

**Cytochromes P450 metabolism studies of [6]-gingerol, [8]-gingerol, and [10]-gingerol by liver microsomes of human and different species of tissue combined with expressed CYP enzymes.**

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## Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

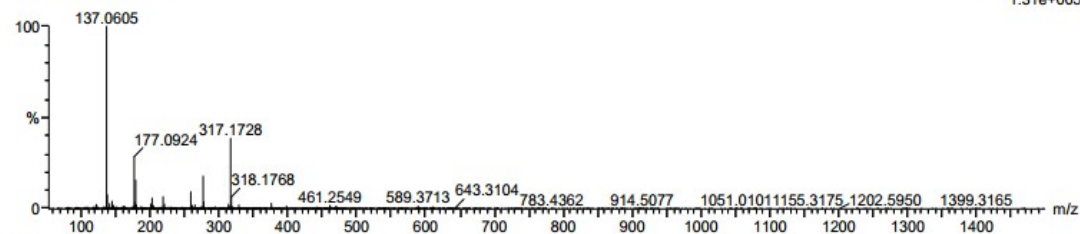
107 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-100 H: 0-100 O: 0-50 Na: 0-1

2L3A-(2L6G)

2017110631 197 (1.597)

1: TOF MS ES+  
1.31e+005Minimum: -1.5  
Maximum: 5.0 5.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
317.1728	317.1729	-0.1	-0.3	4.5	145.1	n/a	n/a	C17 H26 O4 Na

Figure S1-1.

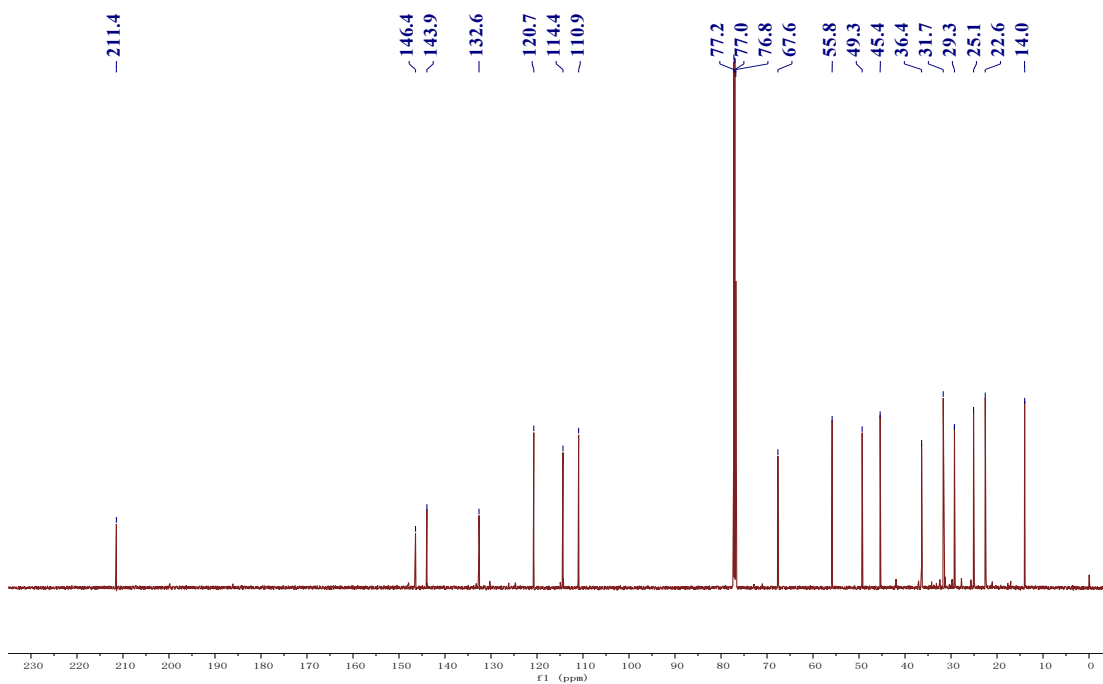


Figure S1-2.

**Figure S1.** The HRESIMS spectrum (S1-1) and <sup>13</sup>C-NMR spectrum (S1-2) of reference standard of [6]-gingerol.

## Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

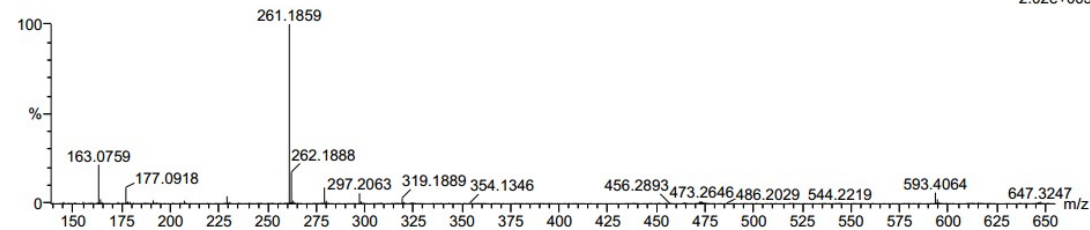
52 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-60 H: 0-100 O: 0-30

ZO209F2

20190610005 194 (1.563)

1: TOF MS ES+  
2.02e+005Minimum: -1.5  
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Conf (%)	Formula
297.2063	297.2066	-0.3	-1.0	3.5	124.4	n/a	C17 H29 O4

Figure S2-1.

