.4 | — | 115([M+H-$H_2O$]$^+$),105([M+H-CO]$^+$),91([$C_7H_7$]$^+$),77([$C_6H_5$]$^+$) | phenylpropanoid | CR |
| 22 | 62.6 | selaginellin | $C_{34}H_{24}O_5$ | 513.4 | 511.4 | 495([M+H-$H_2O$]$^+$),419([M+H-PhOH]$^+$),401([M+H-PhOH-$H_2O$]$^+$), 378([M+H-$H_2O$-$C_8H_5O$•]$^{+\bullet}$),325([M+H-2PhOH]$^+$),297([M+H-$H_2O$-$C_{13}H_{10}O_2$]$^+$) | acetylenic phenol compound | ST |
| 23 | 64.2 | 2-methoxy cinnamaldehyde | $C_{10}H_{10}O_2$ | 163.4 | — | 145([M+H-$H_2O$]$^+$),135([M+H-CO]$^+$), 115 ([M+H-$H_2O$-$CH_2O$]$^+$),107([M+H-CO-$C_2H_4$]$^+$) | phenylpropanoid | CR |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 24 | 70.1 | amentoflavone* | $C_{30}H_{18}O_{10}$ | 539.4 | 537.2 | 497([M+H-$C_2H_2O$]$^+$),403($^{1,3}$IIA$^+$-$H_2O$),377($^{0,4}$IIA$^+$), 347 ($^{1,3}$IIA$^+$-$H_2O$-2CO), 335($^{0,4}$IIA$^+$-$C_2H_2O$) | biflavonoid | ST |
| 25 | 70.8 | 2'',3''-dihydroamentoflavone | $C_{30}H_{20}O_{10}$ | 541.2 | 539.2 | 421($^{1,3}$IIA$^+$),403($^{1,3}$IIA$^+$-$H_2O$), 337($^{1,3}$IIA$^+$-2$C_2H_2O$), 311($^{1,3}$IIA$^+$-$C_2H_2O$-$C_3O_2$),283($^{1,3}$IIA$^+$-$C_2H_2O$-$C_3O_2$-CO) | biflavonoid | ST |
| 26 | 73.6 | 2,3-dihydrorobustaflavone | $C_{30}H_{20}O_{10}$ | 541.4 | 539.2 | 415 ($^{1,4}$IB$^+$), 389 ($^{1,3}$IB$^+$), 153($^{1,3}$IA$^+$),121($^{0,2}$IIB$^+$) | biflavonoid | ST |
| 27 | 74.2 | robustaflavone* | $C_{30}H_{18}O_{10}$ | 539.4 | 537.2 | 521([M+H-$H_2O$]$^+$), 465([M+H-$H_2O$-2CO]$^+$), 413($^{1,4}$IB$^+$), 403($^{1,3}$IIA$^+$-$H_2O$), 387($^{1,3}$IB$^+$), 153($^{1,3}$IA$^+$),121($^{0,2}$IIB$^+$) | biflavonoid | ST |
| 28 | 82.9 | 7''-*O*-methylamentoflavone | $C_{31}H_{20}O_{10}$ | 553.4 | 551.2 | 521([M+H-$CH_3OH$]$^+$),417($^{1,3}$IIA$^+$-$H_2O$), 401($^{1,3}$IB$^+$), 391($^{0,4}$IIA$^+$), 361($^{1,3}$IIA$^+$-$H_2O$-2CO),153($^{1,3}$IA$^+$), 121($^{0,2}$IIB$^+$) | biflavonoid | ST |
| 29 | 87.2 | atractylenolide III* | $C_{15}H_{20}O_3$ | 249.1 | 247.2 | 231([M+H-$H_2O$]$^+$), 213([M+H-2$H_2O$]$^+$), 203([M+H-$H_2O$-CO]$^+$), 189([M+H-$H_2O$-$C_3H_6$]$^+$),185([M+H-2$H_2O$-CO]$^+$),175([M+H-$H_2O$-$C_4H_6$]$^+$),163([M+H-$H_2O$-$C_5H_8$]$^+$) | sesquiterpene | AM |
| 30 | 88.0 | 7-*O*-methylamentoflavone* | $C_{31}H_{20}O_{10}$ | 553.4 | 551.2 | 417($^{1,3}$IIA$^+$-$H_2O$), 391($^{0,4}$IIA$^+$),361 ($^{1,3}$IIA$^+$-$H_2O$-2CO), 349($^{0,4}$IIA$^+$-$C_2H_2O$), 167($^{1,3}$IA$^+$), 121($^{0,2}$IIB$^+$) | biflavonoid | ST |
| 31 | 89.0 | hinokiflavone* | $C_{30}H_{18}O_{10}$ | 539.2 | 537.2 | 387 ($^{1,3}$IB$^+$),286 ([Flavone II + OH]$^{+\bullet}$), 270 ( [Flavone I]$^{+\bullet}$ or [Flavone II]$^{+\bullet}$), 257 ([Flavone II+O-CO]$^+$), 254 ([Flavone I-O]$^{+\bullet}$) | biflavonoid | ST |
| 32 | 98.0 | unknown | | 437.2 | — | 303 | | AM |
| 33 | 103.5 | isocryptomerin | $C_{31}H_{20}O_{10}$ | 553.4 | 551.2 | 521([M+H-$CH_3OH$]$^+$), 401($^{1,3}$IB$^+$), 299 ([Flavone II+O]$^+$), 284 ([Flavone II]$^{+\bullet}$), 271 ([Flavone II+O-CO]$^+$), 254 ([Flavone I-O]$^{+\bullet}$) | biflavonoid | ST |
| 34 | 105.5 | isoatracytlenolide I | $C_{15}H_{20}O_2$ | 233.4 | — | 215([M+H-$H_2O$]$^+$),187([M+H-$H_2O$-CO]$^+$),177([M+H-$C_4H_8$]$^+$), 159([M+H-CO-$H_2O$-$C_2H_4$]$^+$), 151([M+H-$C_4H_8$-$C_2H_2$]$^+$) | sesquiterpene | AM |
| 35 | 107.6 | atractylenolide I* | $C_{15}H_{20}O_2$ | 233.4 | — | 215([M+H-$H_2O$]$^+$),187([M+H-$H_2O$-CO]$^+$),177([M+H-$C_4H_8$]$^+$), 159([M+H-CO-$H_2O$-$C_2H_4$]$^+$),151([M+H-$C_4H_8$ –$C_2H_2$]$^+$) | sesquiterpene | AM |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 36 | 107.8 | 14-acetoxy-12-senecioyloxytetradeca-2E,8EZ,10E-trien-4,6-diyn-1-ol | $C_{21}H_{24}O_5$ | 379.4[M+Na]$^+$ | — | 279([M+Na-SenOH]$^+$),257([M+Na-SenONa]$^+$),197([M+Na-SenOH-AcONa]$^+$),179[M+Na-SenOH-AcONa-$H_2O$]$^+$ | polyacetylene | AM |
| 37 | 111.4 | neocryptomerin | $C_{31}H_{20}O_{10}$ | 553.2 | 551.2 | 387($^{1,3}$IB$^+$),286([Flavone II+OH]$^{+\bullet}$),270([Flavone II]$^{+\bullet}$), 268([Flavone I-O]$^{+\bullet}$),257 ([Flavone II+O-CO]$^+$) | biflavonoid | ST |
| 38 | 112.2 | 14-acetoxy-12-senecioyloxytetradeca-2E,8EZ,10E-trien-4,6-diyn-1-ol | $C_{21}H_{24}O_5$ | 379.2 [M+Na]$^+$ | 393 | 279([M+Na-SenOH]$^+$),257([M+Na-SenONa]$^+$),197([M+Na-SenOH-AcONa]$^+$),179[M+Na-SenOH-AcONa-$H_2O$]$^+$ | polyacetylene | AM |
| 39 | 113.0 | 14-acetoxy-12-methylbutyryltetradeca-2E,8EZ,10E-trien-4, 6-diyn-1-ol | $C_{21}H_{26}O_5$ | 381.4 [M+Na]$^+$ | — | 279([M+Na-MeBOH]$^+$),257([M+Na-MeBONa]$^+$),197([M+Na-MeBOH-AcONa]$^+$),179[M+Na-MeBOH-AcONa-$H_2O$]$^+$ | polyacetylene | AM |
| 40 | 114.0 | unknown | | | | | | |
| 41 | 115.6 | 14-acetoxy-12-methylbutyryltetradeca-2E,8EZ,10E-trien-4, 6-diyn-1-ol | $C_{21}H_{26}O_5$ | 381.4 [M+Na] | — | 279([M+Na-MeBOH]$^+$),257([M+Na-MeBONa]$^+$),197([M+Na-MeBOH-AcONa]$^+$),179[M+Na-MeBOH-AcONa-$H_2O$]$^+$ | polyacetylene | AM |
| 42 | 116.2 | unknown | | | | | | |
| 43 | 119.2 | 8-methoxyatractylenolide I | $C_{16}H_{22}O_3$ | 263.2 | 261.2 | 231([M+H-$CH_3OH$]$^+$), 213([M+H-$CH_3OH$-$H_2O$]$^+$), 203([M+H-$CH_3OH$-CO]$^+$),189([M+H-$CH_3OH$-$C_3H_6$]$^+$), 185([M+H-$CH_3OH$-$H_2O$-CO]$^+$), 163([M+H-$CH_3OH$-$C_5H_8$]$^+$) | sesquiterpene | AM |

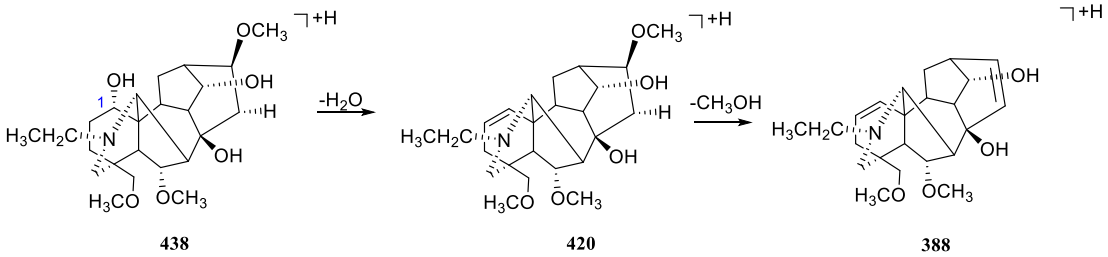
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 44 | 123.0 | atractylenolide II* | $C_{15}H_{18}O_2$ | 231.4 | 229.4 | 213($[M+H-H_2O]^+$),203($[M+H-C_2H_4]^+$),185($[M+H-H_2O-CO]^+$), 157($[M+H-H_2O-CO-C_2H_4]^+$),143($[M+H-H_2O-CO-C_3H_6]^+$) | sesquiterpene | AM |
| 45 | 124.2 | unknown | | 496.4 | — | 478,313,258,284,104 | | AM |
| 46 | 128.1 | 7,7”-di-*O*-methylhinokiflavone | $C_{32}H_{22}O_{10}$ | 567.6 | 565.4 | 401($^{1,3}IB^+$),299([Flavone II+O]$^+$),284 ([Flavone I]$^{+\bullet}$ or [Flavone II]$^{+\bullet}$), 268([Flavone I-O]$^{+\bullet}$),256([Flavone I or II -CO]$^{+\bullet}$ | biflavonoid | ST |
| 47 | 131.2 | unknown | | 219.4 | | 201,159,145,123,95 | | AM |
| 48 | 132.8 | atractylenolide VI | $C_{15}H_{22}$ | 203.2 | — | 161($[M+H-C_3H_6]^+$), 133($[M+H-C_3H_6-C_2H_4]^+$), 105($[M+H-C_3H_6-C_4H_8]^+$) | sesquiterpene | AM |

* Compared with a reference standard.

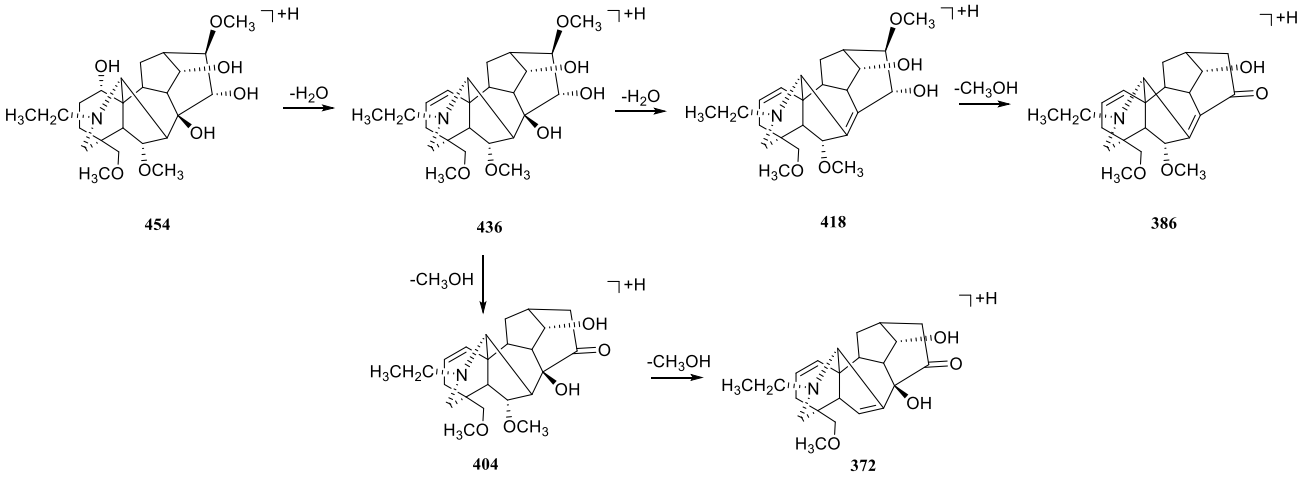
Note: $t_R$, retention time; AM, *Atractylodes macrocephala*; ST, *Selaginella tamariscina*; CR, *Cinnamomi Ramulus*; RA, *Radix Aconiti lateralis Preparata.*

