pharmaceutics

Supplementary Materials: Metabolism of Diterpenoids Derived from the Bark of *Cinnamomum cassia* in Human Liver Microsomes

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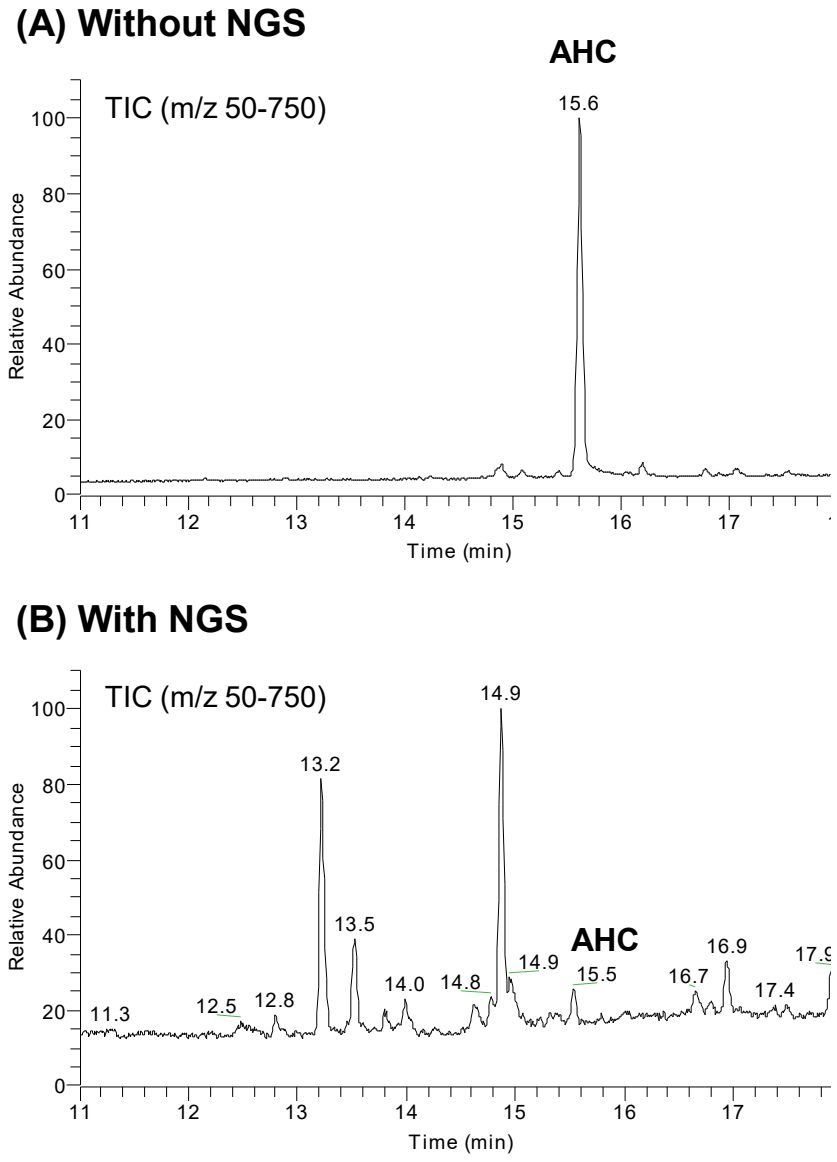


Figure S1. Total ion chromatograms (TIC) for anhydrocinnzeylanine (AHC) and its metabolites. AHC (10 μM) was incubated with 1 mg/mL of pooled human liver microsomes for 60 min in the absence (**A**) or presence (**B**) of a β-NADPH-regenerating system (NGS).