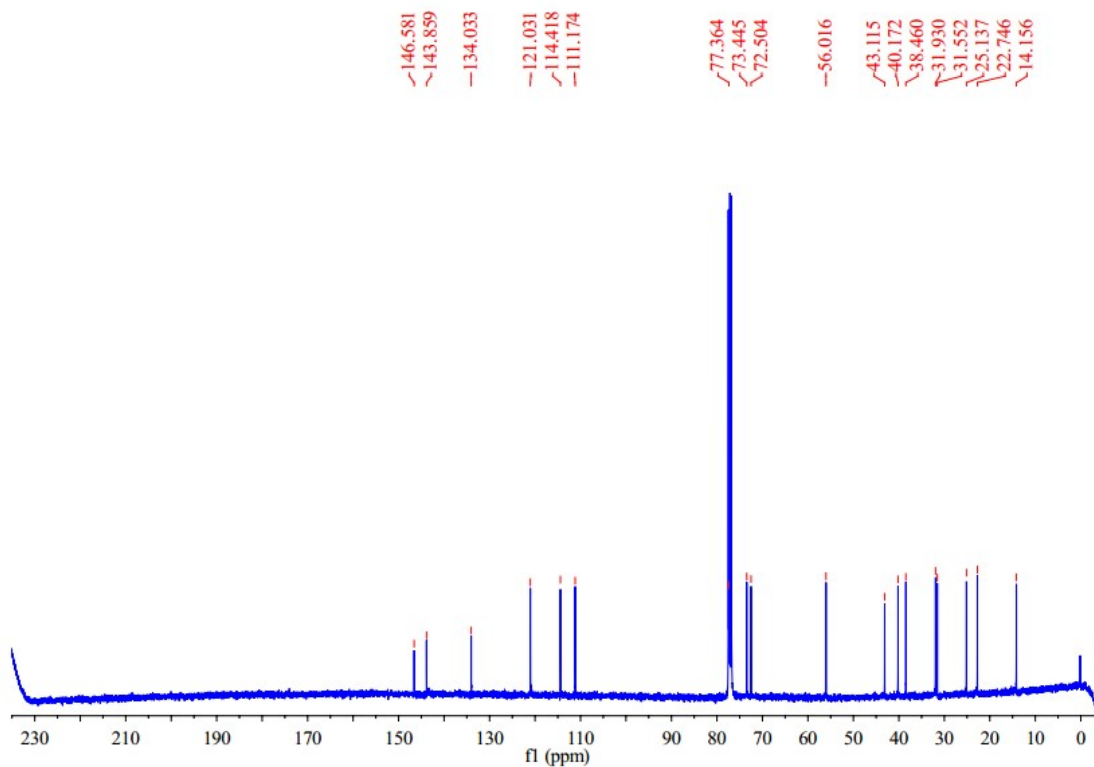


Figure S2-2.

Figure S2. The HRESIMS spectrum (S2-1) and <sup>13</sup>C-NMR spectrum (S2-2) of reference standard of (3R,5S)-[6]-gingerdiol.

## Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

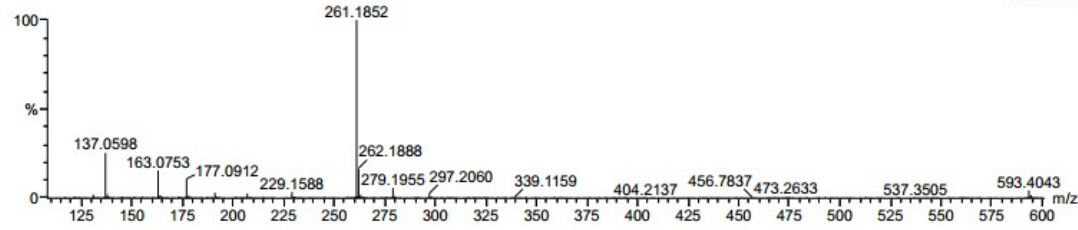
52 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-60 H: 0-100 O: 0-30

ZO2Q8B

20190610014 189 (1.526)

1: TOF MS ES+  
6.56e+005

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Conf (%)	Formula
297.2060	297.2066	-0.6	-2.0	3.5	153.4	n/a	C17 H29 O4

Figure S3-1.