## Figure S1. The fragmentation pathways of the identified compounds

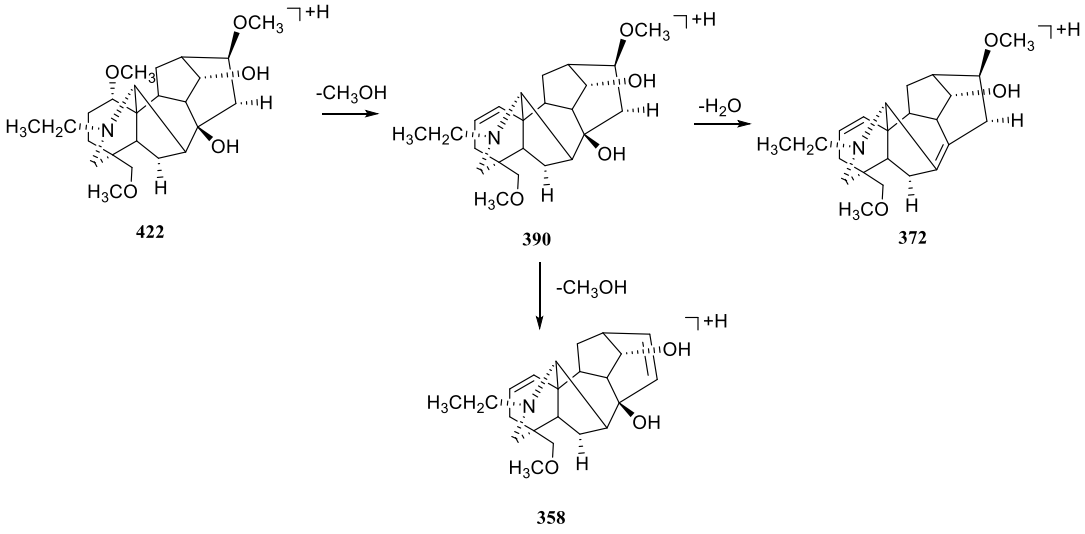
- Peak 1 (neoline)



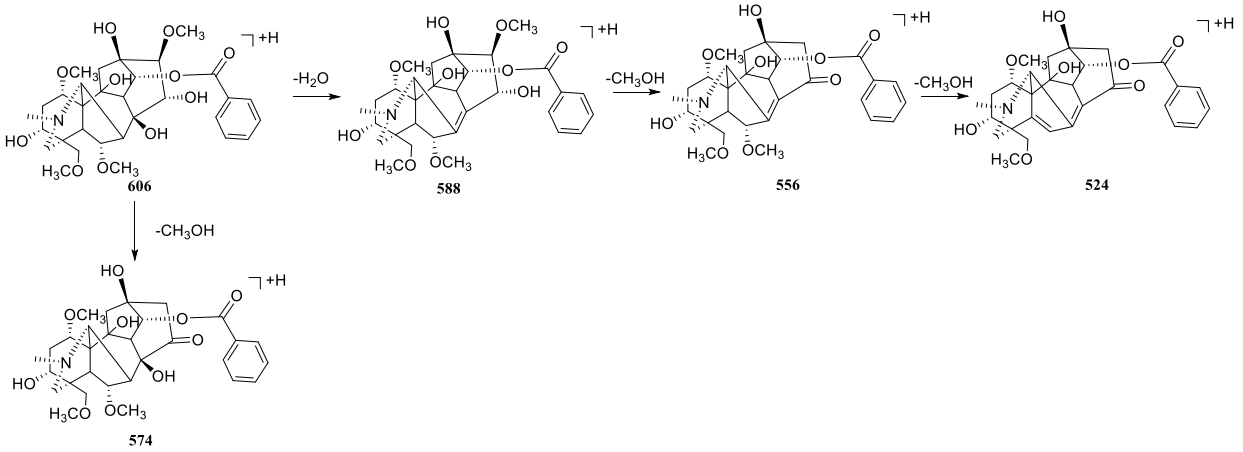

- Peak 2 (fuziline)



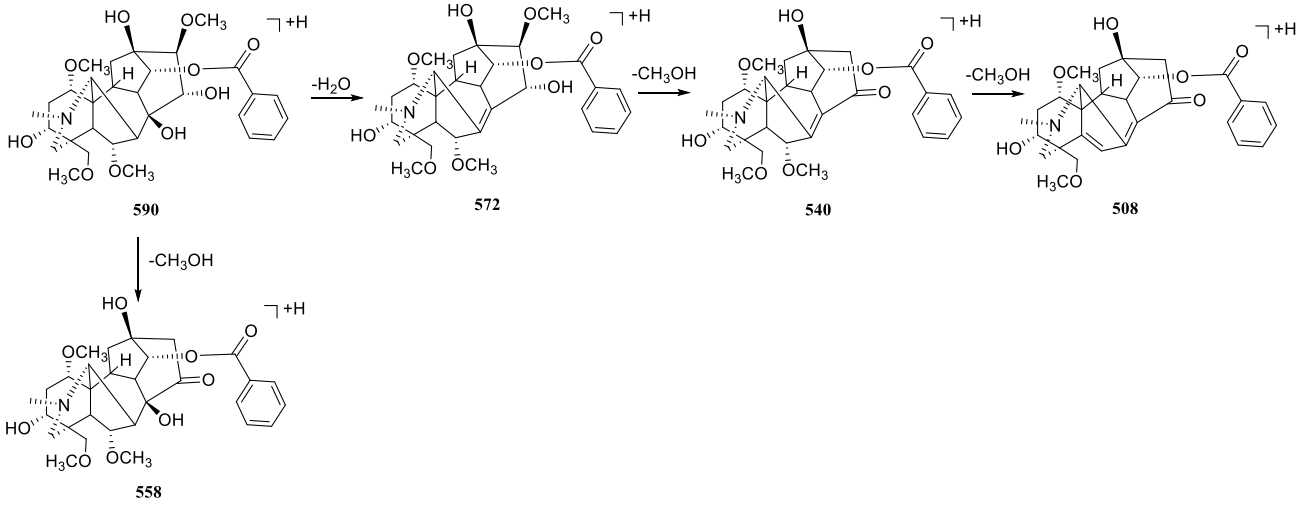

- Peak 3 (talatizamine)



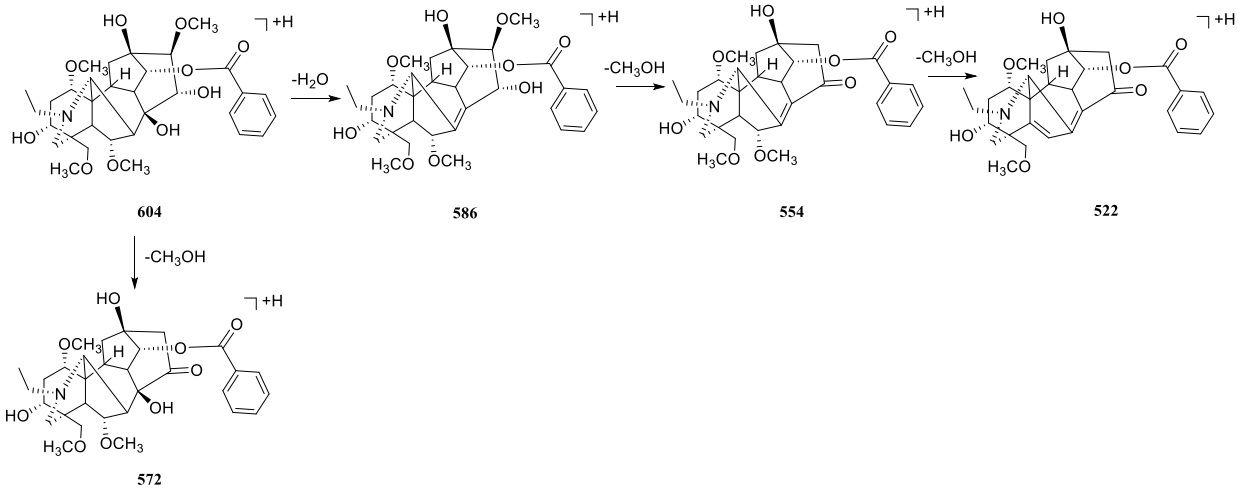

- Peak 4 (14-benzoyl-10-OH-mesaconine)



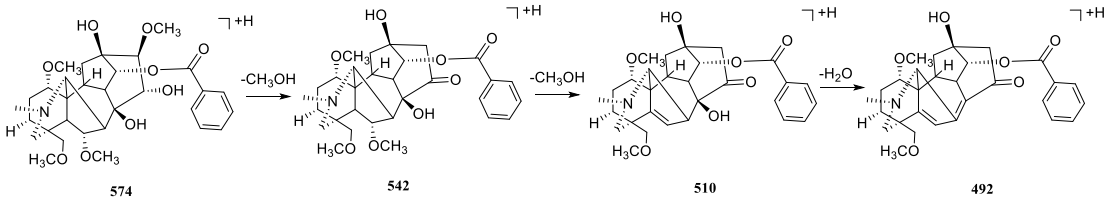

- Peak 5 (benzoylmesaconine)



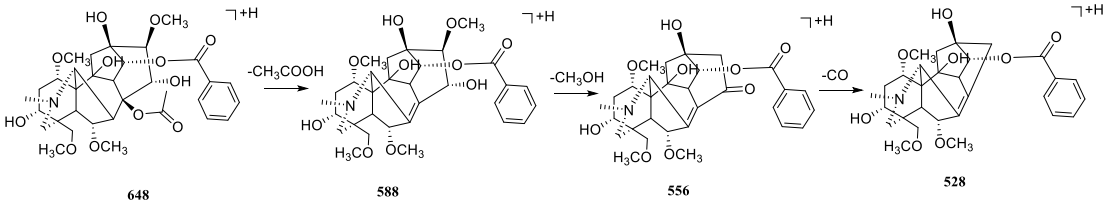

- Peak 6 (14-benzoylaconine)



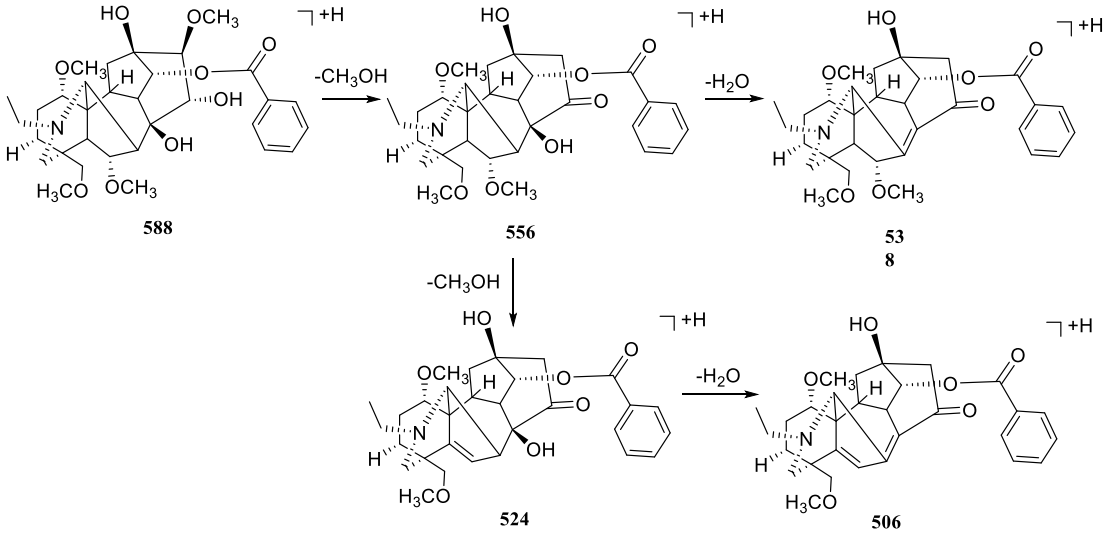

- Peak 7 (14-benzoylhypaconine)



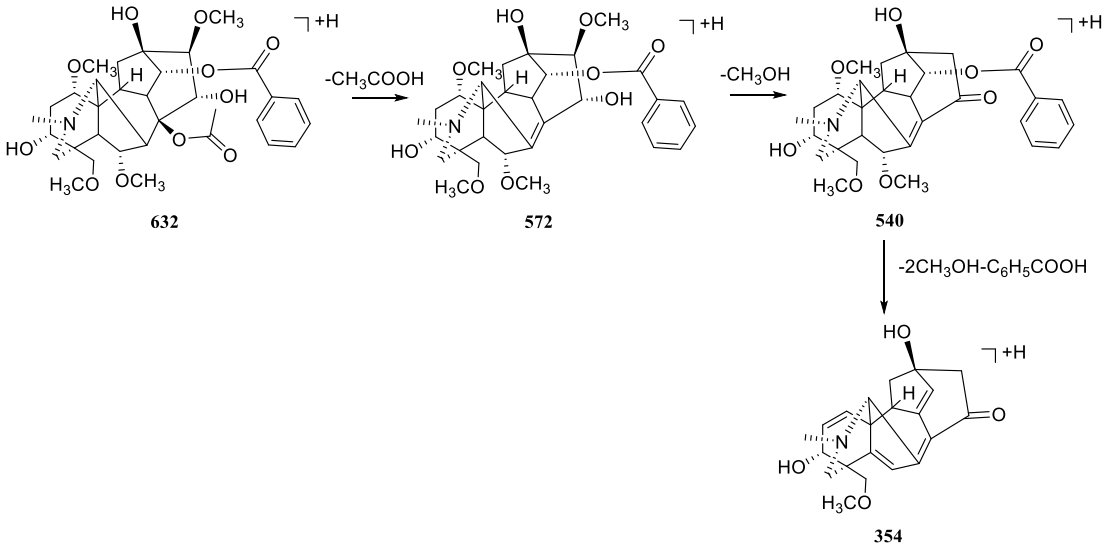

- Peak 9 (10-OH-mesaconitine)