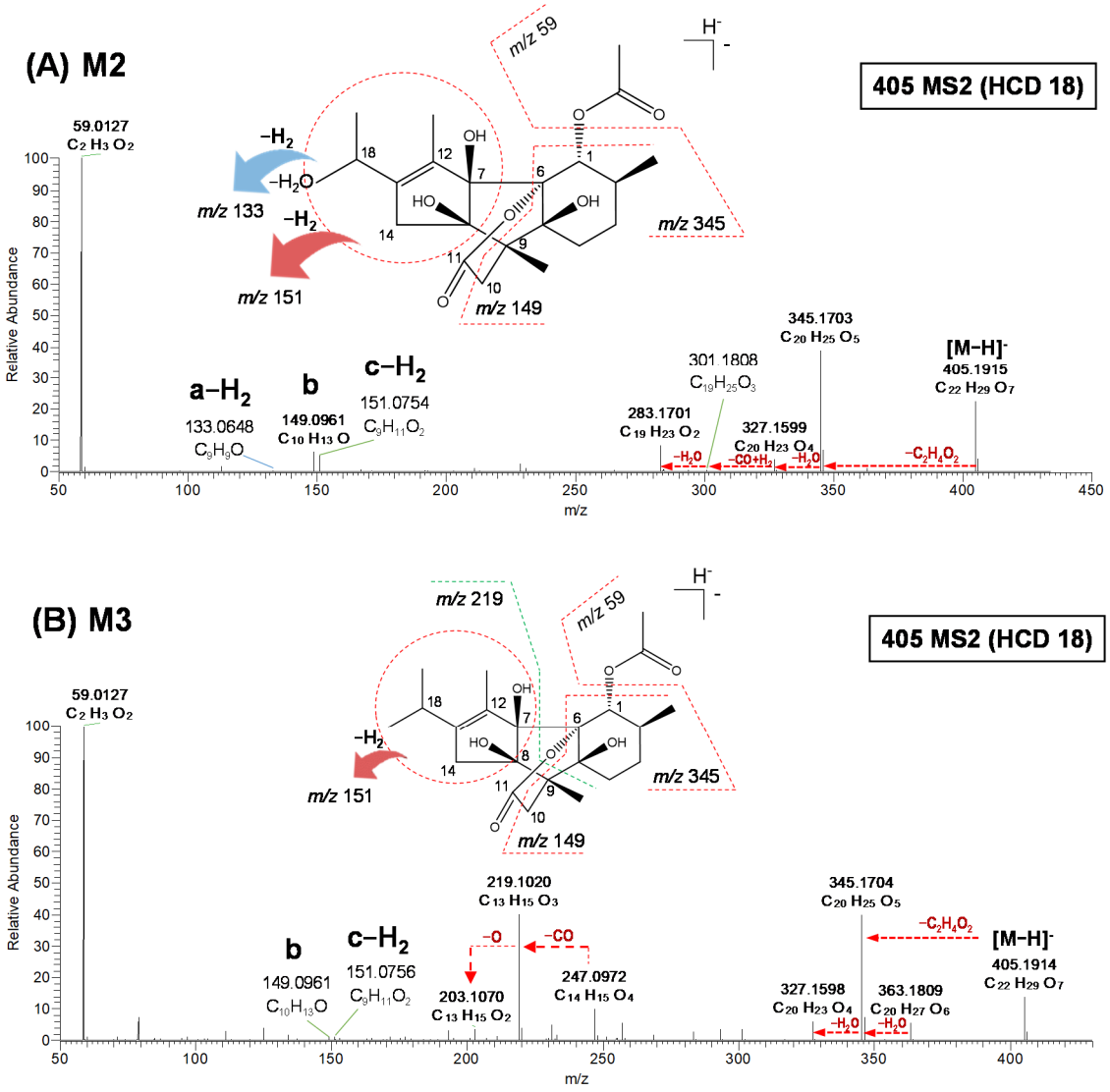
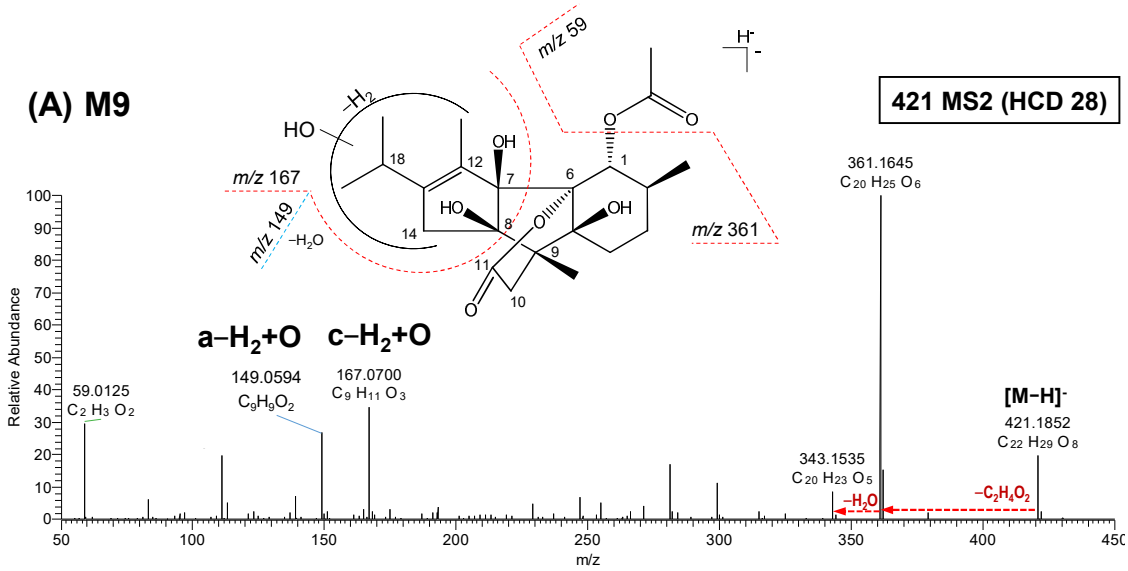
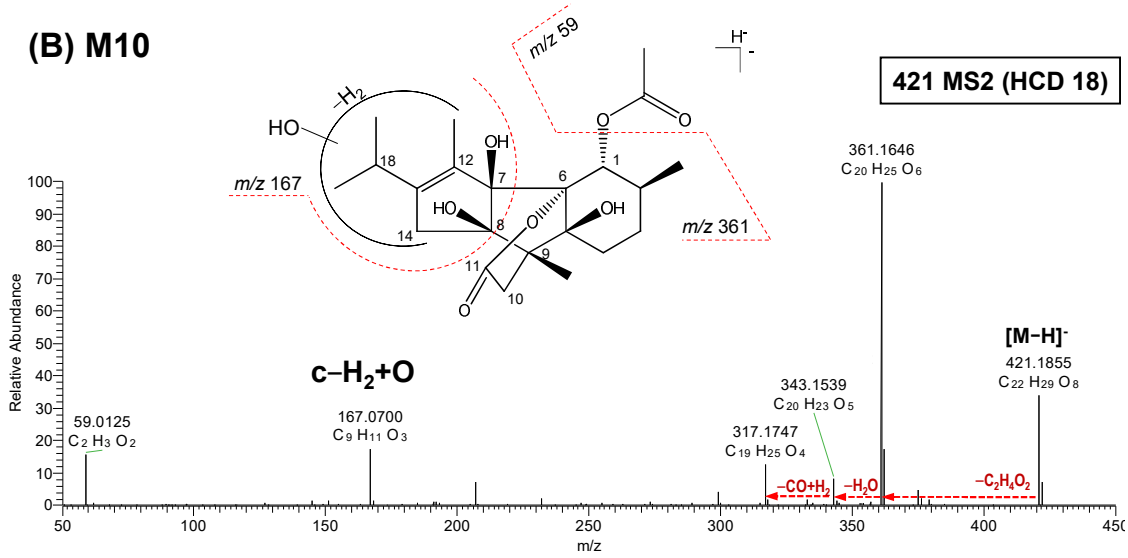


Figure S2. MS/MS spectra of dehydrogenated M2 (**A**) and M3 (**B**) using a high-resolution quadrupole-orbitrap mass spectrometer.