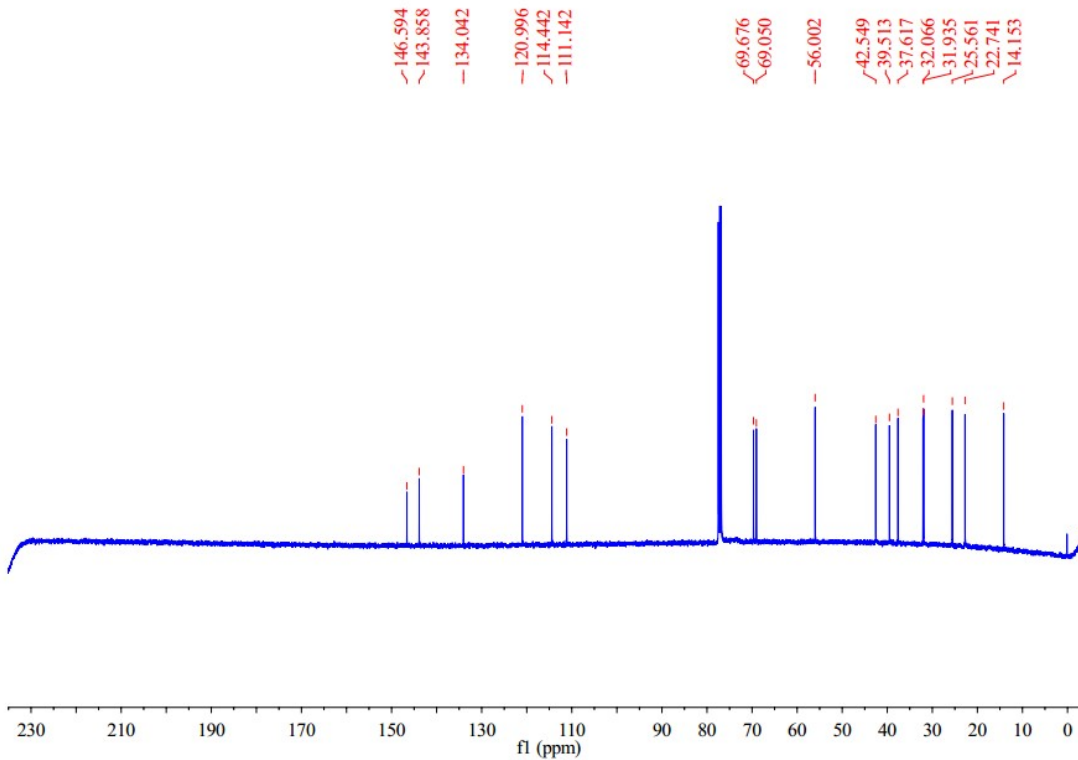


Figure S3-2.

**Figure S3.** The HRESIMS spectrum (S3-1) and  $^{13}\text{C}$ -NMR spectrum (S3-2) of reference standard of (3S,5S)-[6]-gingerdiol.

## Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

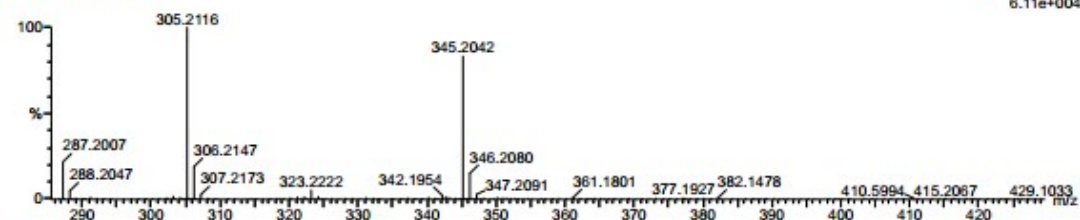
64 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-100 H: 0-100 O: 0-50

2L7C

2017110629 217 (1.758)

1: TOF MS ES+  
6.11e+004

Mass	Calc. Mass	mba	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
323.2222	323.2222	0.0	0.0	4.5	58.6	n/a	n/a	C19 H31 O4

Figure S4-1.

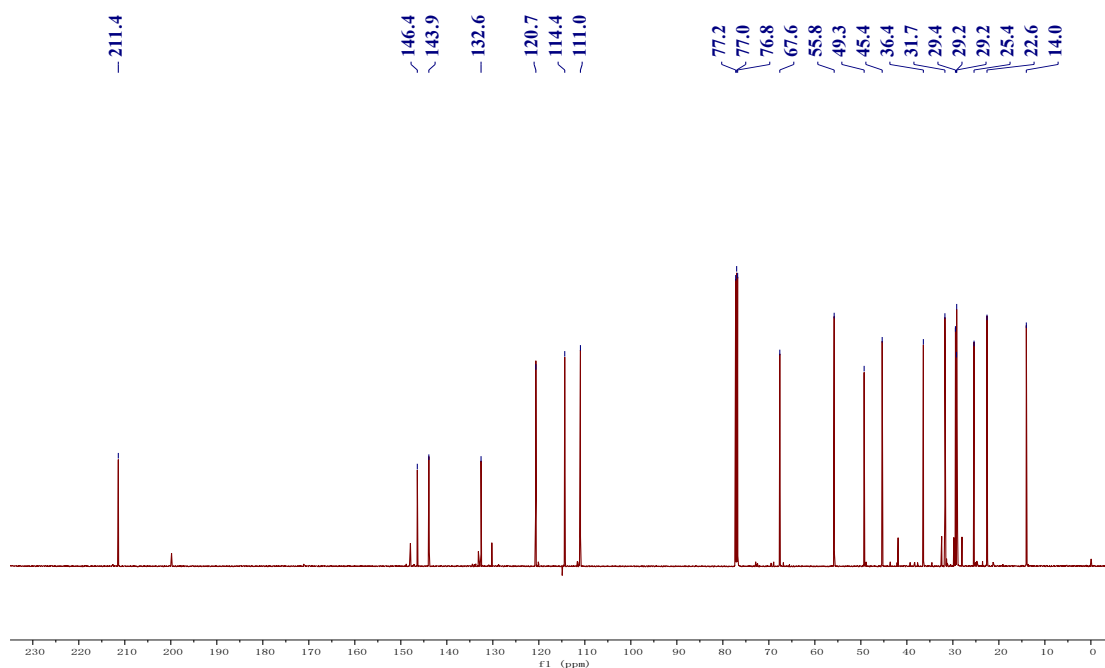


Figure S4-2.

Figure S4. The HRESIMS spectrum (S4-1) and  $^{13}\text{C}$ -NMR spectrum (S4-2) of reference standard of [8]-gingerol.

(3R,5S)-[8]-gingerdiol

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

133 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)

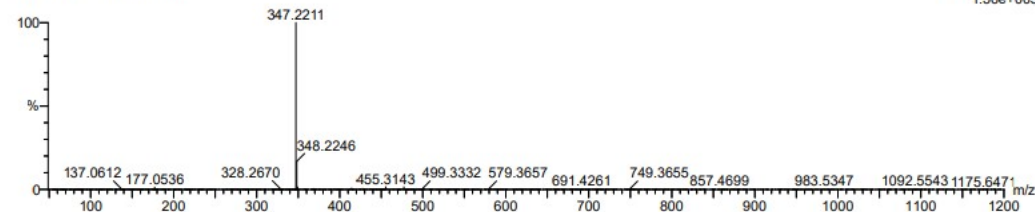
Elements Used:

C: 0-500 H: 0-1000 O: 0-200 Na: 0-1

70%M-GINGER

20170118\_3 1817 (14.552)

1: TOF MS ES+  
1.56e+005



Minimum: 5.0 10.0 -1.5  
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
347.2211	347.2198	1.3	3.7	3.5	130.5	0.426	65.29	C19 H32 O4 Na
	347.2222	-1.1	-3.2	6.5	131.1	1.058	34.71	C21 H31 O4

Figure S5-1.