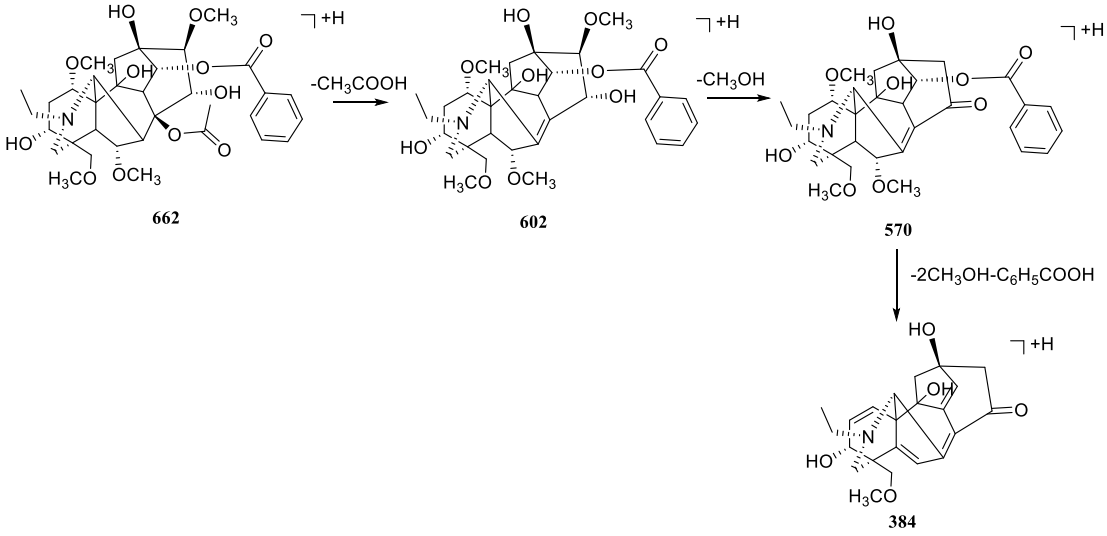

- Peak 10 (14-benzoyldeoxyaconine )



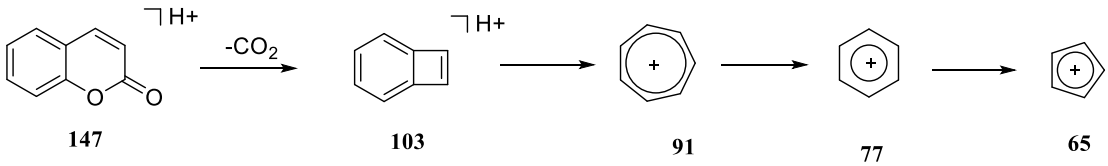

- Peak 11 (mesaconitine)



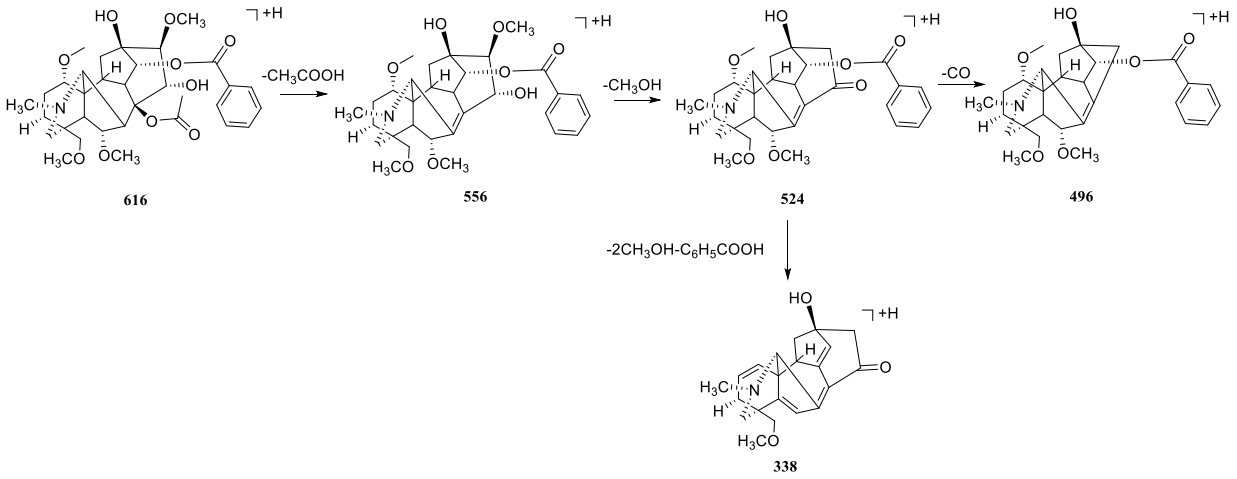

- Peak 12 (10-OH-aconitine)



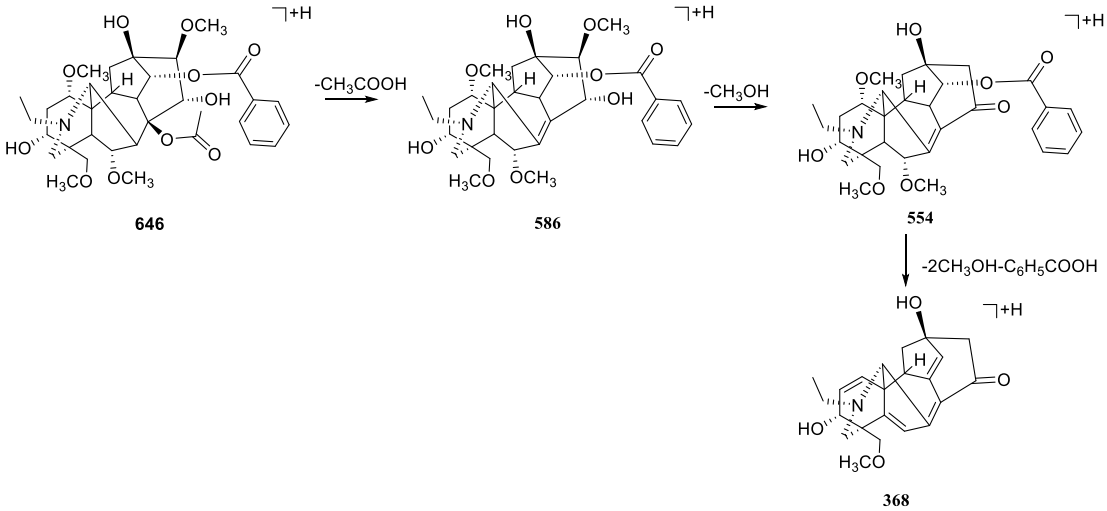

- Peak 13 (coumarin)



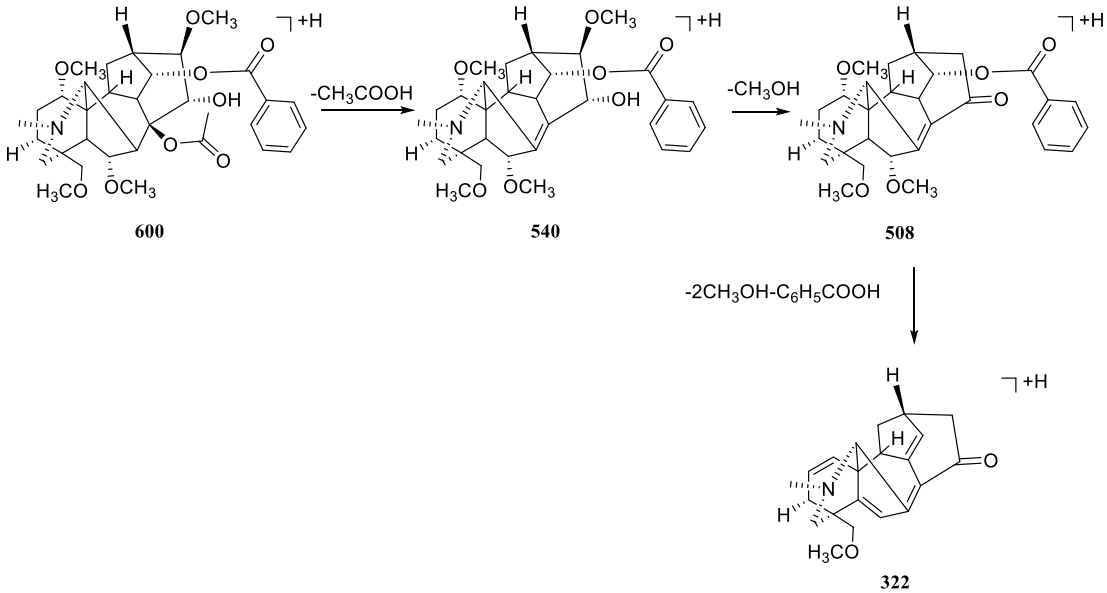

- Peak 14 (hypaconitine)



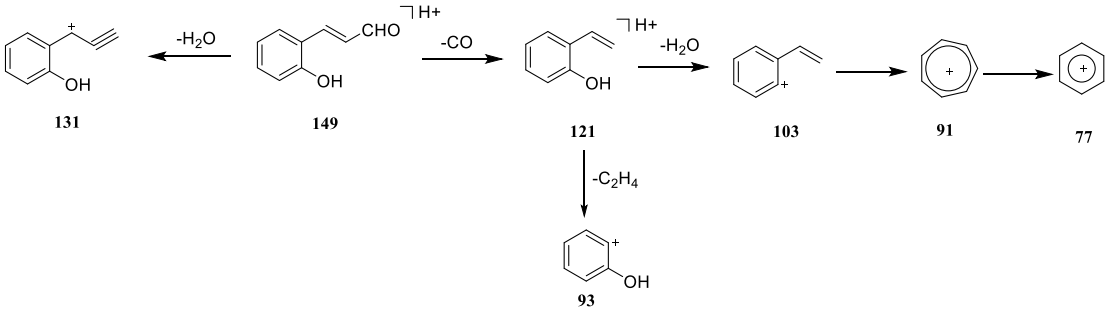

- Peak 15 (aconitine)



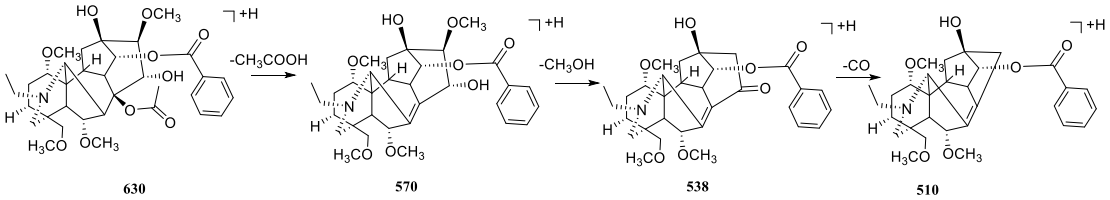

- Peak 16 (13-deoxyhypaconitine)



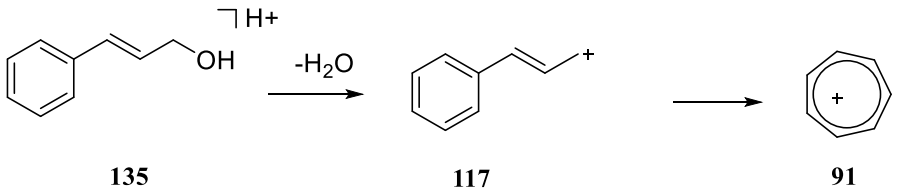

- Peak 17 (2-hydroxy cinnamaldehyde)



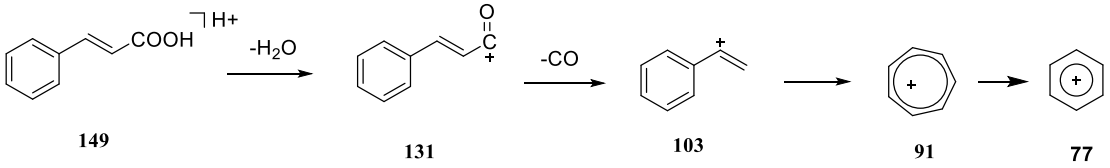

- Peak 18 (deoxyaconitine)



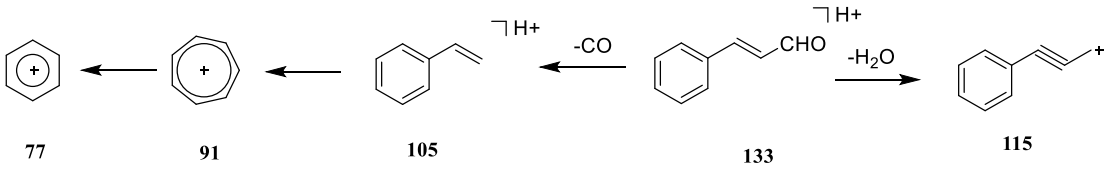

- Peak 19 (cinnamic alcohol)



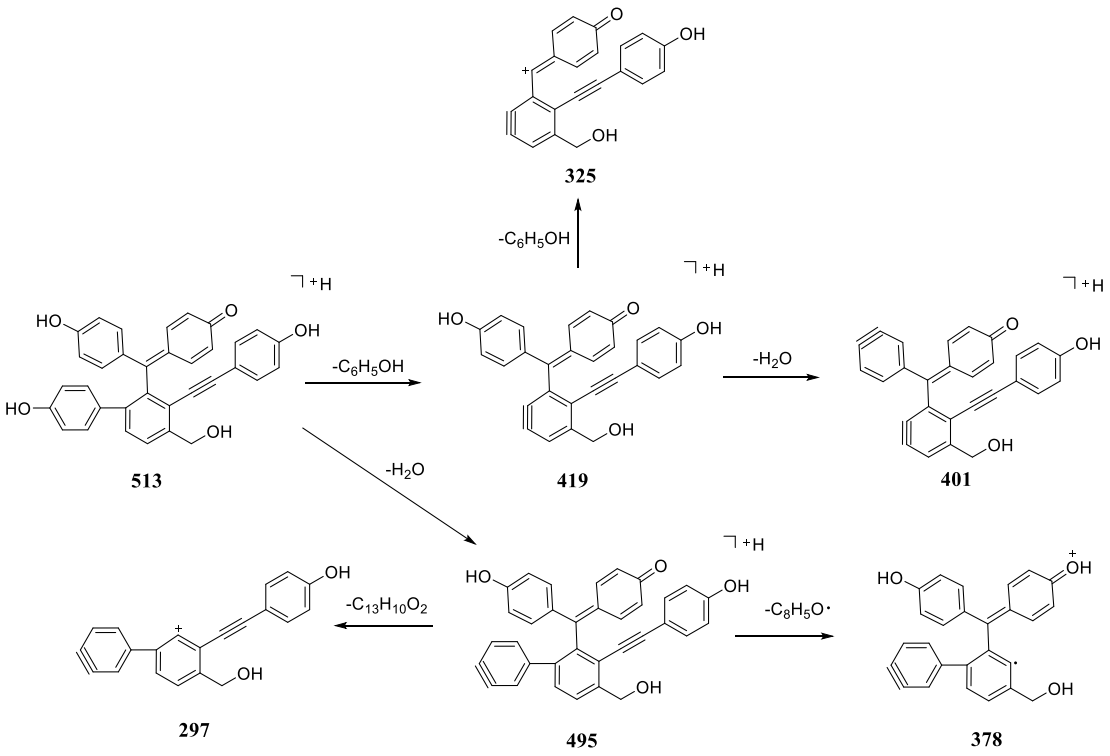

- Peak 20 (cinnamic acid)