(A) M9
421 MS2 (HCD 28)
m/z 59
m/z 167
m/z 149
−H2O
m/z 361
a−H2+O
c−H2+O
149.0594
C9H9O2
167.0700
C9 H11 O3
59.0125
C2 H3 O2
361.1645
C20 H25 O6
[M−H]-
421.1852
C22 H29 O8
343.1535
C20 H23 O5
−H2O
−C2H4O2
Relative Abundance
m/z


(B) M10
421 MS2 (HCD 18)
m/z 59
m/z 167
m/z 361
c−H2+O
59.0125
C2 H3 O2
167.0700
C9 H11 O3
361.1646
C20 H25 O6
[M−H]-
421.1855
C22 H29 O8
343.1539
C20 H23 O5
317.1747
C19 H25 O4
−CO+H2
−H2O
−C2H4O2
Relative Abundance
m/z

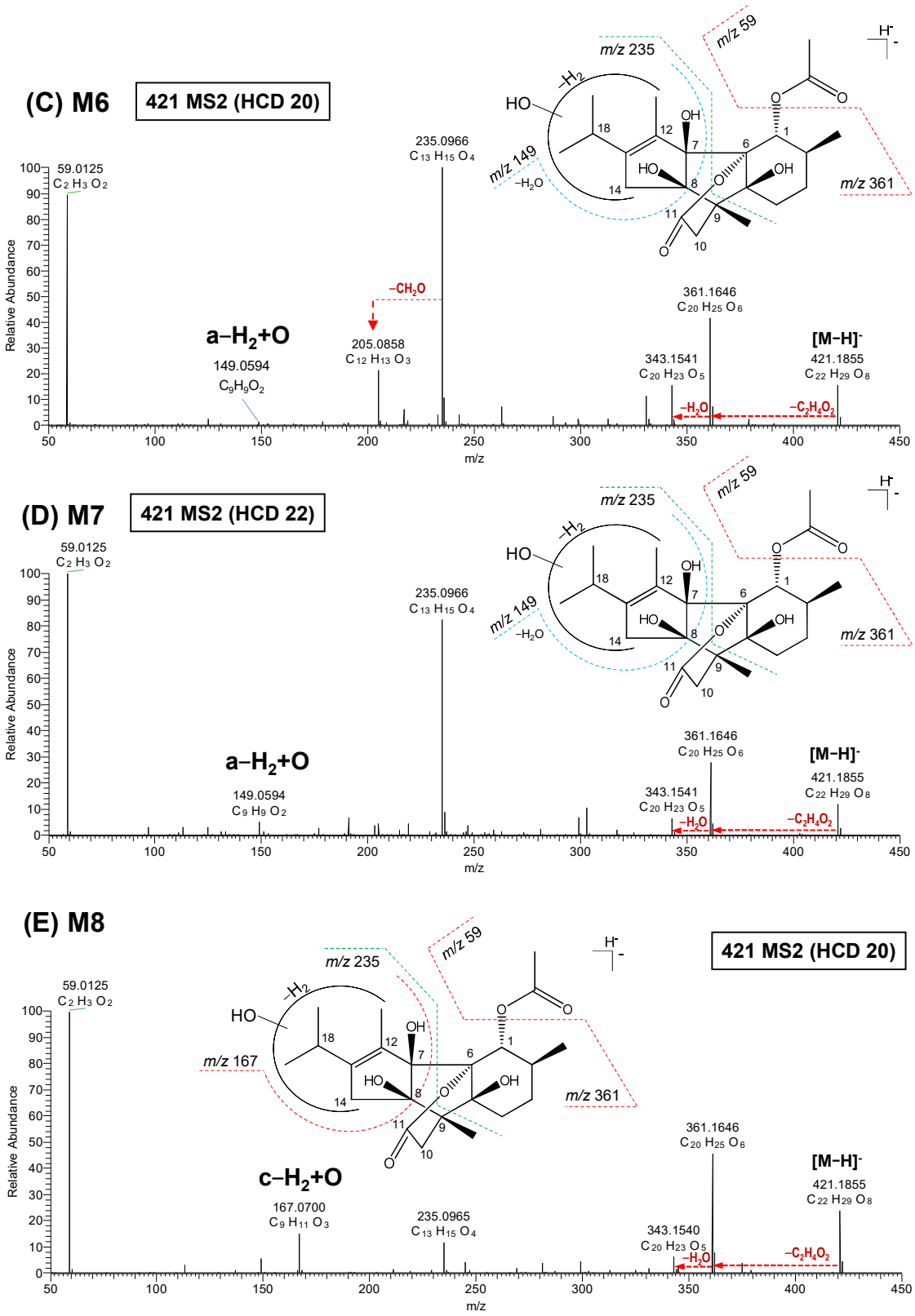


Figure S3. MS/MS spectra of dehydrogenated M9 (**A**), M10 (**B**), M6 (**C**), M7 (**D**) and M8 (**E**) using a high-resolution quadrupole-orbitrap mass spectrometer.

