

FIG. 1

FIG 2A

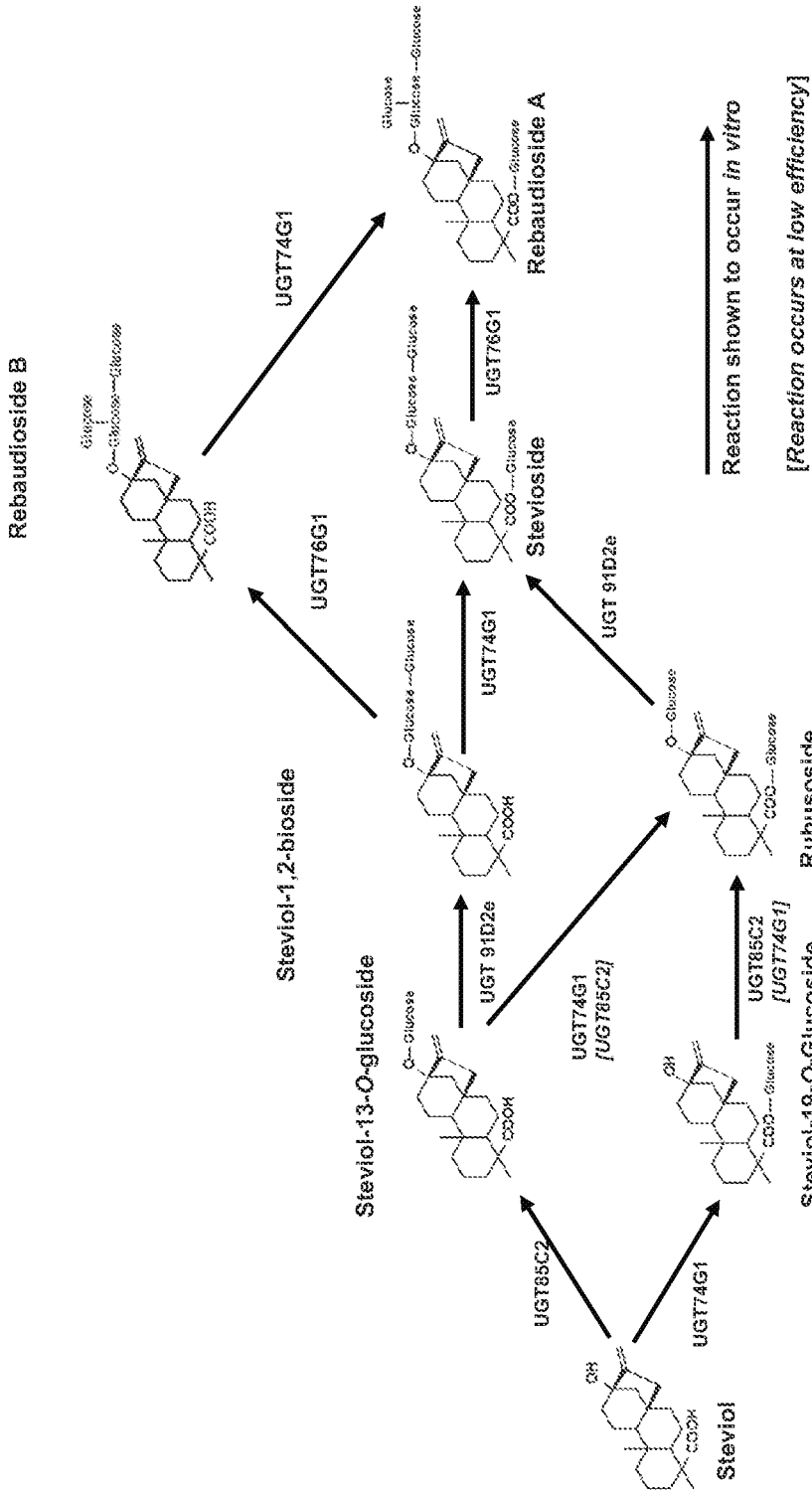


FIG 2B

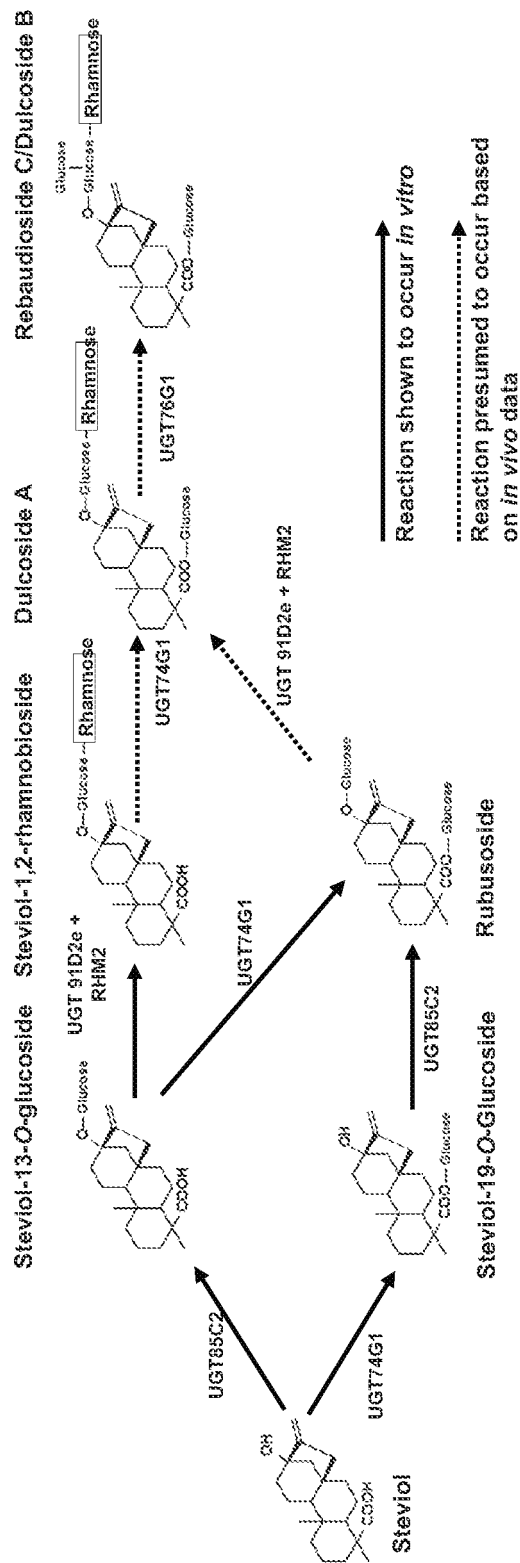


FIG. 2C

Steviol

UGT85C2

UGT74G1

Steviol-13-O-glucoside

UGT91D2e

UGT74G1 [UGT85C2]

Steviol-1,2-bioside

UGT76G1

Rebaudioside A

UGT91D2e

Rebaudioside D

UGT76G1

Rebaudioside E

UGT85C2 [UGT74G1]

Steviol-19-O-glucoside

Rebaudioside A

Rebaudioside E

Rebaudioside D

Reaction shown to occur *in vitro*

Reaction presumed to occur

Reaction presumed to occur

The diagram illustrates the metabolic pathway of Steviol-19-O-glucoside in the liver. The pathway begins with Steviol-19-O-glucoside, which is converted to Steviol-13-O-glucoside by the enzyme UGT85C2. Steviol-13-O-glucoside can then be converted to Steviol-1,2-xylobioside by UGT74G1, or to Steviol-1,2-xylobioside by UGT74G1. Steviol-1,2-xylobioside is further converted to 1,2-Stevioxyxylobioside by UGT74G1. 1,2-Stevioxyxylobioside is then converted to Rebaudioside F by UGT78G1. The diagram also shows the conversion of Steviol-19-O-glucoside to Steviol-13-O-glucoside by UGT85C2, and Steviol-13-O-glucoside to Steviol-1,2-xylobioside by UGT74G1. The diagram includes chemical structures for Steviol-19-O-glucoside, Steviol-13-O-glucoside, Steviol-1,2-xylobioside, 1,2-Stevioxyxylobioside, and Rebaudioside F. The diagram also includes a legend indicating that solid arrows represent reactions shown to occur *in vitro*, and dotted arrows represent reactions presumed to occur.