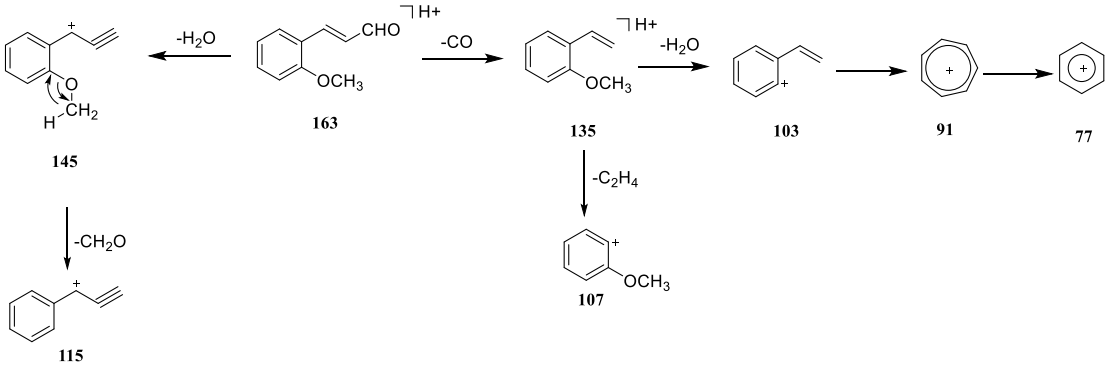

- Peak 21 (cinnamic aldehyde)



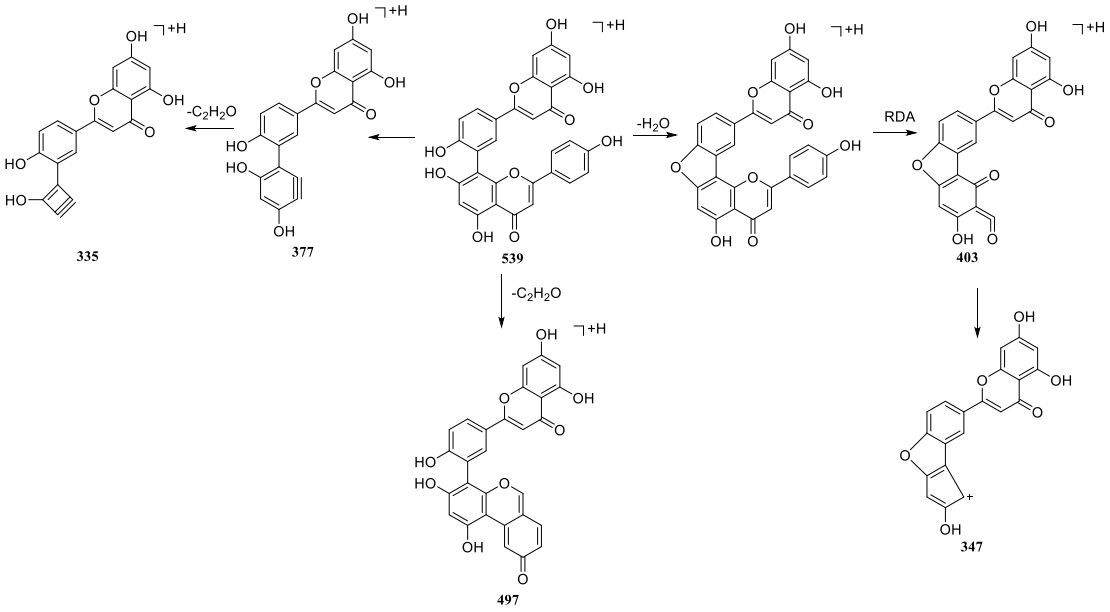

- Peak 22 (selaginellin)



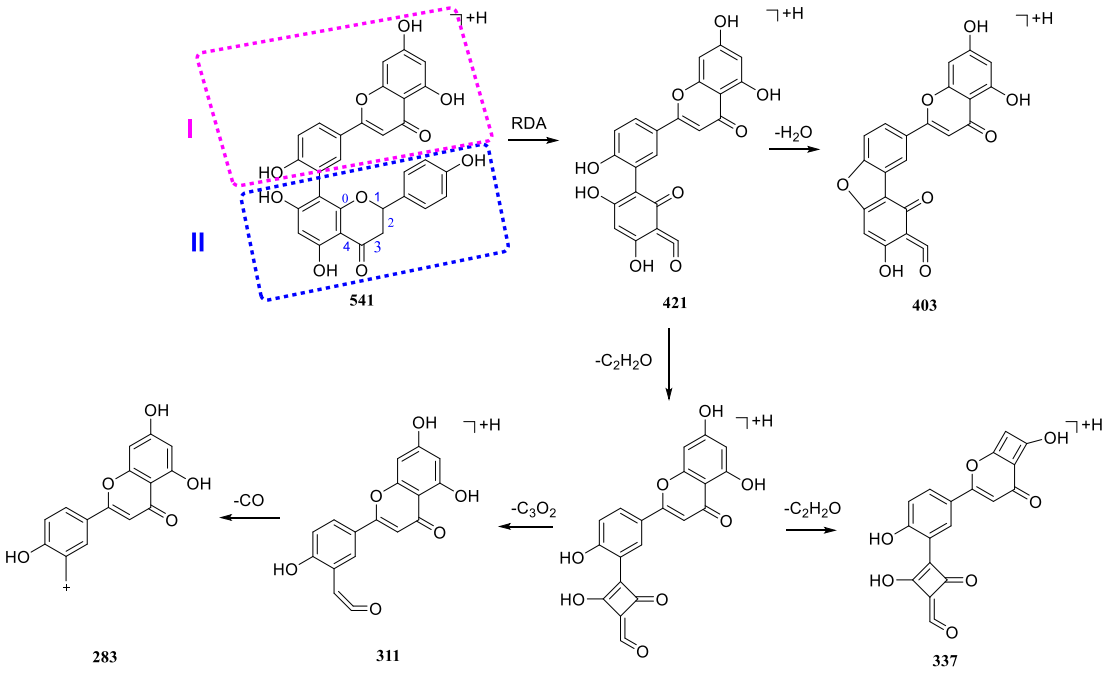

- Peak 23 (2-methoxy cinnamaldehyde)



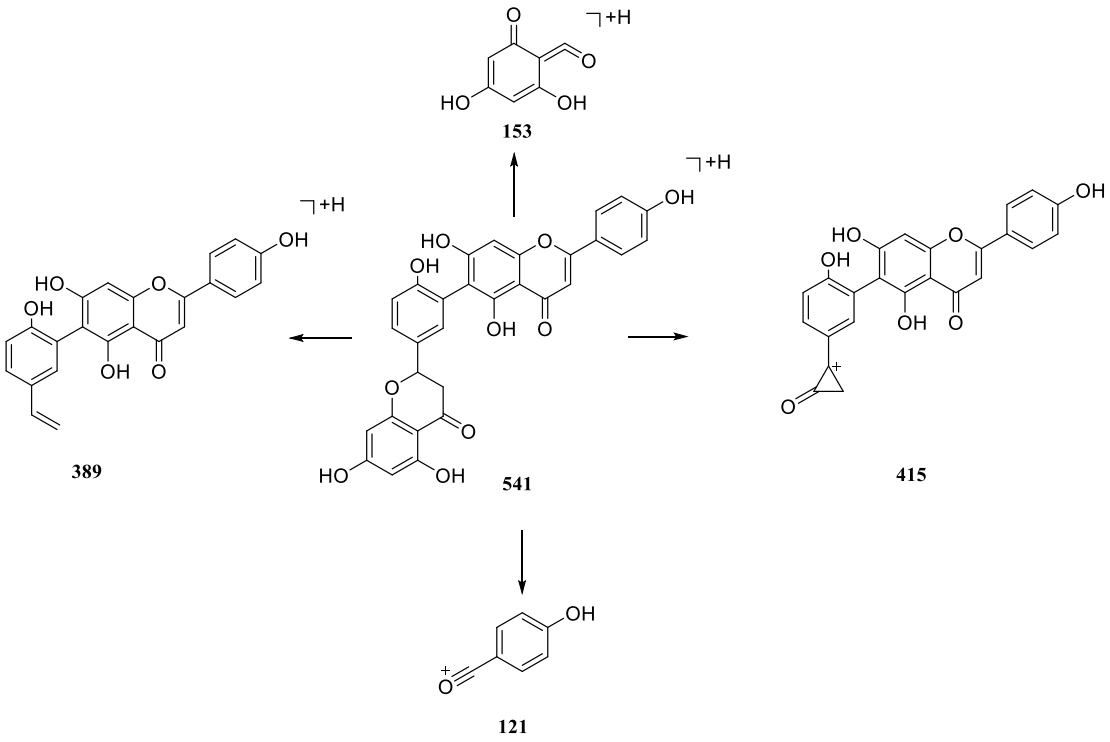

- Peak 24 (amentoflavone)



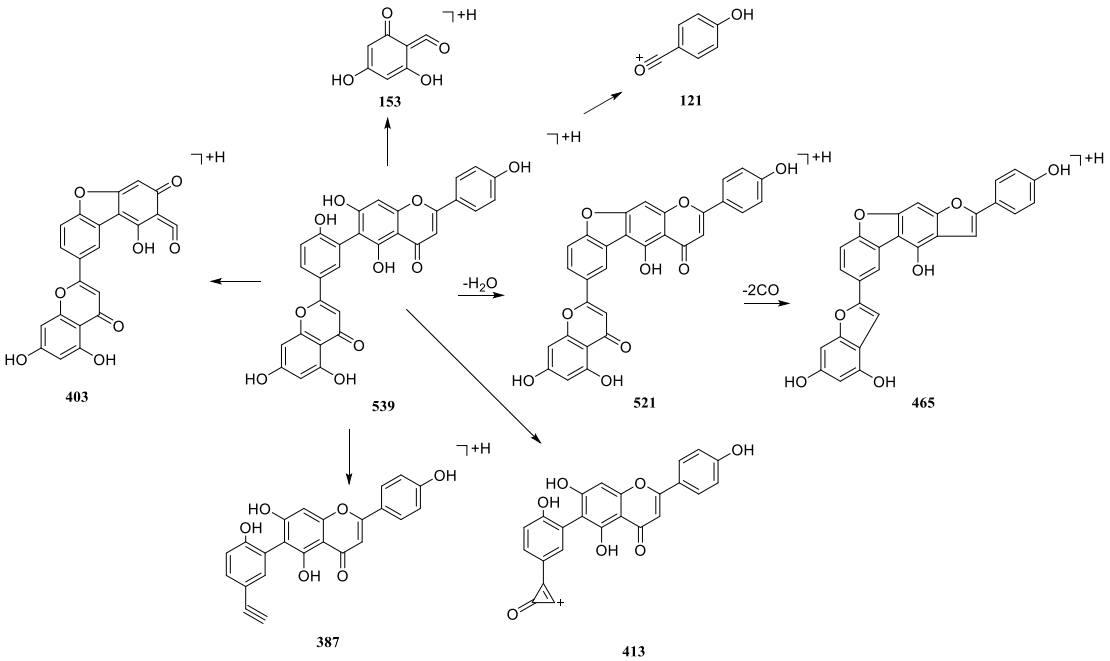

- Peak 25 (2″,3″-dihydroamentoflavone)



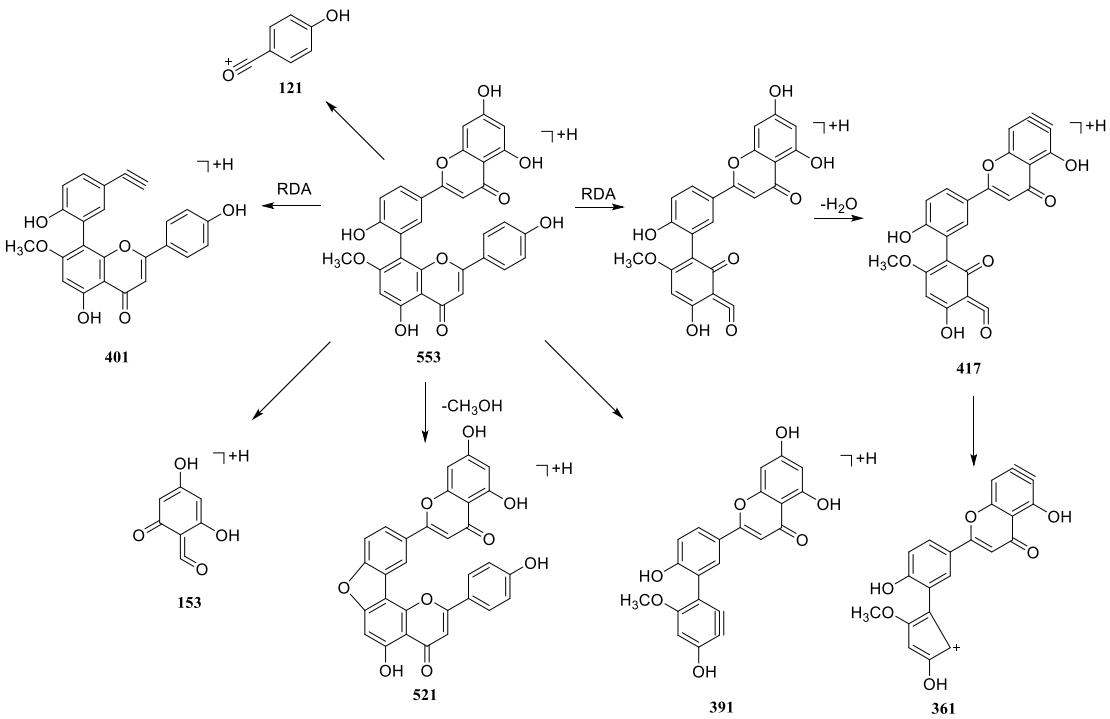

- Peak 26 (2,3-dihydrorobustaflavone)