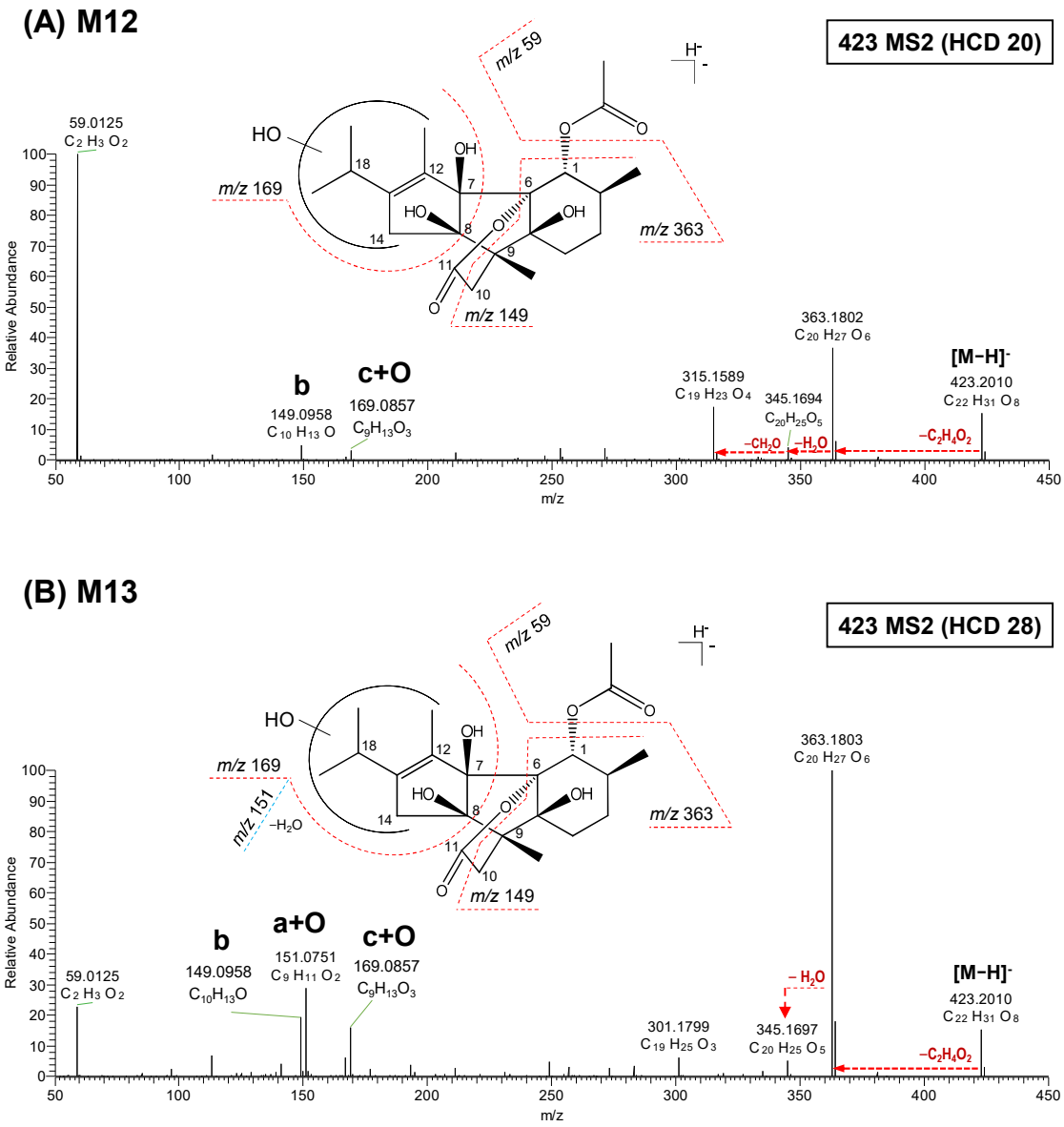


Figure S4. MS/MS spectra of dehydrogenated M12 (**A**) and M13 (**B**) using a high-resolution quadrupole-orbitrap mass spectrometer.