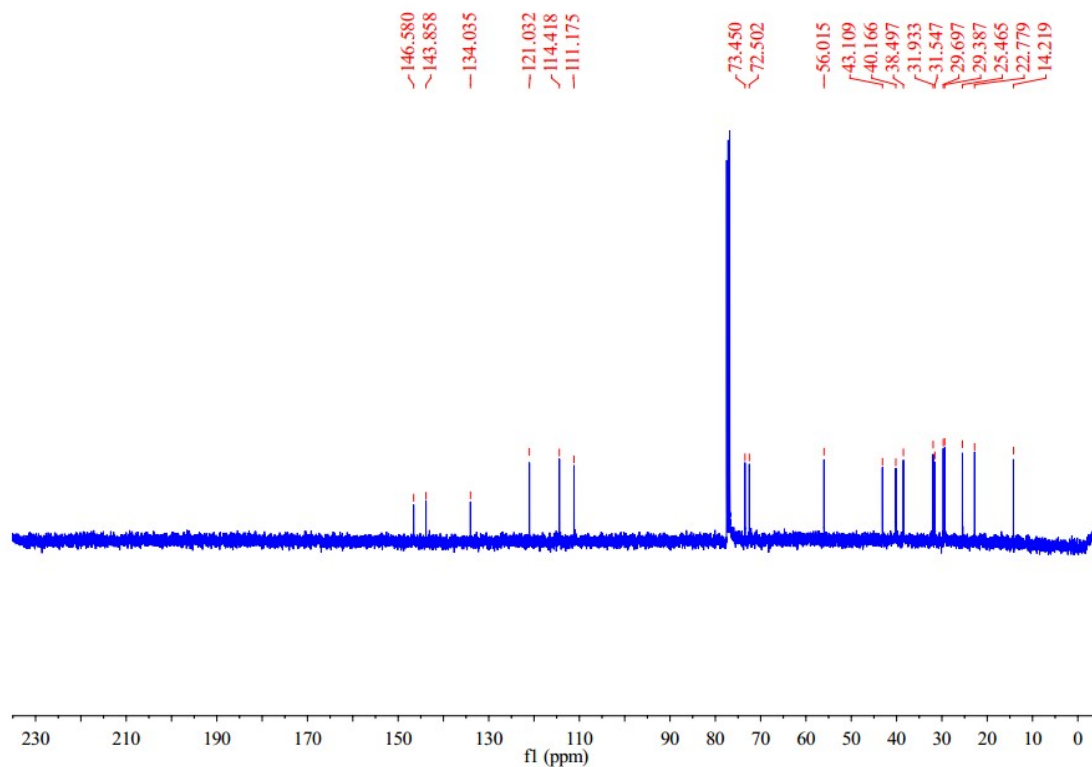


Figure S5-2.

Figure S5. The HRESIMS spectrum (S5-1) and <sup>13</sup>C-NMR spectrum (S5-2) of reference standard of (3R,5S)-[8]-gingerdiol.

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

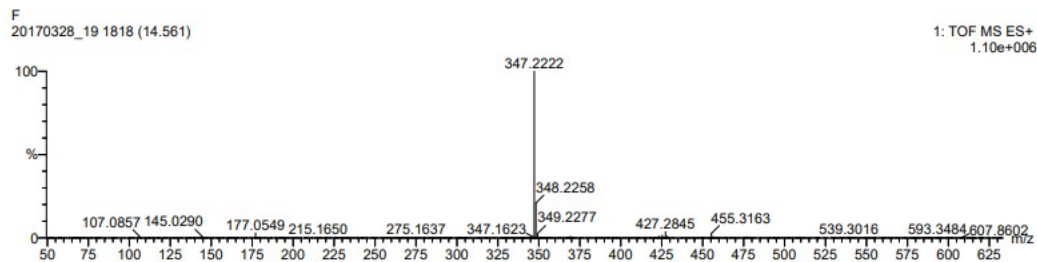
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

133 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 0-500 H: 0-1000 O: 0-200 Na: 0-1



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
347.2222	347.2198	2.4	6.9	3.5	603.9	0.052	94.93	C19 H32 O4 Na
	347.2222	0.0	0.0	6.5	606.8	2.981	5.07	C21 H31 O4

Figure S6-1.