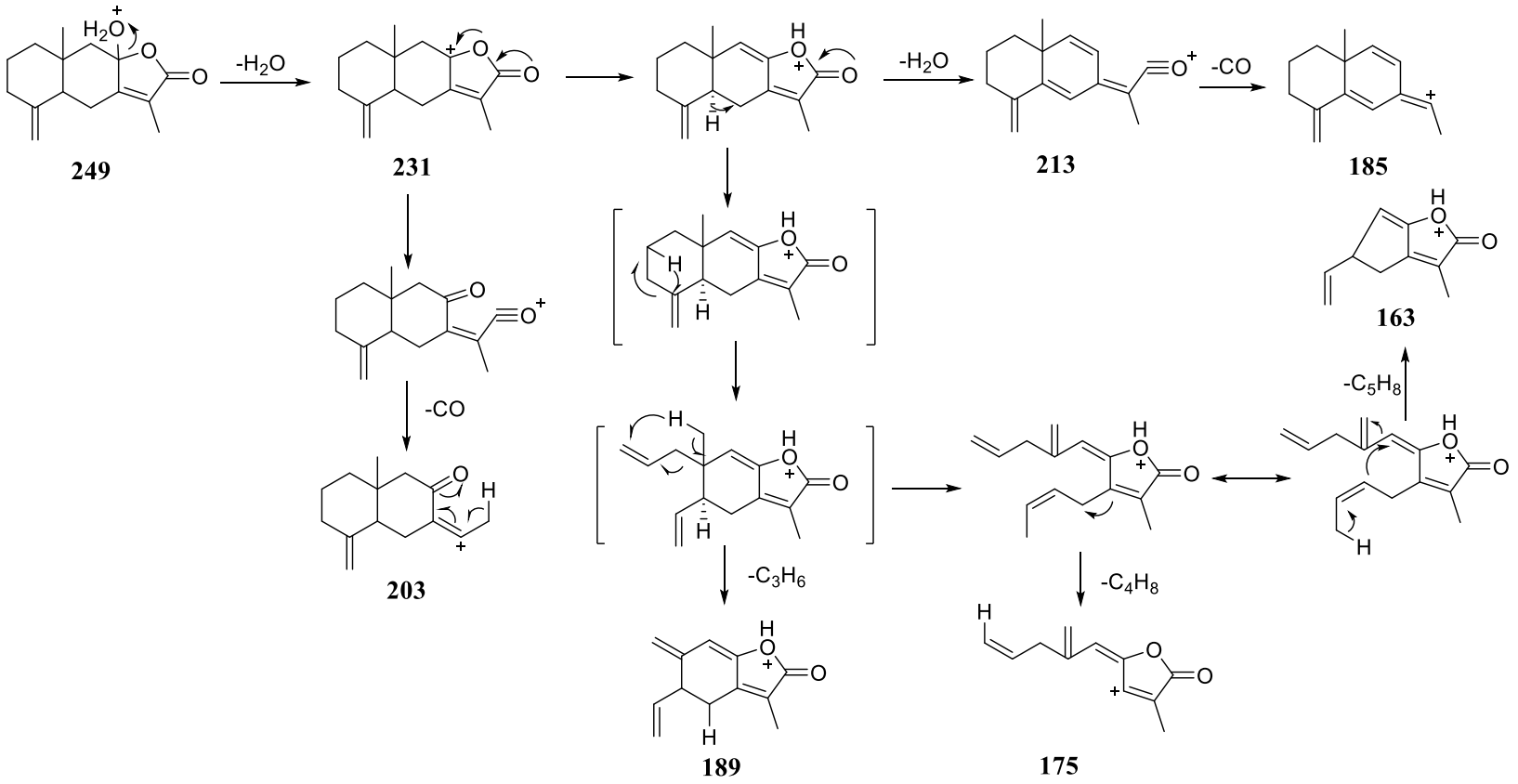

- Peak 27 (robustaflavone)



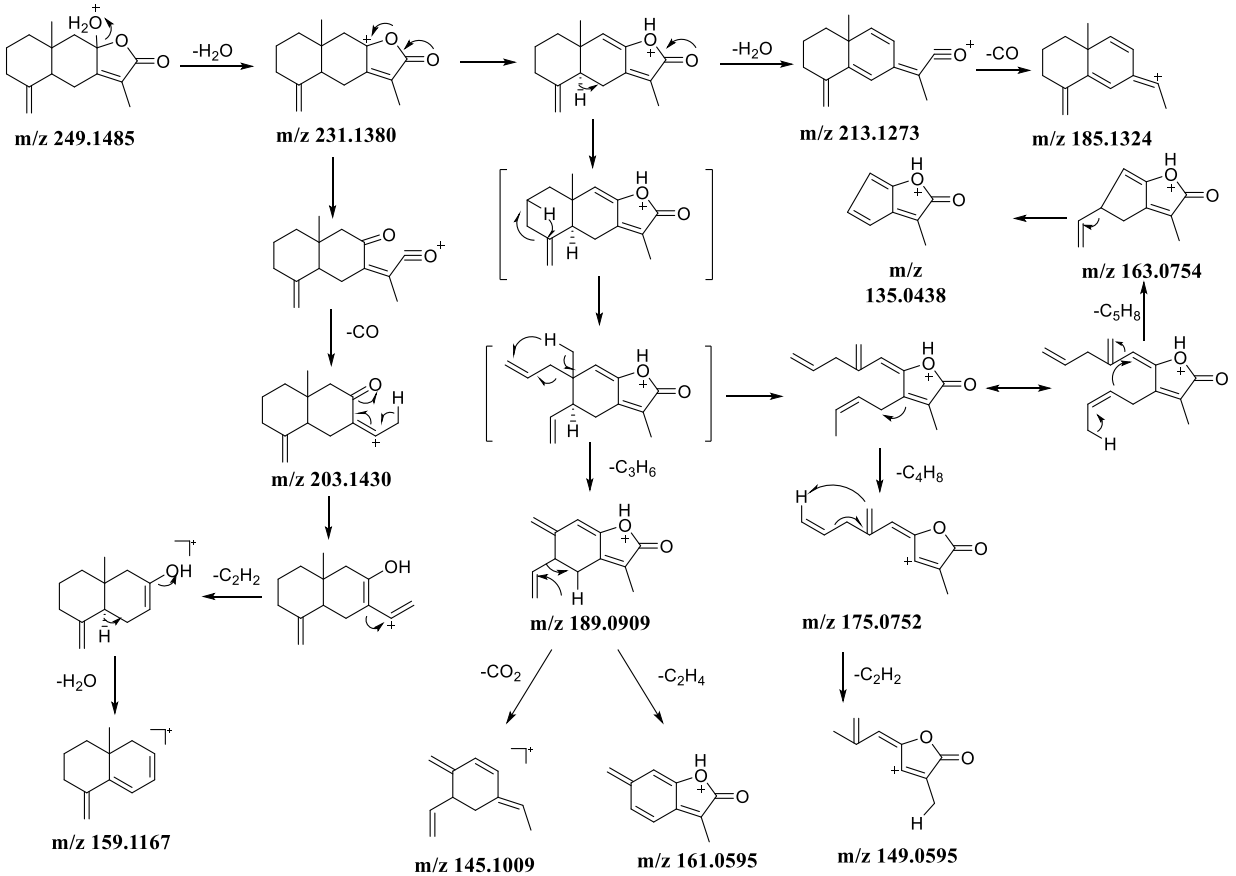

- Peak 28 (7″-O-methylamentoflavone)


121
401
RDA
553
RDA
-H2O
417
153
-CH3OH
521
391
361

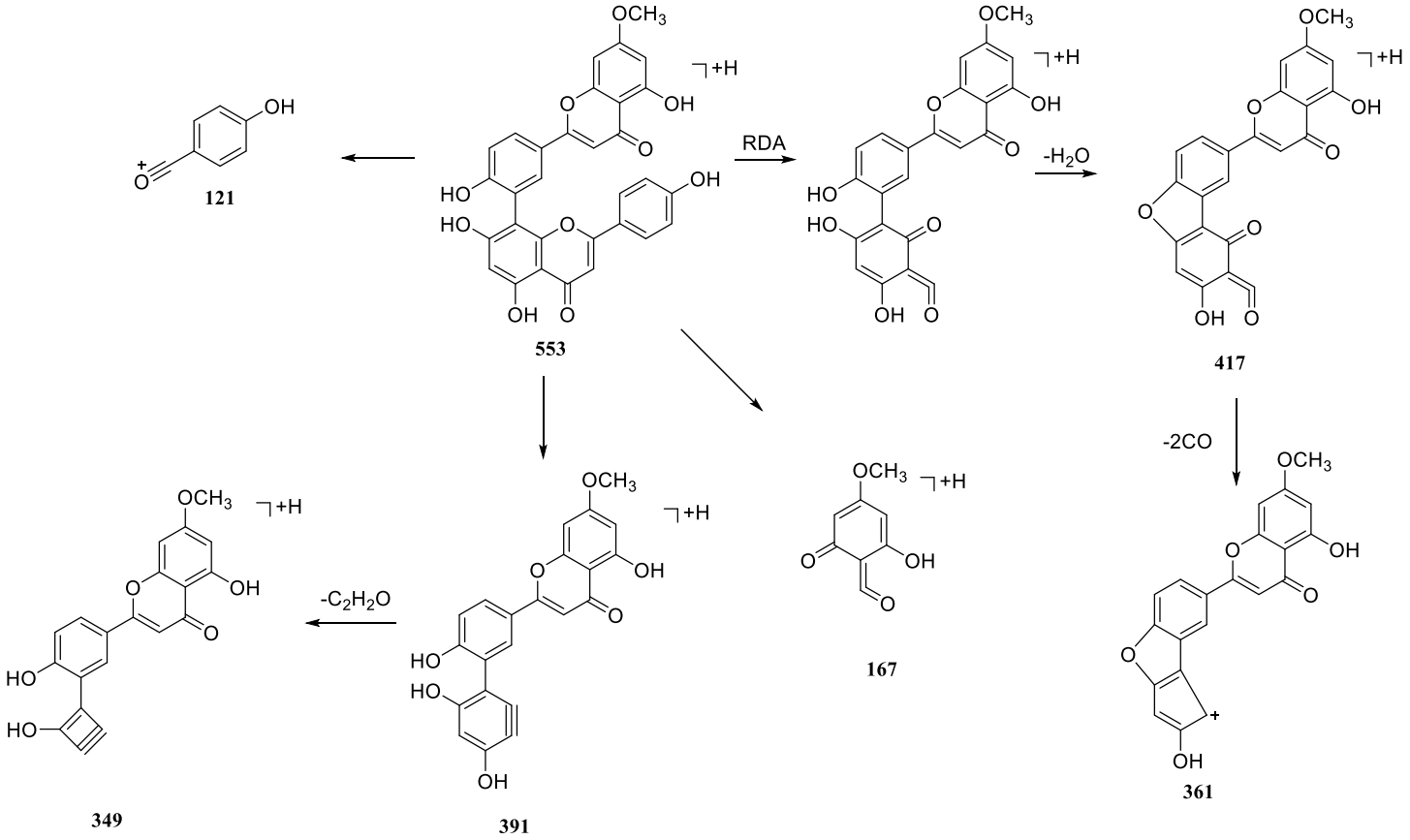
- Peak 29 (atractylenolide III)



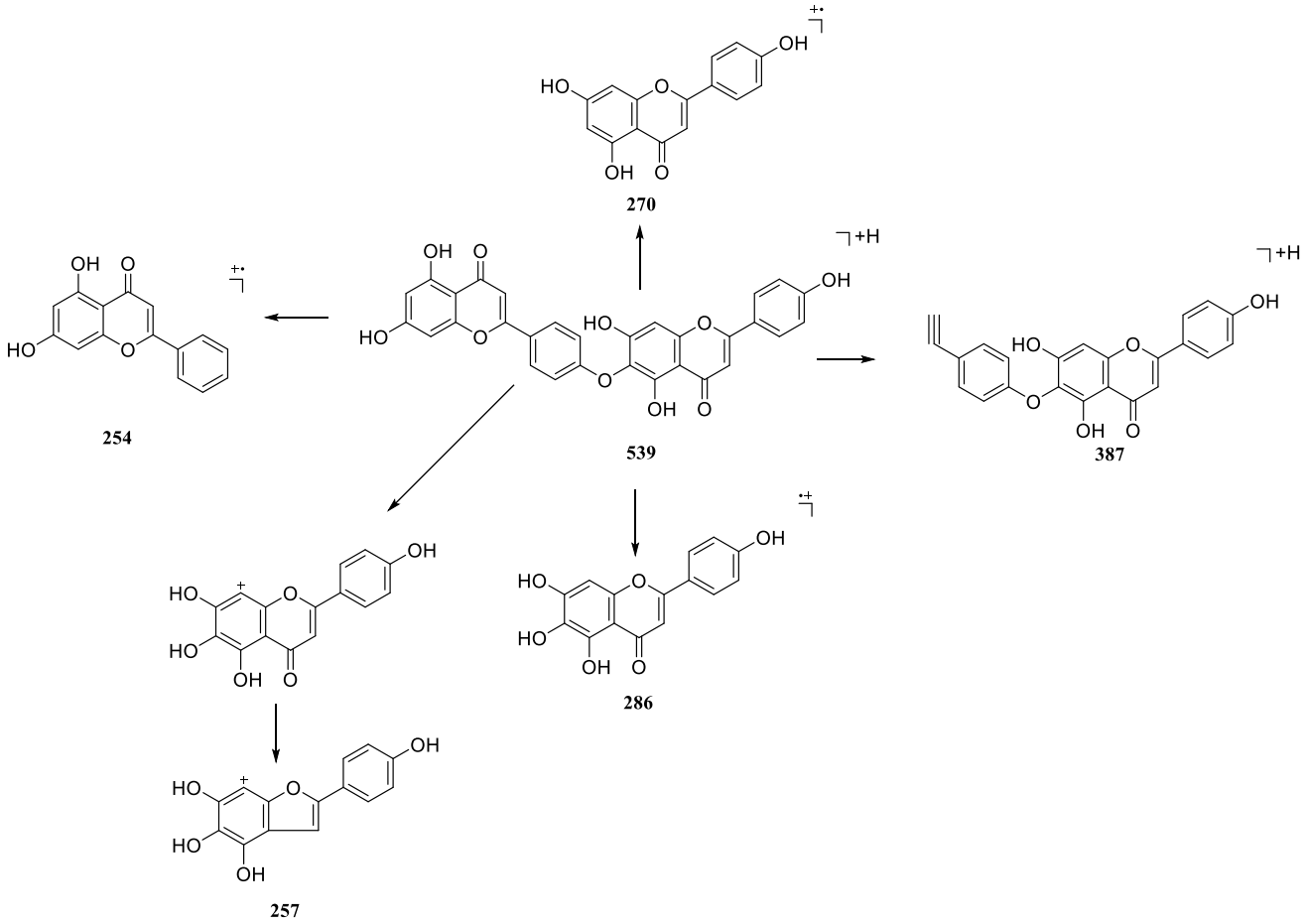
The fragmentation pathway of atractylenolide III were analyzed using a high-resolution linear ion trap Orbitrap mass spectrometer