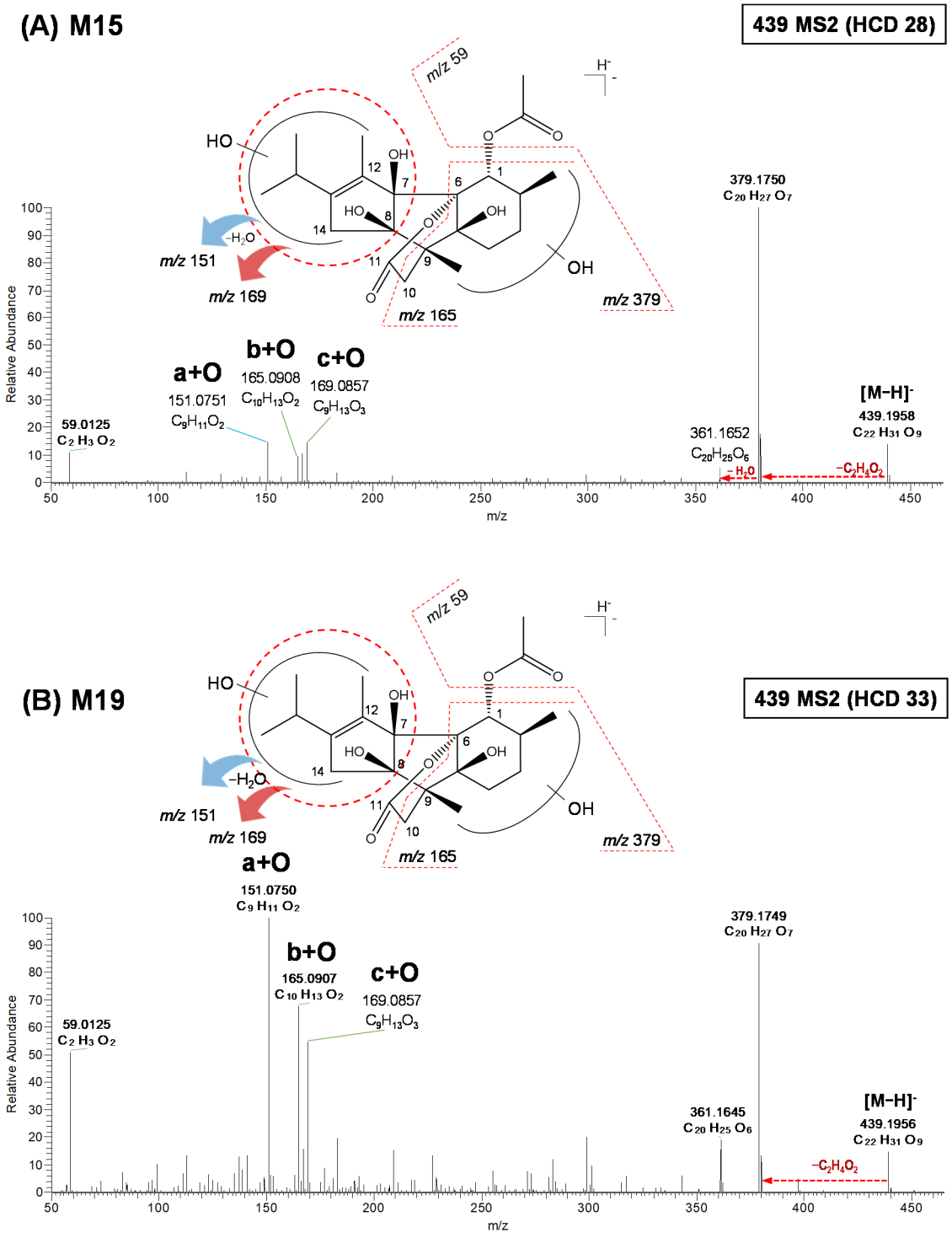


Figure S5. MS/MS spectra of dehydrogenated M15 (**A**) and M19 (**B**) using a high-resolution quadrupole-orbitrap mass spectrometer.

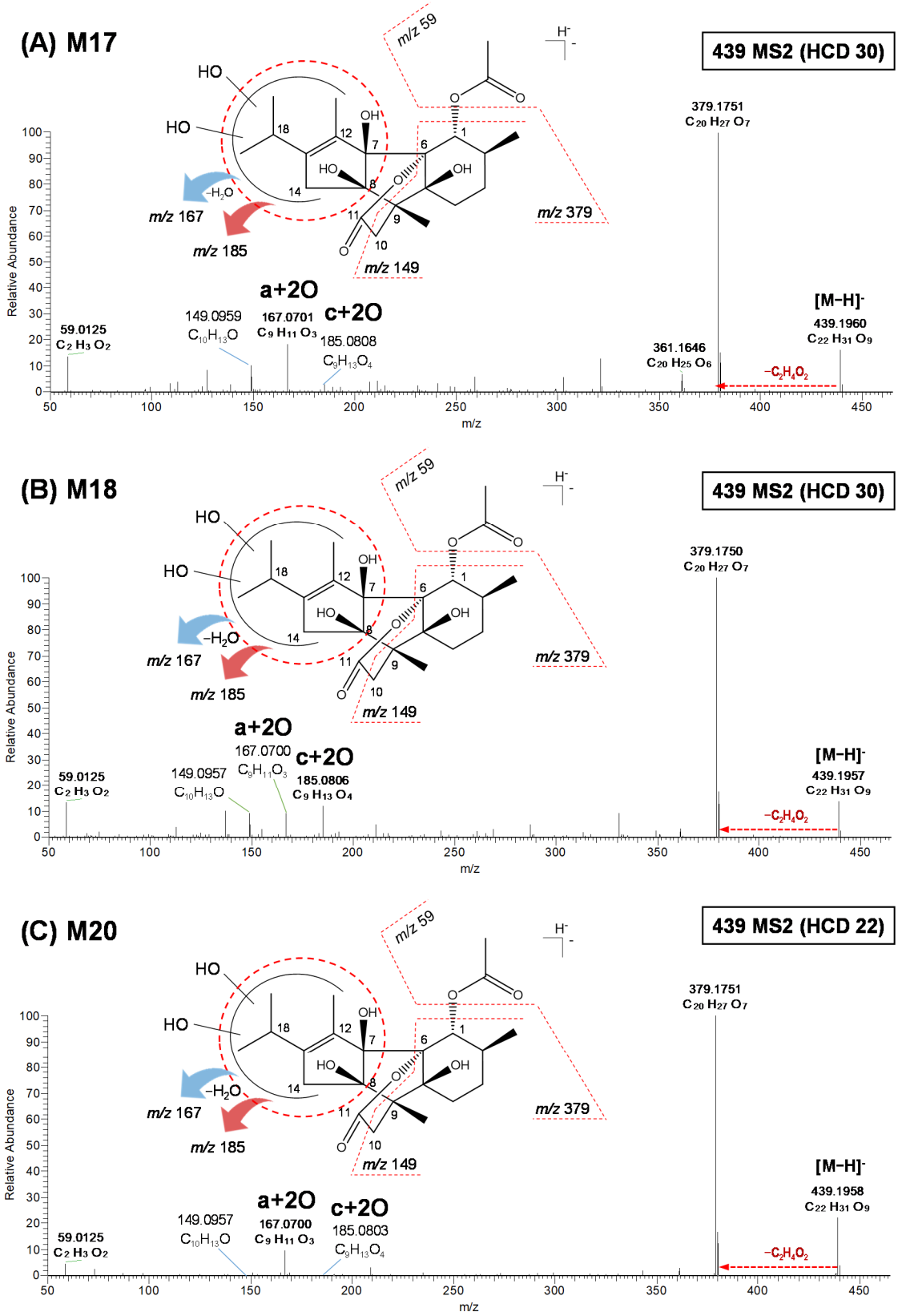


Figure S6. MS/MS spectra of dehydrogenated M17 (**A**), M18 (**B**) and M20 (**C**) using a high-resolution quadrupole-orbitrap mass spectrometer.