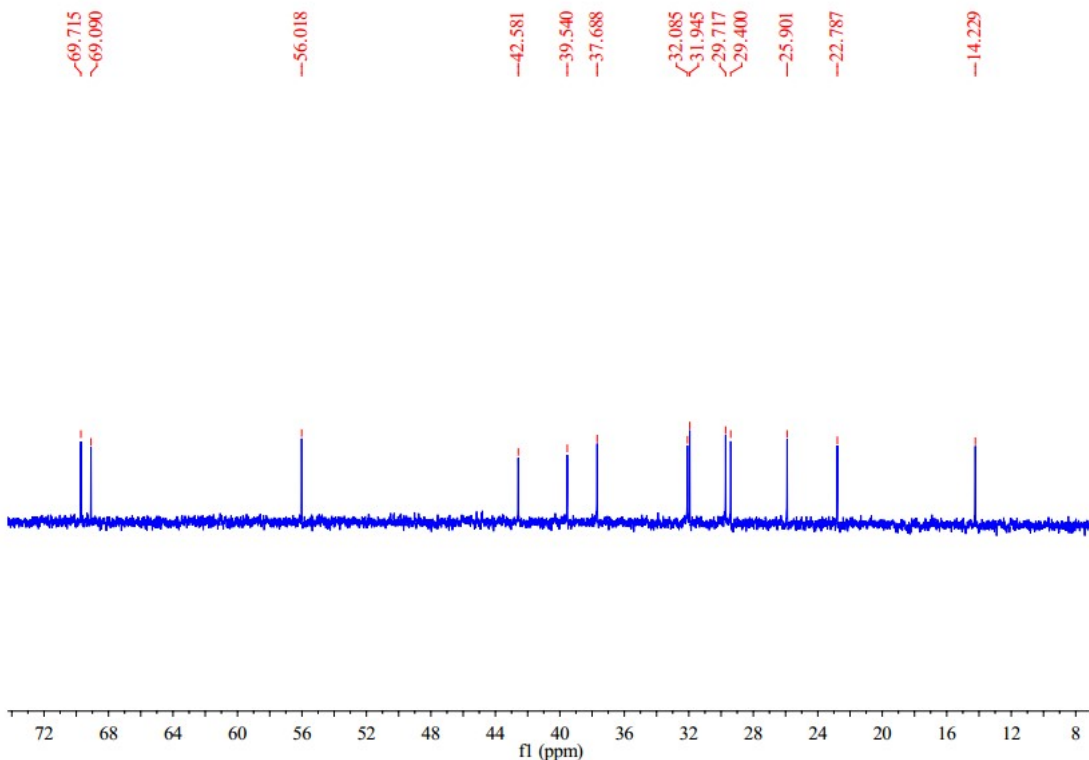


Figure S6-2.

Figure S6. The HRESIMS spectrum (S6-1) and <sup>13</sup>C-NMR spectrum (S6-2) of reference standard of (3S,5S)-[8]-gingerdiol.

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

142 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

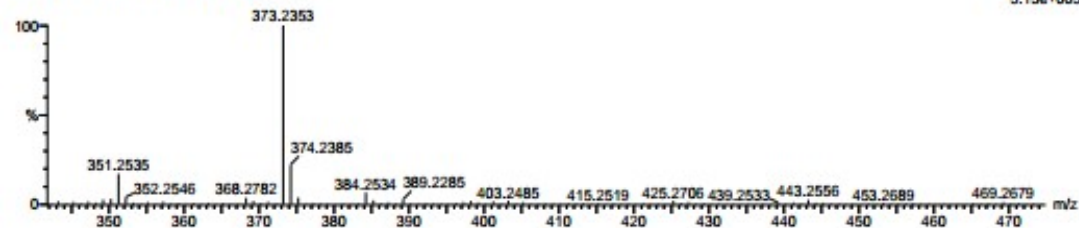
Elements Used:

C: 0-60 H: 0-100 O: 0-50 23Na: 0-1

ZL13

20171017-8 251 (2.023) Cm (248:256)

1: TOF MS ES+  
5.13e+005



Minimum: -1.5  
Maximum: 10.0 5.0 100.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
373.2353	373.2355	-0.2	-0.5	4.5	15.3	n/a	n/a	C21 H34 O4 23Na

Figure S7-1.

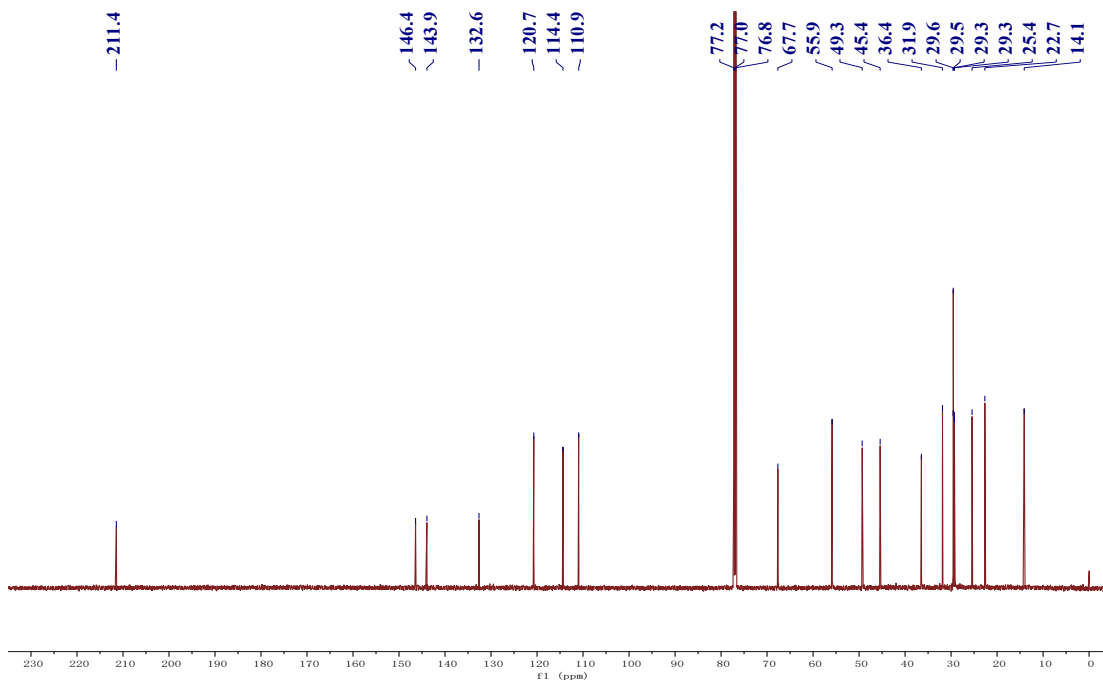


Figure S7-2.