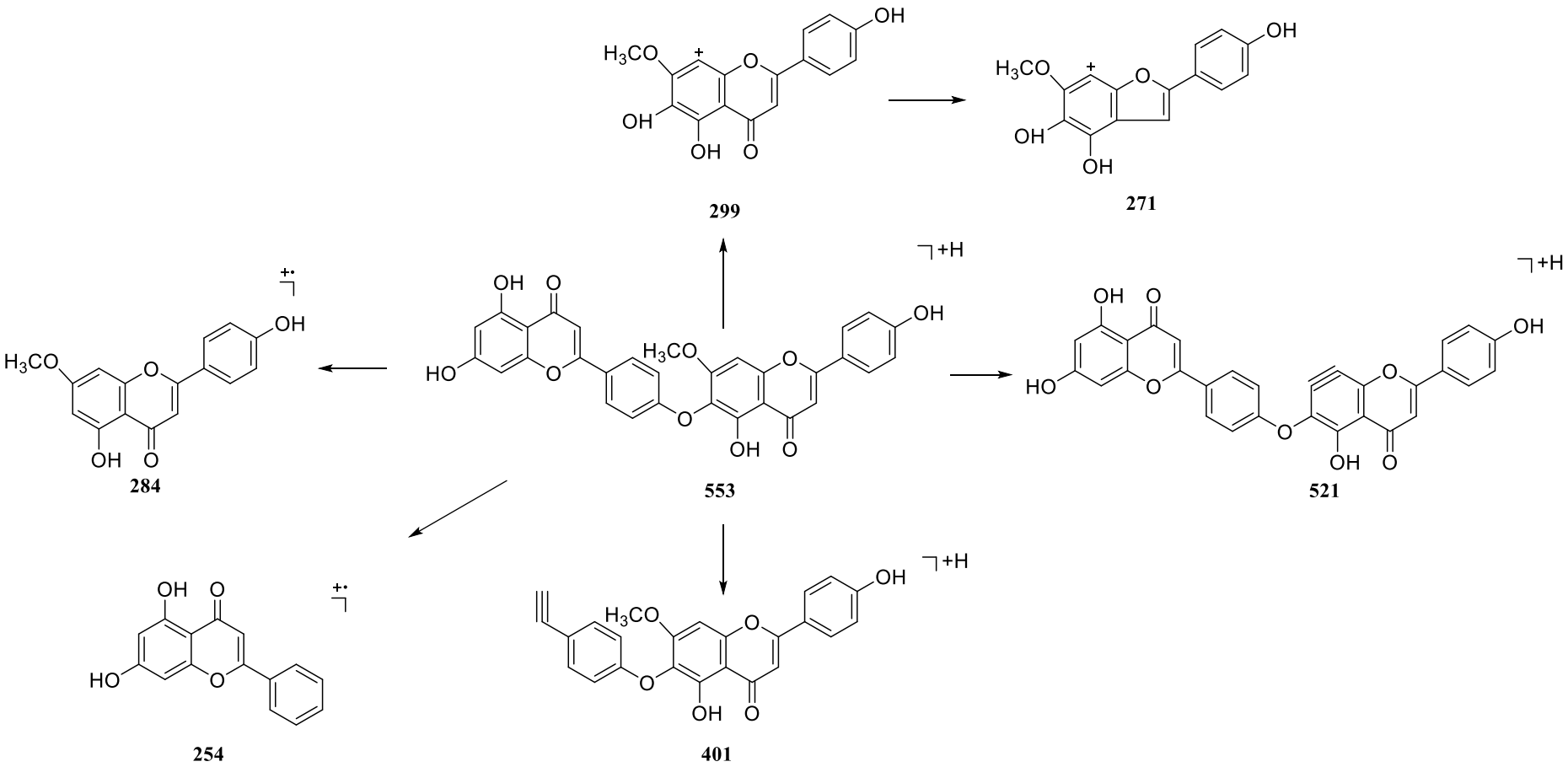
- Peak 30 (7-O-methylamentoflavone)



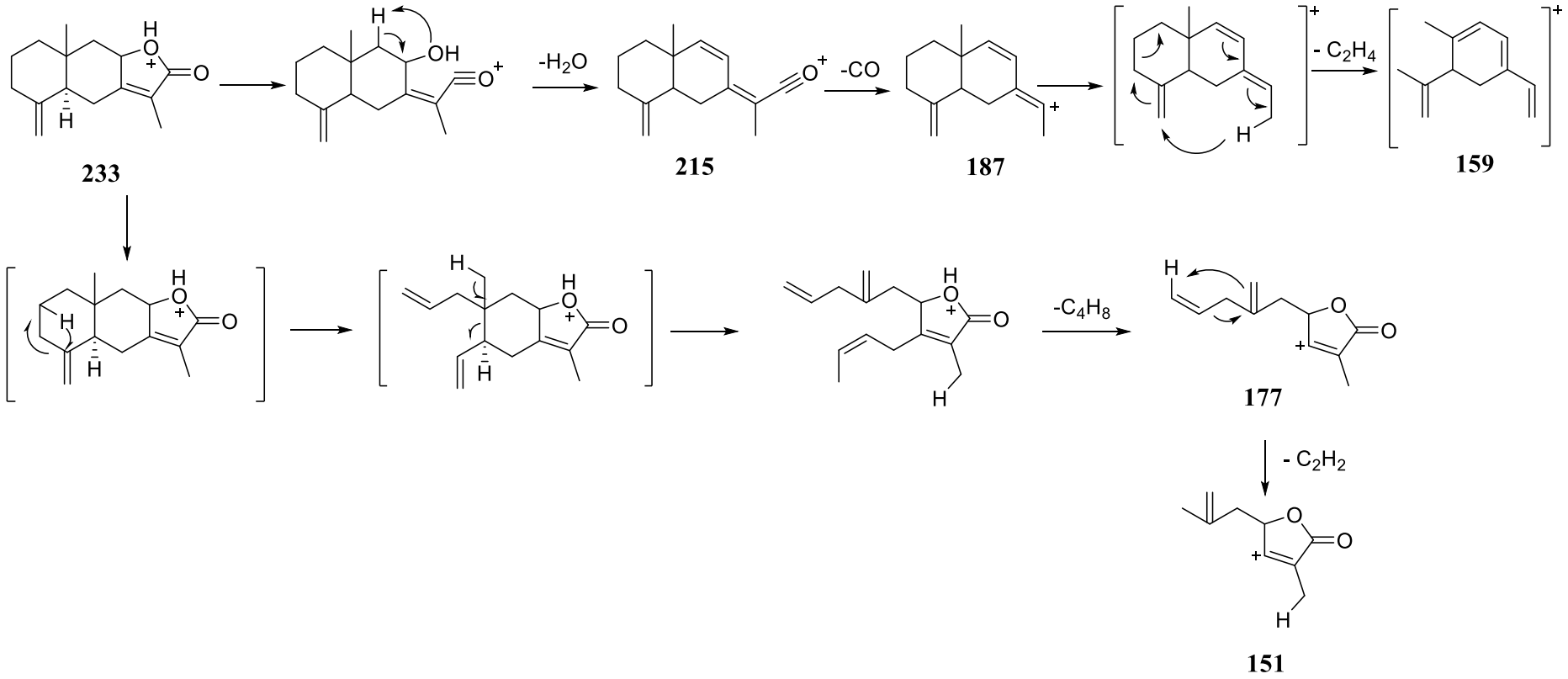
- Peak 31 (hinokiflavone)



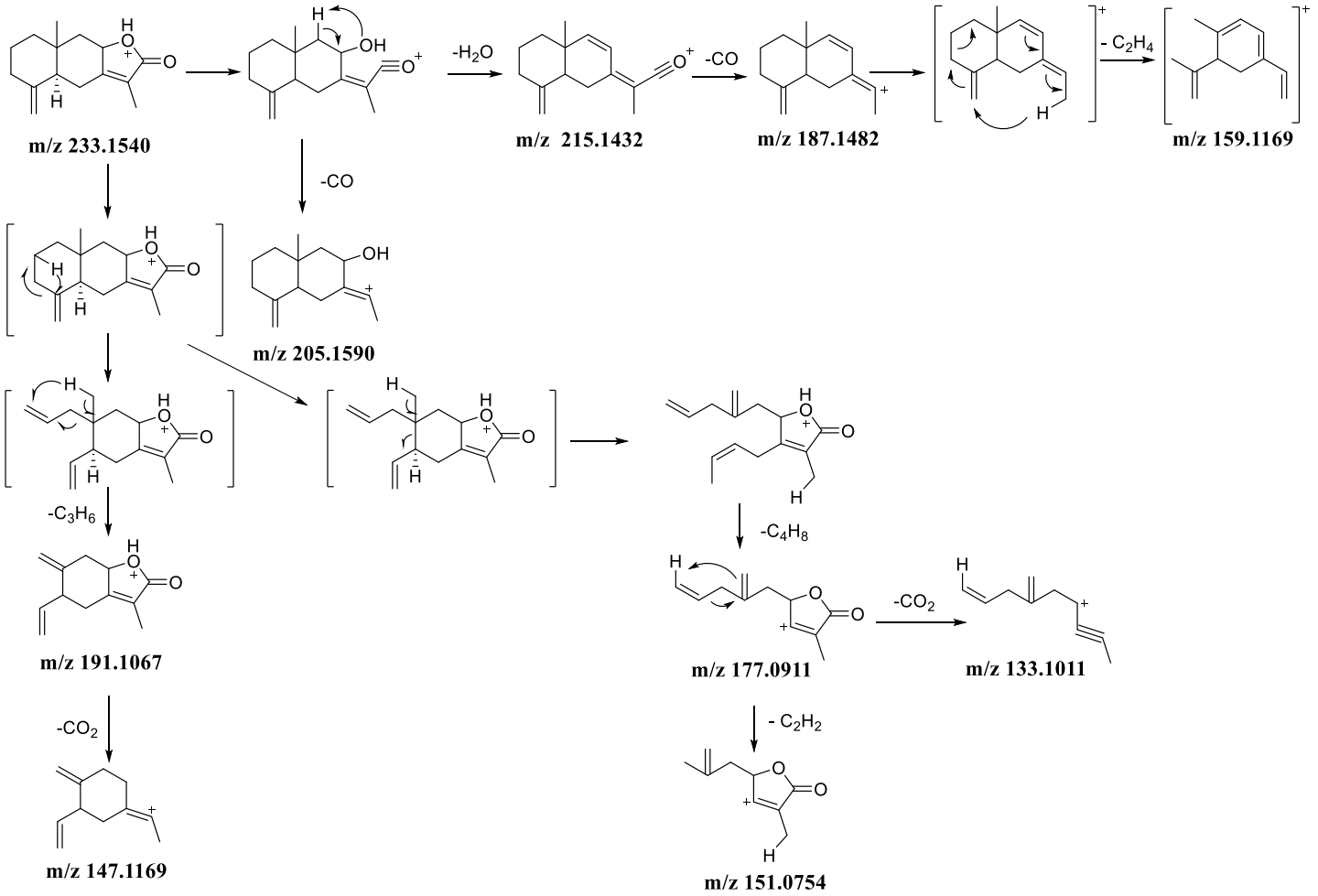
- Peak 33 (isocryptomerin)