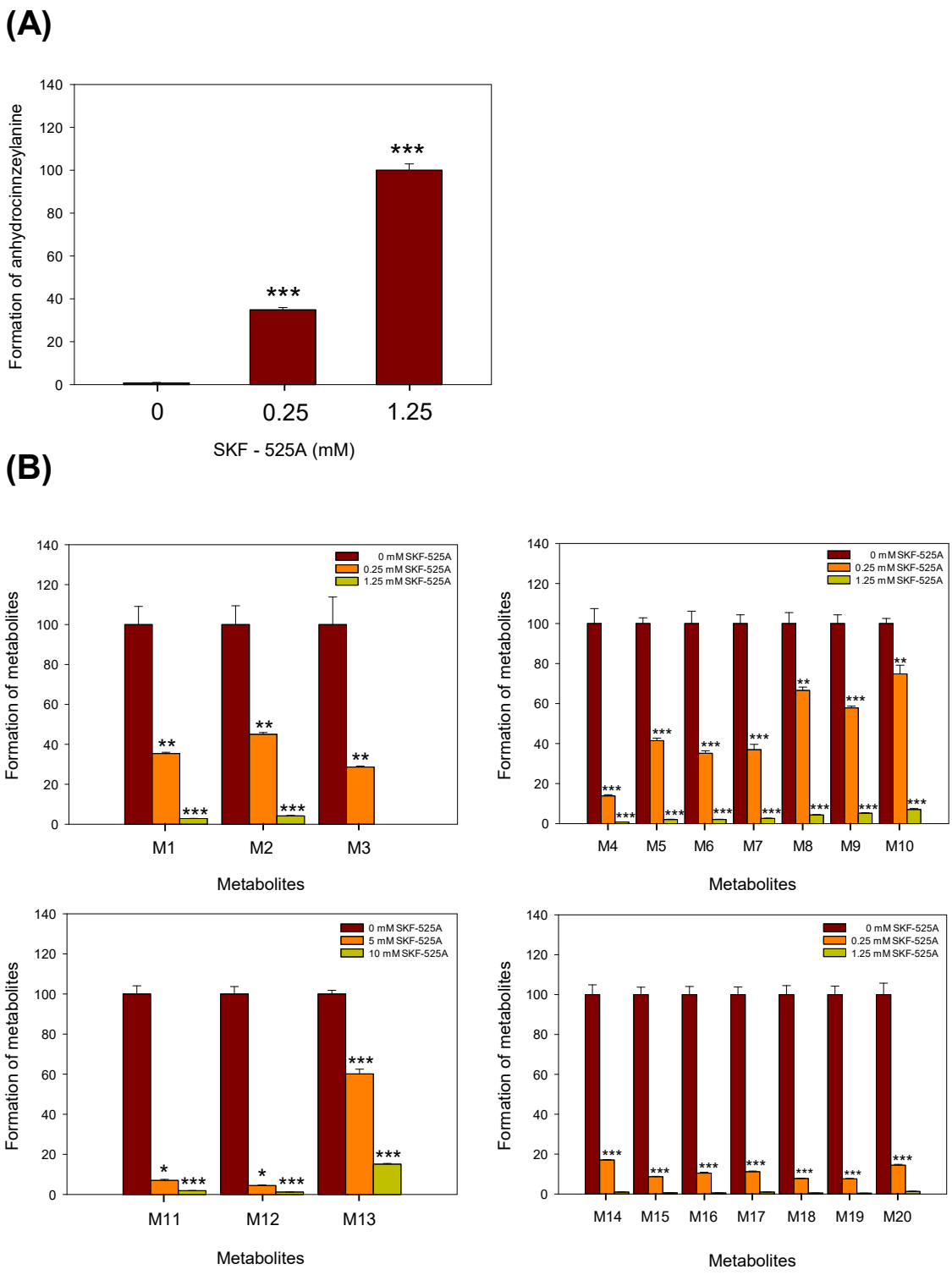


Figure S7. Effect of non-specific CYP inhibitor, SKF 525-A in AHC metabolism in HLMs. Inhibition of AHC metabolism by SKF-525 treatment (0, 0.25 and 1.25 mM) (**A**). Decreased of formation of M1, M2 and M3 (**B**), M4–M10 (**C**), M11–M13 (**D**) and M14–M20 (E). The data are expressed as mean ± standard errors (SE) of the triplicate samples. Bars indicate standard error ($n = 3$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *vs* 0 mM SKF-525A.