Figure S7. The HRESIMS spectrum (S7-1) and <sup>13</sup>C-NMR spectrum (S7-2) of reference standard of [10]-gingerol.



## Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

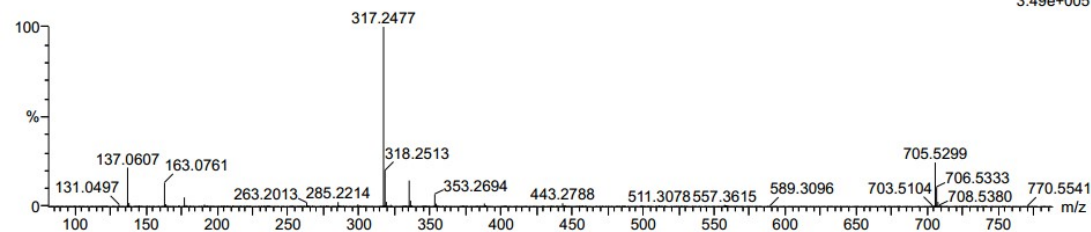
68 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-80 H: 0-100 O: 0-20

Z02Q16D

20190715-6 259 (2.094)

1: TOF MS ES+  
3.49e+005Minimum: -1.5  
Maximum: 10.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Conf (%)	Formula
353.2694	353.2692	0.2	0.6	3.5	159.7	n/a	C21 H37 O4

Figure S8-1.

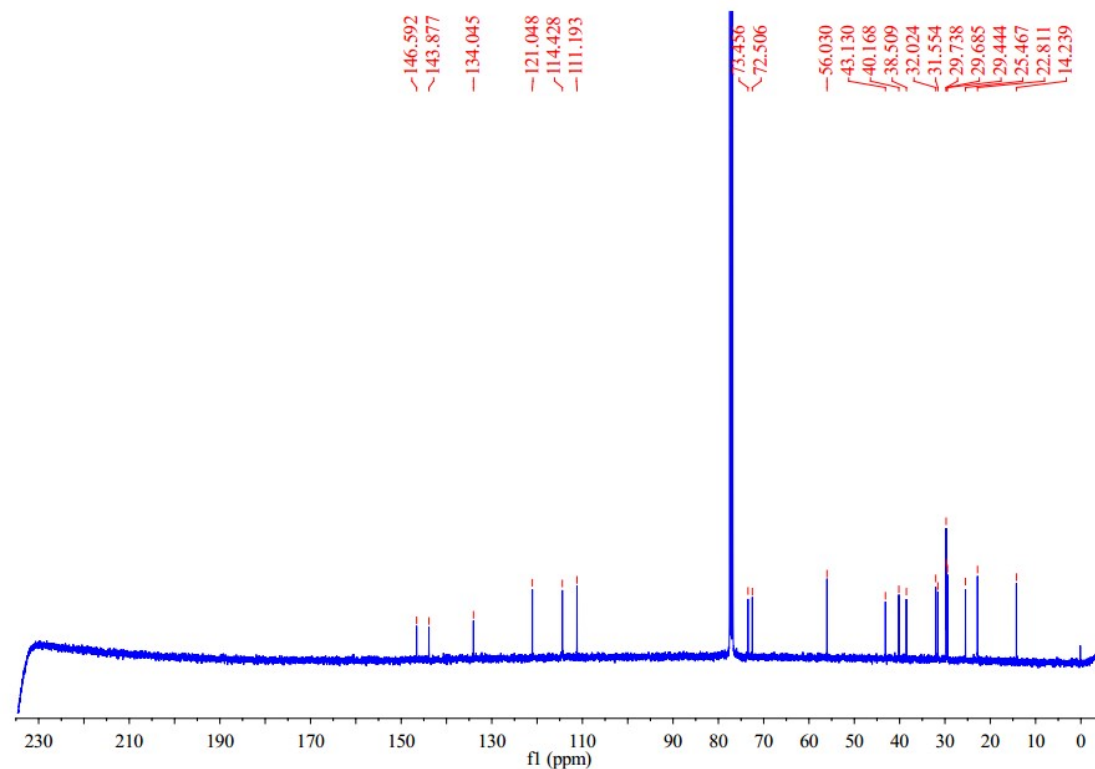


Figure S8-2.

Figure S8. The HRESIMS spectrum (S8-1) and <sup>13</sup>C-NMR spectrum (S8-2) of reference standard of (3R,5S)-[10]-gingerdiol.

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

68 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

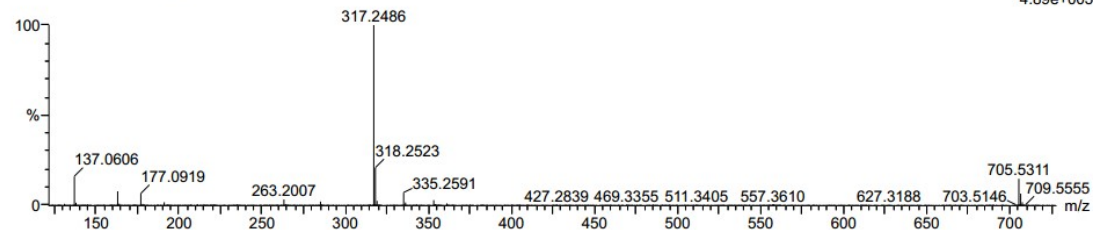
Elements Used:

C: 0-80 H: 0-100 O: 0-20

Z02Q16C

2019070103 256 (2.061)

1: TOF MS ES+  
4.89e+005



Minimum: -1.5  
Maximum: 10.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Conf (%)	Formula
353.2687	353.2692	-0.5	-1.4	3.5	97.1	n/a	C21 H37 O4

Figure S9-1.