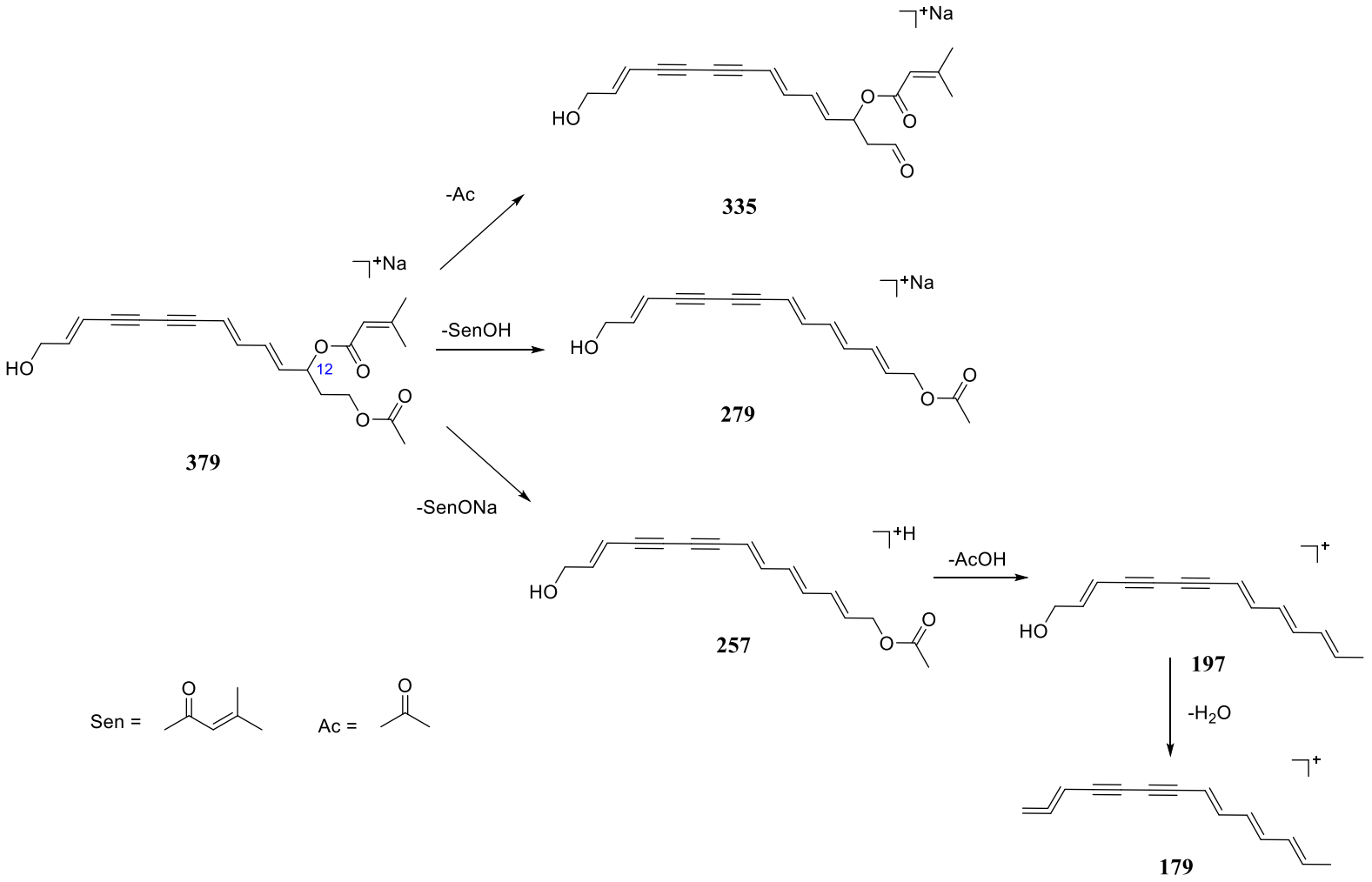

- Peaks 34 and 35 (isoatracytlenolide I and atractylenolide I)



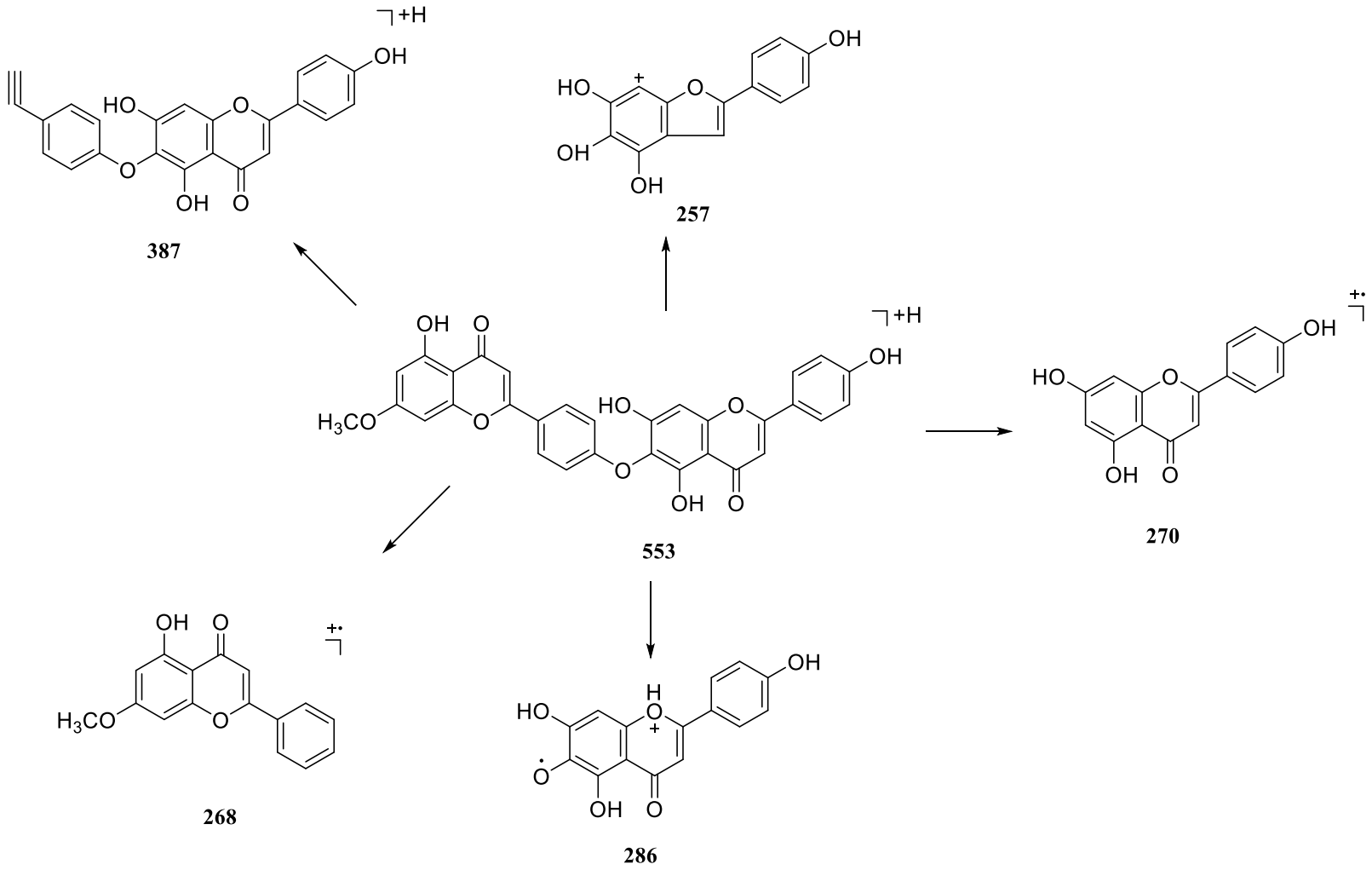

The fragmentation pathway of atractylenolide I were analyzed using a high-resolution linear ion trap Orbitrap mass spectrometer



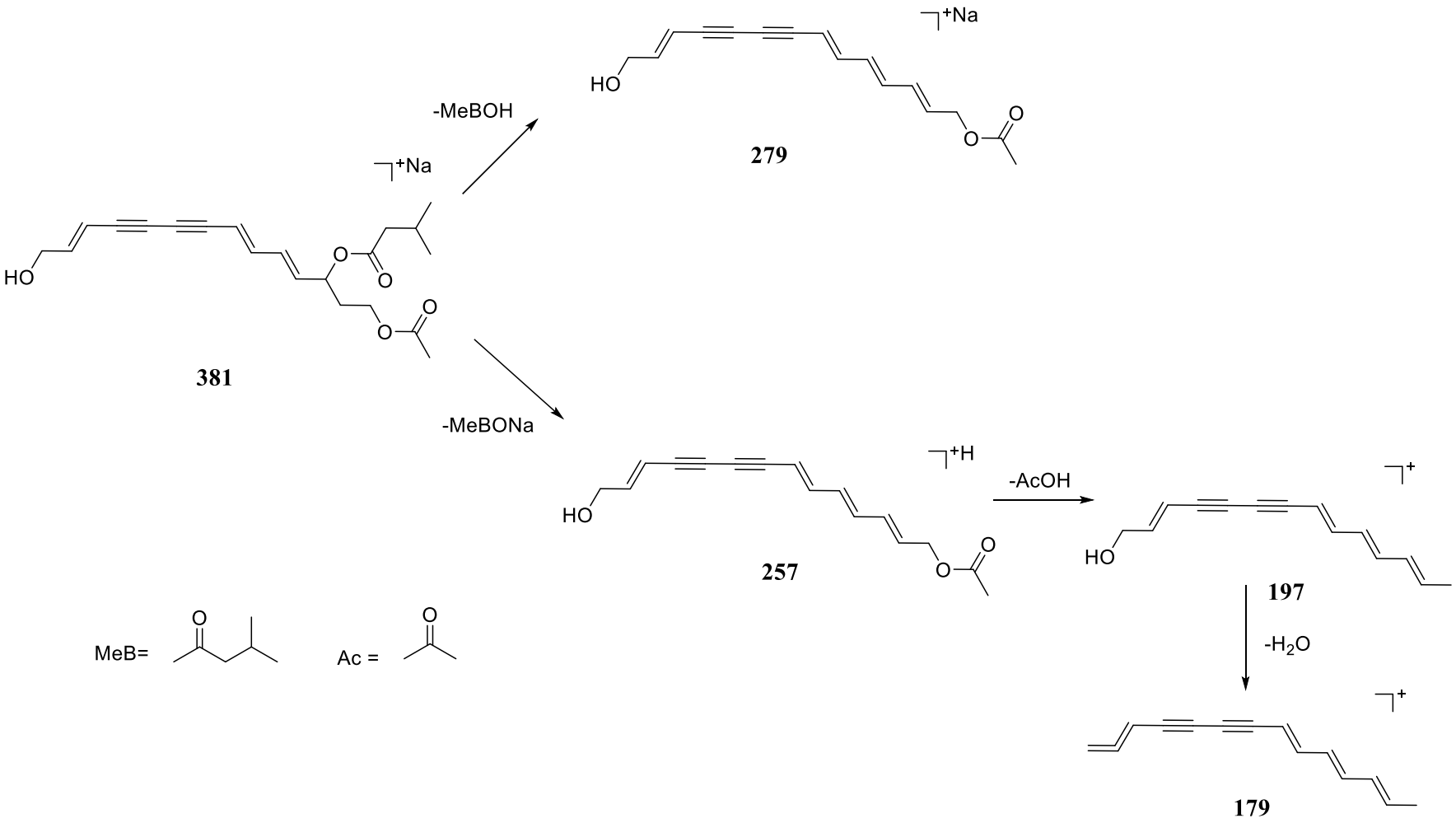

- Peaks 36 and 38 (14-acetoxy-12-senecioyloxytetradeca-2E,8EZ,10E-trien-4,6-diyn-1-ol)



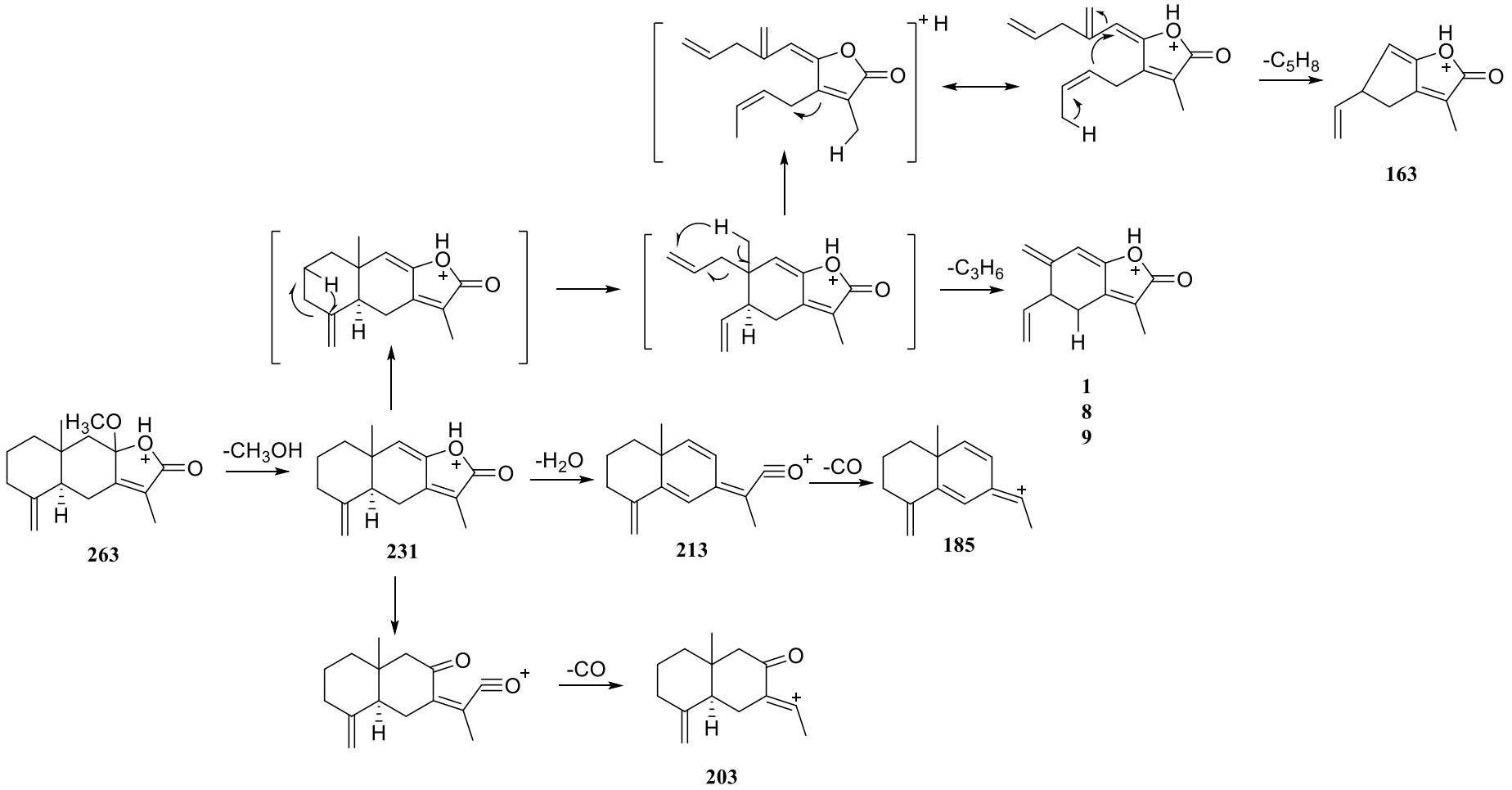

- Peak 37 (neocryptomerin)