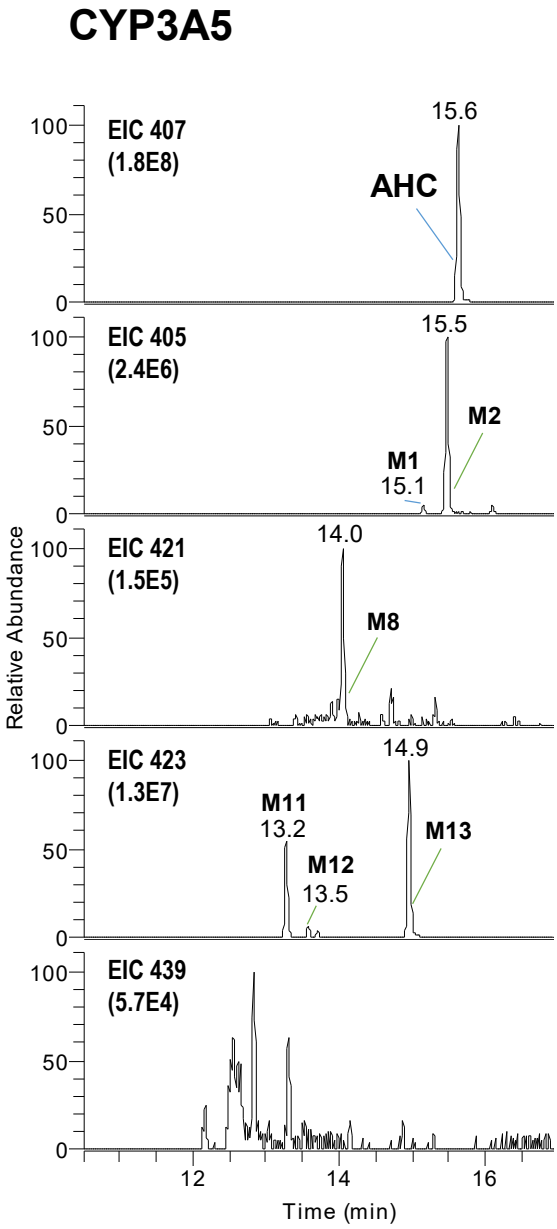


Figure S8. The formation of AHC metabolites in recombinant *cDNA-expressed* CYP3A5 after incubation at 37 °C for 60 min.

Table S1. Elemental composition of key product ions of anhydrocinnzeylanine and its metabolites in human liver microsomes using high-resolution quadrupole-orbitrap mass spectrometry.

Compound	Precursor Ions (m/z)			CE (eV)	Product Ion (m/z)	Elemental Comp. (exp.)	Error (ppm)
	[M-H]$^-$	**Elemental Comp. (exp.)**	**Error (ppm)**				
Anhydro-cinnzeylanine	**407.2072**	**$C_{22}H_{31}O_7$**	**1.7**	18	347.1861	$C_{20}H_{27}O_5$	2.4
				18	329.1757	$C_{20}H_{25}O_4$	2.3
				18	303.1963	$C_{19}H_{27}O_3$	2.6
				18	285.1857	$C_{19}H_{25}O_2$	2.7
				18	153.0910	$C_9H_{13}O_2$	0.0
				18	149.0962	$C_{10}H_{13}O$	0.3
				18	135.0802	$C_9H_{11}O$	0.9
				18	59.0127	$C_2H_3O_2$	−1.2
M1	405.1914	$C_{22}H_{29}O_7$	1.6	20	345.1703	$C_{20}H_{25}O_5$	2.0
				20	327.1598	$C_{20}H_{23}O_4$	2.2
				20	301.1805	$C_{19}H_{25}O_3$	2.3
				20	283.1698	$C_{19}H_{23}O_2$	2.0
				20	151.0754	$C_9H_{11}O_2$	0.1
				20	149.0962	$C_{10}H_{13}O$	0.2
				20	59.0126	$C_2H_3O_2$	−1.8
M2	405.1915	$C_{22}H_{29}O_7$	1.6	18	345.1703	$C_{20}H_{25}O_5$	2.0
				18	327.1599	$C_{20}H_{23}O_4$	2.4
				18	301.1808	$C_{19}H_{25}O_3$	3.4
				18	283.1701	$C_{19}H_{23}O_2$	2.9
				18	151.0754	$C_9H_{11}O_2$	0.1
				18	149.0961	$C_{10}H_{13}O$	0.2
				18	133.0648	C_9H_9O	−0.2
				18	59.0127	$C_2H_3O_2$	−1.6
M3	405.1914	$C_{22}H_{29}O_7$	1.5	18	363.1809	$C_{20}H_{27}O_6$	1.9
				18	345.1704	$C_{20}H_{25}O_5$	2.2
				18	327.1598	$C_{20}H_{23}O_4$	2.2
				18	247.0972	$C_{14}H_{15}O_4$	2.8
				18	219.1020	$C_{13}H_{15}O_3$	1.9
				18	203.1070	$C_{13}H_{15}O_2$	1.5
				18	151.0753	$C_9H_{11}O_2$	−0.1
				18	149.0961	$C_{10}H_{13}O$	0.0
				18	59.0127	$C_2H_3O_2$	−1.6
M4	421.1856	$C_{22}H_{29}O_8$	−0.1	22	361.1646	$C_{20}H_{25}O_6$	0.0
				22	343.1534	$C_{20}H_{23}O_5$	−0.5
				22	303.1227	$C_{17}H_{19}O_5$	0.0
				22	167.0699	$C_9H_{11}O_3$	−0.3
				22	149.0596	$C_9H_9O_2$	−0.1
				22	59.0125	$C_2H_3O_2$	−0.2
M5	421.1855	$C_{22}H_{29}O_8$	−0.2	20	361.1647	$C_{20}H_{25}O_6$	0.1
				20	343.1540	$C_{20}H_{23}O_5$	0.0
				20	167.0701	$C_9H_{11}O_3$	−0.1
				20	149.0958	$C_{10}H_{13}O$	−0.2
				20	59.0125	$C_2H_3O_2$	−0.2
M6	421.1855	$C_{22}H_{29}O_8$	−0.1	20	361.1646	$C_{20}H_{25}O_6$	0.0
				20	343.1541	$C_{20}H_{23}O_5$	0.0
				20	235.0966	$C_{13}H_{15}O_4$	0.1