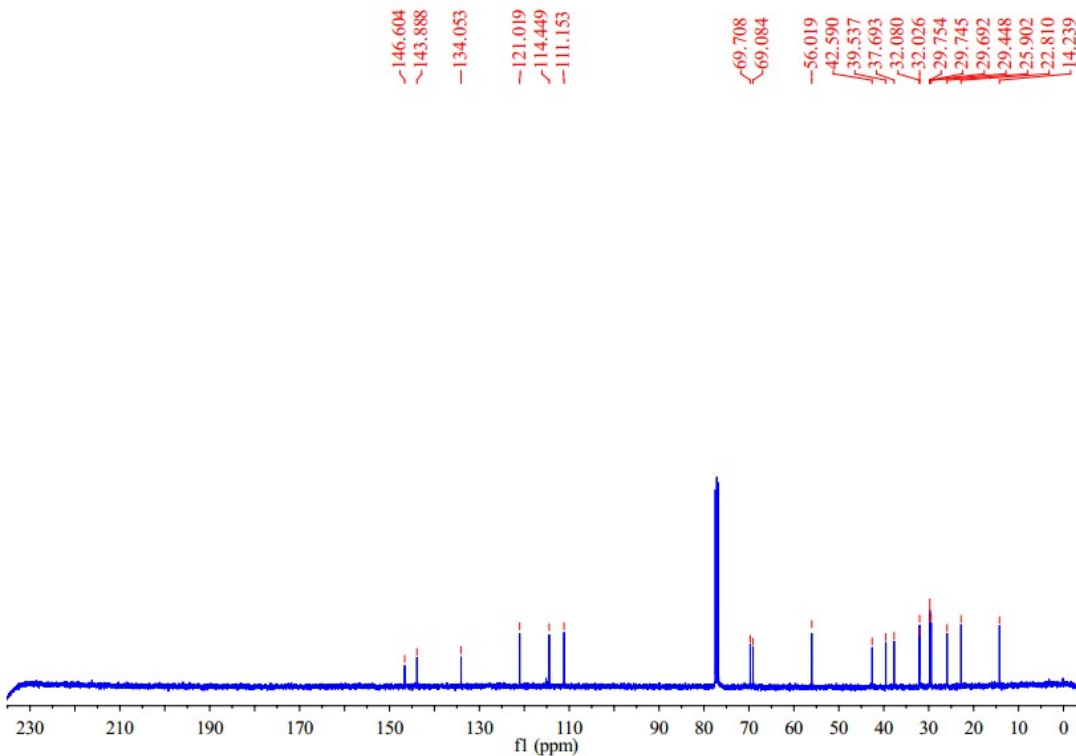


Figure S9-2.

Figure S9. The HRESIMS spectrum (S9-1) and <sup>13</sup>C-NMR spectrum (S9-2) of reference standard of (3S,5S)-[10]-gingerdiol.

**Table S1. Kinetic parameters derived for gingerols-related metabolites by HLM, DLM, MkLM, MLM, MpLM, and RLM (Mean ± SD).**