387
257
553
270
268
286

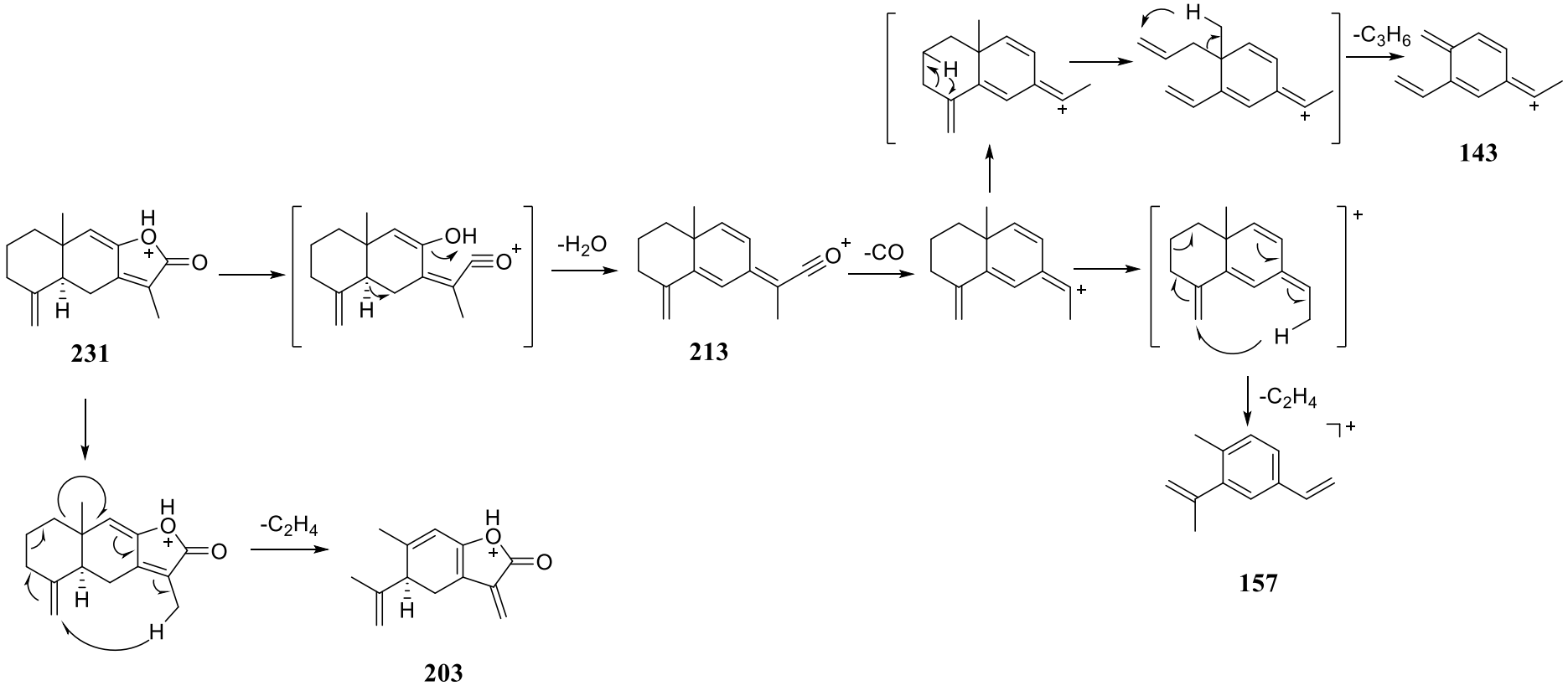
- Peaks 39 and 41 (14-acetoxy-12-methylbutyryltetradeca-2E,8EZ,10E-trien-4,6-diyn-1-ol)



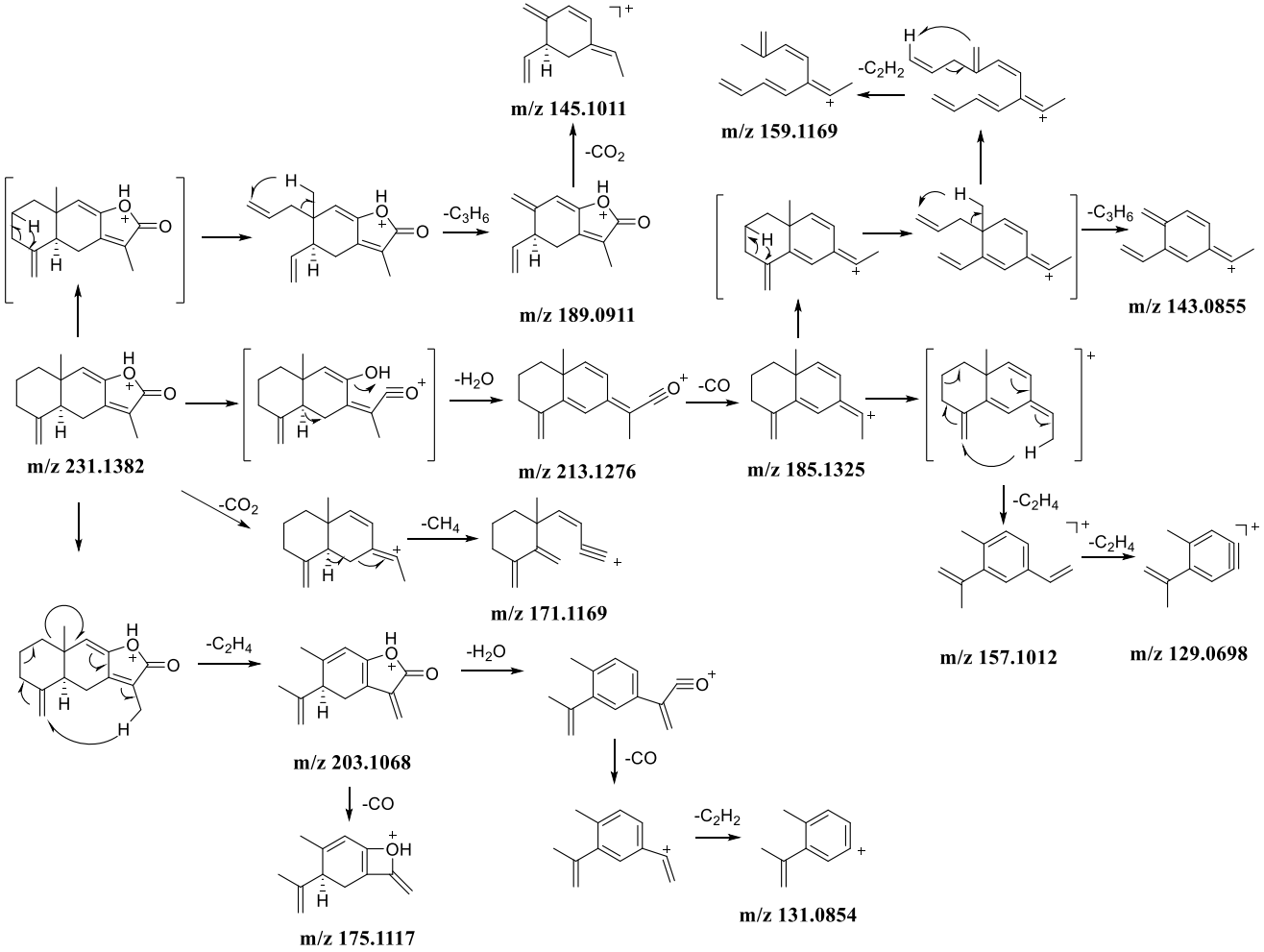
- Peaks 43 (8-methoxyatractylenolide I)



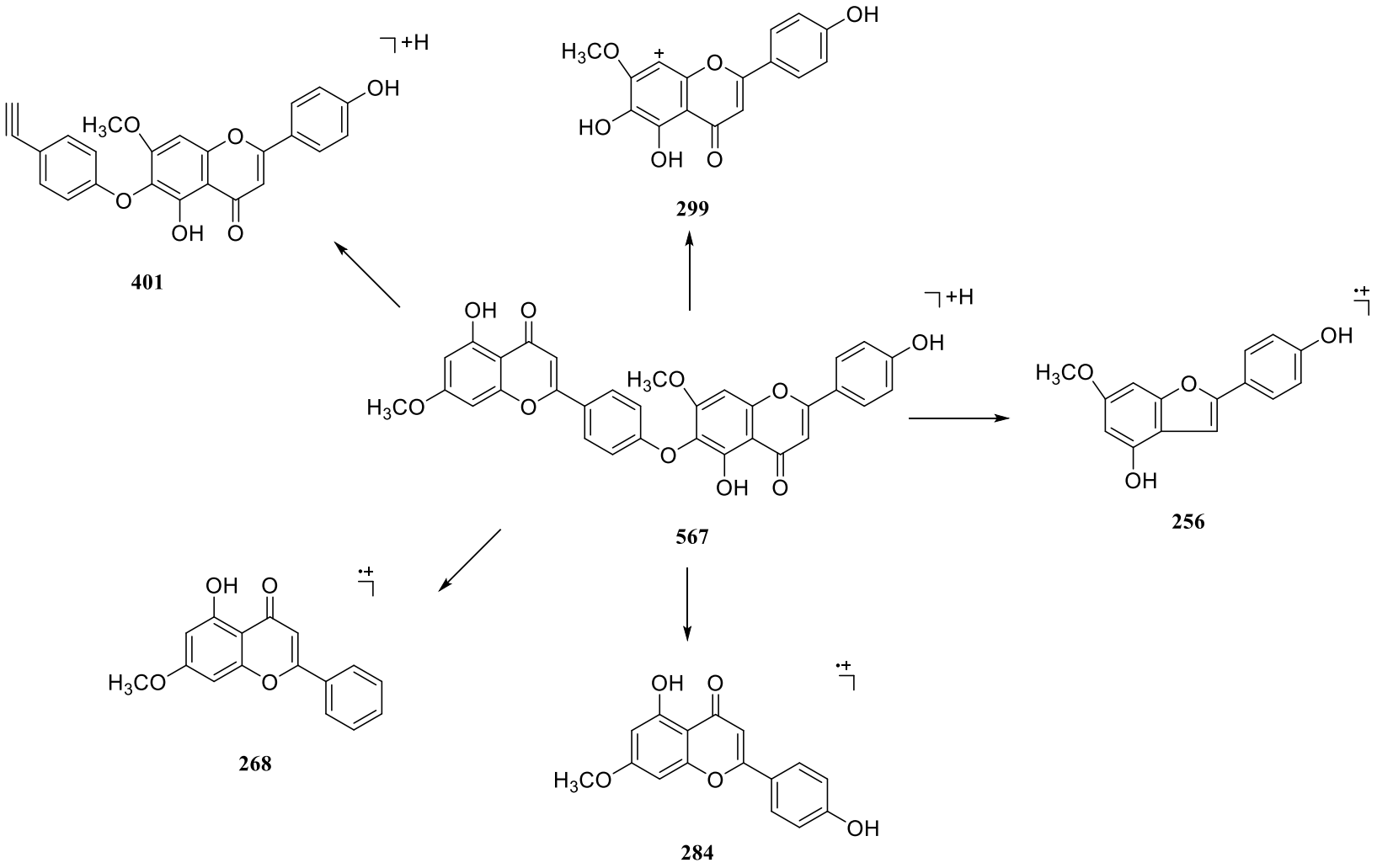
- Peak 44 (atractylenolide II)



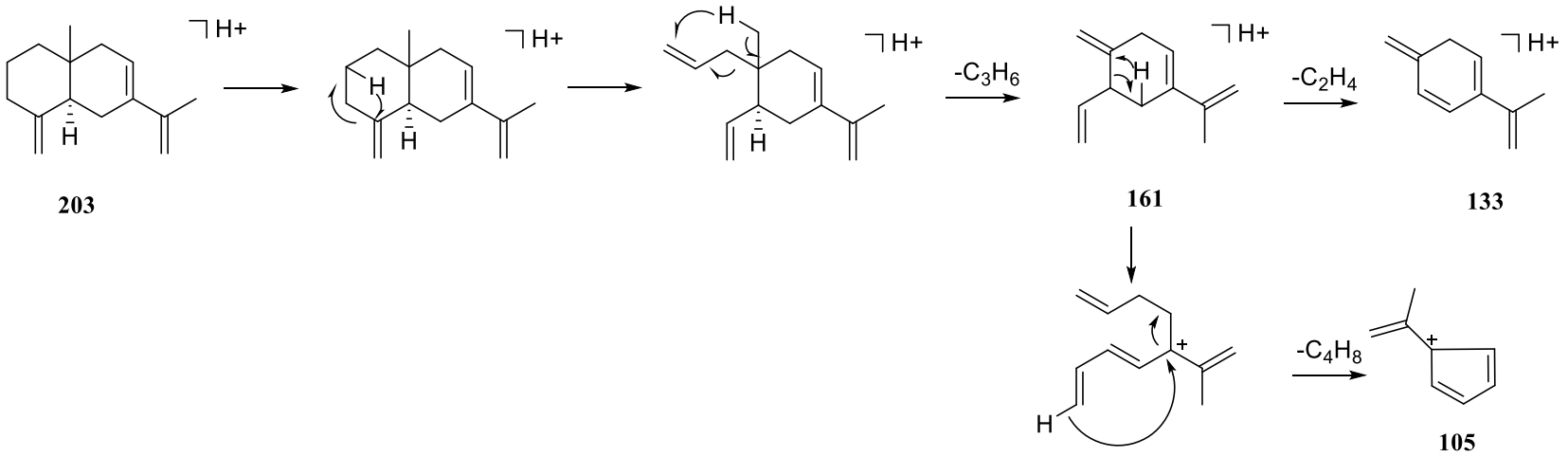
The fragmentation pathway of atractylenolide II were analyzed using a high-resolution linear ion trap Orbitrap mass spectrometer




- Peak 46 (7,7″-di-*O*-methylhinokiflavone)




- Peak 48 (atractylenolide VI)




**203** **161** **133** **105**