				20	205.0858	$C_{12}H_{13}O_3$	−0.1
				20	149.0594	$C_9H_9O_2$	−0.2
				20	59.0125	$C_2H_3O_2$	−0.2
M7	421.1855	$C_{22}H_{29}O_8$	−0.1	22	361.1646	$C_{20}H_{25}O_6$	0.0
				22	343.1541	$C_{20}H_{23}O_5$	0.1
				22	235.0966	$C_{13}H_{15}O_4$	0.1
				22	149.0594	$C_9H_9O_2$	−0.3
				22	59.0125	$C_2H_3O_2$	−0.2
M8	421.1855	$C_{22}H_{29}O_8$	−0.1	20	361.1646	$C_{20}H_{25}O_6$	0.0
				20	343.1540	$C_{20}H_{23}O_5$	0.0
				20	235.0965	$C_{13}H_{15}O_4$	0.0
				20	167.0700	$C_9H_{11}O_3$	−0.2
				20	59.0125	$C_2H_3O_2$	−0.2
M9	421.1852	$C_{22}H_{29}O_8$	−0.4	28	361.1645	$C_{20}H_{25}O_6$	0.0
				28	343.1535	$C_{20}H_{23}O_5$	−0.4
				28	167.0700	$C_9H_{11}O_3$	−0.2
				28	149.0594	$C_9H_9O_2$	−0.3
				28	59.0125	$C_2H_3O_2$	-0.2
M10	421.1855	$C_{22}H_{29}O_8$	−0.2	18	361.1646	$C_{20}H_{25}O_6$	0.0
				18	343.1539	$C_{20}H_{23}O_5$	0.0
				18	317.1747	$C_{19}H_{25}O_4$	0.0
				18	167.0700	$C_9H_{11}O_3$	−0.2
				18	59.0125	$C_2H_3O_2$	−0.2
M11	423.2011	$C_{22}H_{31}O_8$	−0.2	20	363.1803	$C_{20}H_{27}O_6$	0.1
				20	345.1696	$C_{20}H_{25}O_5$	0.0
				20	301.1798	$C_{19}H_{25}O_3$	0.0
				20	151.0750	$C_9H_{11}O_2$	−0.3
				20	149.0959	$C_{10}H_{13}O$	−0.2
				20	59.0125	$C_2H_3O_2$	−0.2
M12	423.2010	$C_{22}H_{31}O_8$	−0.3	20	363.1802	$C_{20}H_{27}O_6$	0.0
				20	345.1694	$C_{20}H_{25}O_5$	−0.2
				20	315.1589	$C_{19}H_{23}O_4$	−0.1
				20	169.0857	$C_9H_{13}O_3$	−0.2
				20	149.0958	$C_{10}H_{13}O$	−0.3
				20	59.0125	$C_2H_3O_2$	−0.2
M13	423.2010	$C_{22}H_{31}O_8$	−0.2	28	363.1803	$C_{20}H_{27}O_6$	0.1
				28	345.1697	$C_{20}H_{25}O_5$	0.0
				28	301.1799	$C_{19}H_{25}O_3$	0.0
				28	169.0857	$C_9H_{13}O_3$	−0.1
				28	151.0751	$C_9H_{11}O_2$	−0.2
				28	149.0958	$C_{10}H_{13}O$	−0.2
				28	59.0125	$C_2H_3O_2$	−0.2
M14	439.1958	$C_{22}H_{31}O_9$	−0.4	25	379.1750	$C_{20}H_{27}O_7$	−0.1
				25	361.1645	$C_{20}H_{25}O_6$	0.0
				25	317.1745	$C_{19}H_{25}O_4$	−0.2
				25	169.0857	$C_9H_{13}O_3$	−0.2
				25	165.0908	$C_{10}H_{13}O_2$	−0.2
				25	151.0751	$C_9H_{11}O_2$	−0.2
				25	59.0125	$C_2H_3O_2$	−0.2
M15	439.1958	$C_{22}H_{31}O_9$	−0.4	28	379.1750	$C_{20}H_{27}O_7$	0.0
				28	169.0857	$C_9H_{13}O_3$	−0.2
				28	165.0908	$C_{10}H_{13}O_2$	−0.2

				28	151.0751	$C_9H_{11}O_2$	−0.2
				28	59.0125	$C_2H_3O_2$	−0.2
M16	439.1958	$C_{22}H_{31}O_9$	−0.4	28	379.1750	$C_{20}H_{27}O_7$	0.0
				28	185.0807	$C_9H_{13}O_4$	−0.1
				28	167.0701	$C_9H_{11}O_3$	−0.1
				28	149.0958	$C_{10}H_{13}O$	−0.2
				28	59.0125	$C_2H_3O_2$	−0.2
M17	439.1960	$C_{22}H_{31}O_9$	−0.2	30	379.1751	$C_{20}H_{27}O_7$	0.0
				30	361.1646	$C_{20}H_{25}O_6$	0.0
				30	185.0808	$C_9H_{13}O_4$	0.0
				30	167.0701	$C_9H_{11}O_3$	−0.1
				30	149.0959	$C_{10}H_{13}O$	−0.2
				30	59.0125	$C_2H_3O_2$	−0.2
M18	439.1957	$C_{22}H_{31}O_9$	−0.3	30	379.1750	$C_{20}H_{27}O_7$	0.0
				30	185.0806	$C_9H_{13}O_4$	−0.2
				30	167.0700	$C_9H_{11}O_3$	−0.2
				30	149.0957	$C_{10}H_{13}O$	−0.2
				30	59.0125	$C_2H_3O_2$	−0.2
M19	439.1956	$C_{22}H_{31}O_9$	−0.6	33	379.1749	$C_{20}H_{27}O_7$	−0.2
				33	361.1645	$C_{20}H_{25}O_6$	0.0
				33	169.0857	$C_9H_{13}O_3$	−0.2
				33	165.0907	$C_{10}H_{13}O_2$	−0.2
				33	151.0750	$C_9H_{11}O_2$	−0.3
				33	59.0125	$C_2H_3O_2$	−0.2
M20	439.1958	$C_{22}H_{31}O_9$	-0.4	22	379.1751	$C_{20}H_{27}O_7$	0.0
				22	185.0803	$C_9H_{13}O_4$	−0.5
				22	167.0700	$C_9H_{11}O_3$	−0.2
				22	149.0957	$C_{10}H_{13}O$	−0.4
				22	59.0125	$C_2H_3O_2$	−0.2

CE, collision energy; comp., composition; exp., experiment.