Compound	Enzyme	Metabolite	Vmax (pmol/min/mg)	Km (μM)	Ksi (μM)	Clint (μL/min/mg)	Model
6G	HLM	M <sub>6G</sub> -1	123.82±3.47	181.30±11.30	NA	0.88±0.05	MM
		M <sub>6G</sub> -5	432.80±7.02	149.10±5.82	NA	2.90±0.12	MM
	DLM	M <sub>6G</sub> -5	343.4±18.48	138.9±17.7	NA	2.47±0.34	MM
	MkLM	M <sub>6G</sub> -5	415.2±12.88	47.88±5.008	NA	8.71±0.95	MM
		M <sub>6G</sub> -8	297.8±11.30	18.45±2.583	NA	22.13±4.3	MM
	MLM	M <sub>6G</sub> -3	131.3±8.753	118.2±15.21	NA	1.11±0.15	MM
		M <sub>6G</sub> -5	1558±84.13	188.7±21.73	NA	8.24±1.05	MM
	MpLM	M <sub>6G</sub> -5	97.04±4.497	173.4±17.83	NA	0.58±0.08	MM
	RLM	M <sub>6G</sub> -1	254.82±7.72	180.90±12.21	NA	1.41±0.10	MM
		M <sub>6G</sub> -4	1280.00±181.70	170.80±28.48	80.57±9.48	7.49±1.50	SI
	M <sub>6G</sub> -5	531.50±54.44	409.90±88.52	NA	1.30±0.25	MM	
8G	HLM	M <sub>8G</sub> -1	81.41±1.85	8.78±0.88	820.3±88.35	9.08±0.92	SI
		M <sub>8G</sub> -2	390.40±4.88	1.93±0.005	805.2±28.80	202.00±13.11	SI
		M <sub>8G</sub> -6	848.90±38.23	109.00±13.39	NA	7.79±1.01	MM
	DLM	M <sub>8G</sub> -2	121.8±8.199	98.93±20.05	NA	1.23±0.28	MM
		M <sub>8G</sub> -6	398.2±7.538	94.85±5.458	NA	4.21±0.28	MM
		M <sub>8G</sub> -7	95.03±4.818	88.05±2.059	NA	1.44±0.11	MM
	MkLM	M <sub>8G</sub> -2	38.39±2.527	80.01±13.83	NA	0.84±0.15	MM
		M <sub>8G</sub> -6	284.2±12.42	18.18±3.912	NA	18.35±4.05	MM
	MLM	M <sub>8G</sub> -4	23.5±0.484	25.21±2.102	NA	0.93±0.08	MM
		M <sub>8G</sub> -6	470.4±51.45	72.59±12.89	252.8±48.2	8.48±1.33	SI
	MpLM	M <sub>8G</sub> -2	182.1±14.83	243.4±14.83	NA	0.87±0.15	MM
		M <sub>8G</sub> -6	437.8±28.88	102.4±20.08	NA	4.27±0.88	MM
	RLM	M <sub>8G</sub> -1	98.07±8.53	25.88±3.88	433.10±80.09	3.72±0.81	SI
	M <sub>8G</sub> -2	841.20±17.13	8.53±0.45	225.9±17.09	98.19±7.22	SI	
	M <sub>8G</sub> -6	103±13.42	249.5±82.7	NA	0.41±0.12	MM	
10G	HLM	M <sub>10G</sub> -1	88.42±8.47	29.82±8.08	73.83±14.08	2.24±0.54	SI
		M <sub>10G</sub> -2	348.10±11.83	5.27±0.898	897.50±101.50	88.04±9.02	SI
		M <sub>10G</sub> -4	1957.00±233.90	138.70±23.38	233.90±47.12	14.11±2.91	SI
	DLM	M <sub>10G</sub> -2	157.0±14.74	137.7±23.3	NA	1.14±0.22	MM
		M <sub>10G</sub> -4	225.1±17.34	148.9±20.17	NA	1.51±0.23	MM
	MkLM	M <sub>10G</sub> -1	95.15±5.87	107.7±18.84	NA	0.88±0.15	MM
		M <sub>10G</sub> -4	1074±150.9	294.5±88.19	NA	3.85±0.98	MM
	MLM	M <sub>10G</sub> -1	88.27±4.27	33.98±8.20	NA	2.80±0.49	MM
		M <sub>10G</sub> -2	24.85±1.00	9.72±1.83	NA	2.54±0.49	MM
		M <sub>10G</sub> -4	1022±83.82	88.89±14.97	NA	11.78±2.243	MM

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MpLM	M <sub>10G</sub> -1	99.54±3.535	28.09±3.994	NA	3.54±0.52	MM
	M <sub>10G</sub> -2	204.9±3.885	28.07±2.01	NA	7.88±0.82	MM
	M <sub>10G</sub> -3	830.8±83.58	1239±155.4	NA	0.51±0.08	MM
	M <sub>10G</sub> -4	1292±82.78	228.4±29	NA	5.71±0.82	MM
RLM	M <sub>10G</sub> -1	189.50±7.81	7.04±1.083	433.10±80.09	24.07±3.71	SI
	M <sub>10G</sub> -2	250.00±8.53	8.48±0.811	828.40±35.00	38.58± 5.00	SI
	M <sub>10G</sub> -4	78.33±3.35	39.43±8.82	NA	1.94±0.35	MM

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**Table S2. Kinetic parameters derived for gingerols-related metabolites by expressed CYP enzymes (Mean±SD).**

Compound	Enzyme	Metabolite	Vmax	Km (μM)	Ksi (μM)	Clint	Mode
d		s	(pmol/min/mg)			(μL/min/mg)	l
8G	CYP1A2	M <sub>6G</sub> -1	79.78 ±2.98	212.83±18.5	NA	0.38±0.03	MM
		M <sub>6G</sub> -3	288.40±7.93	135.80±9.43	NA	2.12±0.18	MM
	CYP2C1	M <sub>6G</sub> -1	287.55±11.38	30.21±2.84	549.21±88.57	8.85±0.88	SI
		M <sub>6G</sub> -4	3308.40±129.0	52.22±73.84	558.80±80.74	83.31±5.08	SI
8G	CYP1A2	M <sub>8G</sub> -1	142.00±11.48	28.27±4.98	495.20±109.8	5.02±0.97	SI
		M <sub>8G</sub> -5	284.30±27.51	100.80±15.5	258.80±47.91	2.83±0.49	SI
	CYP2C1	M <sub>8G</sub> -1	398.90±19.88	8.71±0.80	131.50±15.50	59.19±7.89	SI
		M <sub>8G</sub> -5	588.00±81.30	13.00±3.17	59.44±14.27	45.20±12.8	SI
	CYP2E1	M <sub>8G</sub> -1	340.50±48.83	121.30±25.1	389.90±109.8	2.81±0.70	SI
	CYP2D6	M <sub>8G</sub> -4	5.23±0.25	14.52±3.78	NA	0.38±0.09	MM
		M <sub>8G</sub> -8	14.92±0.87	3.48±1.12	3135±2078	4.29±1.4	SI
	10G	CYP1A2	M <sub>10G</sub> -2	120.90±23.58	77.84±23.77	129.20±33.70	1.58±0.57
CYP2B8		M <sub>10G</sub> -2	82.81±5.94	25.35±4.81	110.80±17.74	2.48±0.51	SI
		M <sub>10G</sub> -4	47.99±7.42	38.8±11.09	389.3±142	1.31±0.45	SI
CYP2C1		M <sub>10G</sub> -1	887.70±237.80	9.90±4.54	5.48±2.48	87.48±39.1	SI
CYP2E1		M <sub>10G</sub> -1	719.50±74.71	73.55±12.02	170.80±83.59	9.83±1.89	SI
		M <sub>10G</sub> -2	112.90±14.44	20.03±5.23	102.90±22.49	5.84±1.84	SI