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graph TD
    S19[Steviol-19-O-glucoside] -- UGT85C2 --> S13[Steviol-13-O-glucoside]
    S13 -- UGT74G1 --> S12[Steviol-1,2-xylobioside]
    S13 -- UGT74G1 --> S12
    S12 -- UGT74G1 --> S11[1,2-Stevioxyxylobioside]
    S11 -- UGT78G1 --> RF[Rebaudioside F]
    S19 -.-> S11
    S13 -.-> S11
    S12 -.-> S11
    S11 -.-> RF
    
```

Reaction shown to occur *in vitro*

Reaction presumed to occur

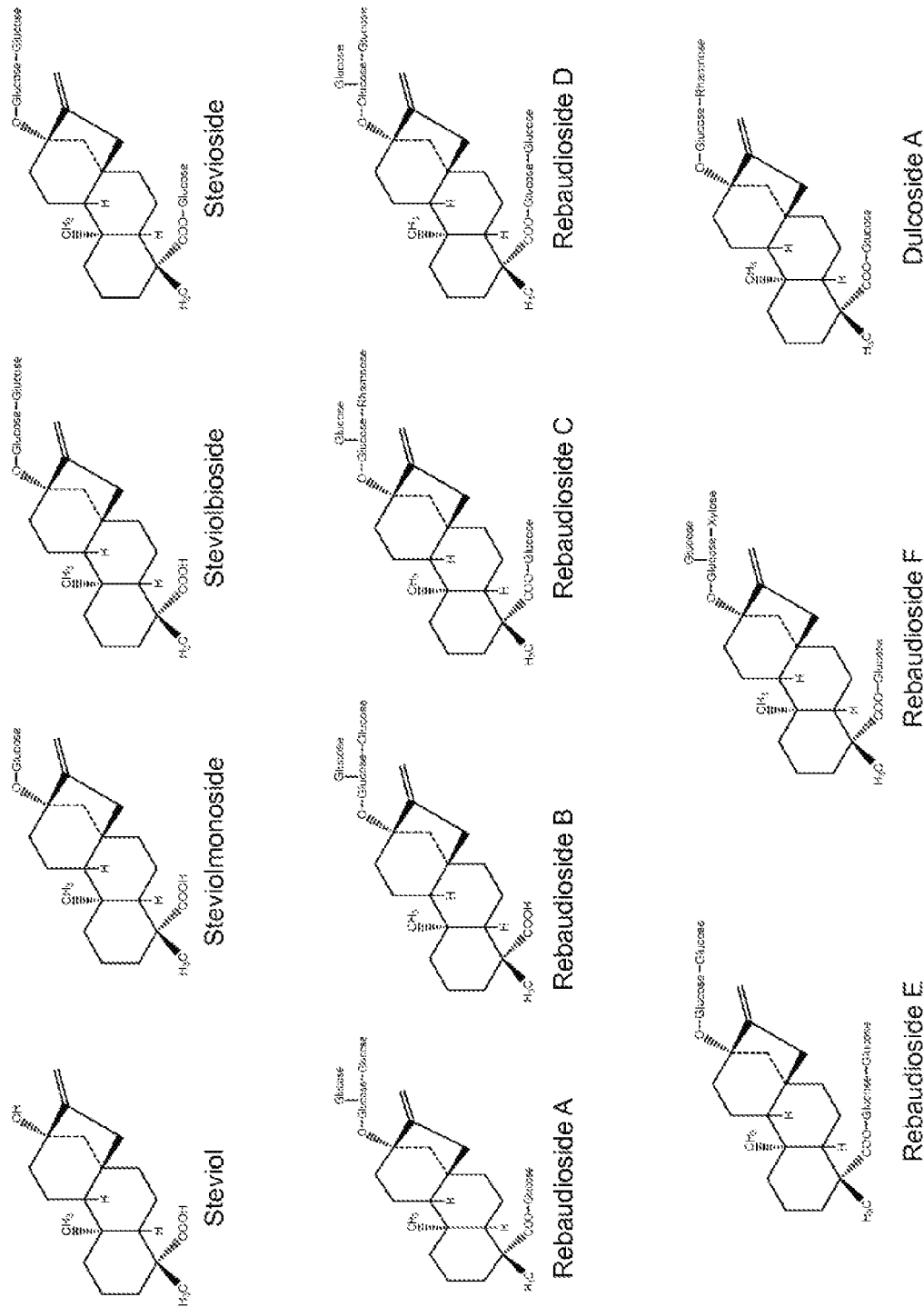


FIG. 3

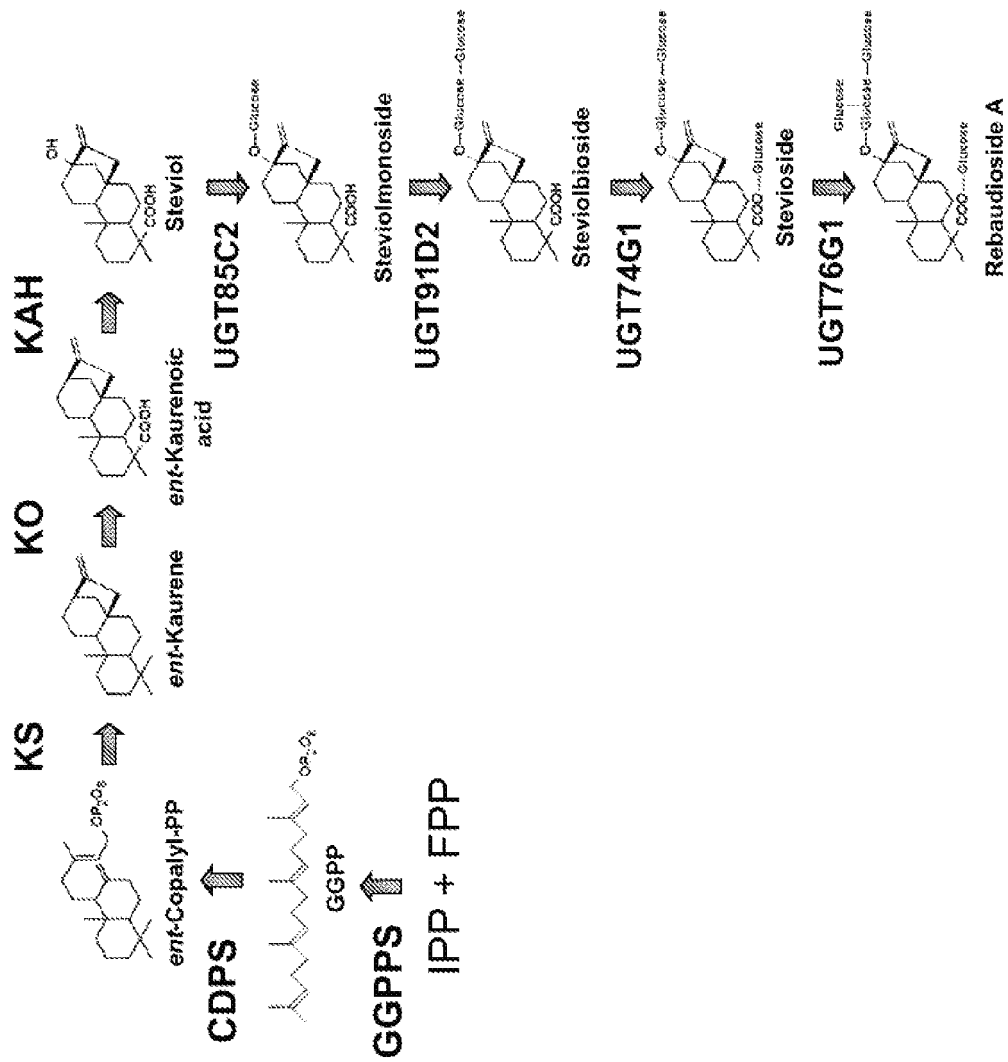


FIG. 4

eYAC system

- Entry Vector (4000 bp) contains: AmpR, ori, Promoter, Gene (cDNA), Terminator, E coli Ori.
- cDNA cassettes are liberated en masse from Entry libraries.
- Concatenated by ligation.
- Chromosome arms are added.
- Optimal size eYACs selected by PFGE (100-300 kb).
- These will contain from 50-150 expression cassettes.

RUBUSOSIDE PRODUCTION UNDER VARIOUS CULTURE CONDITIONS

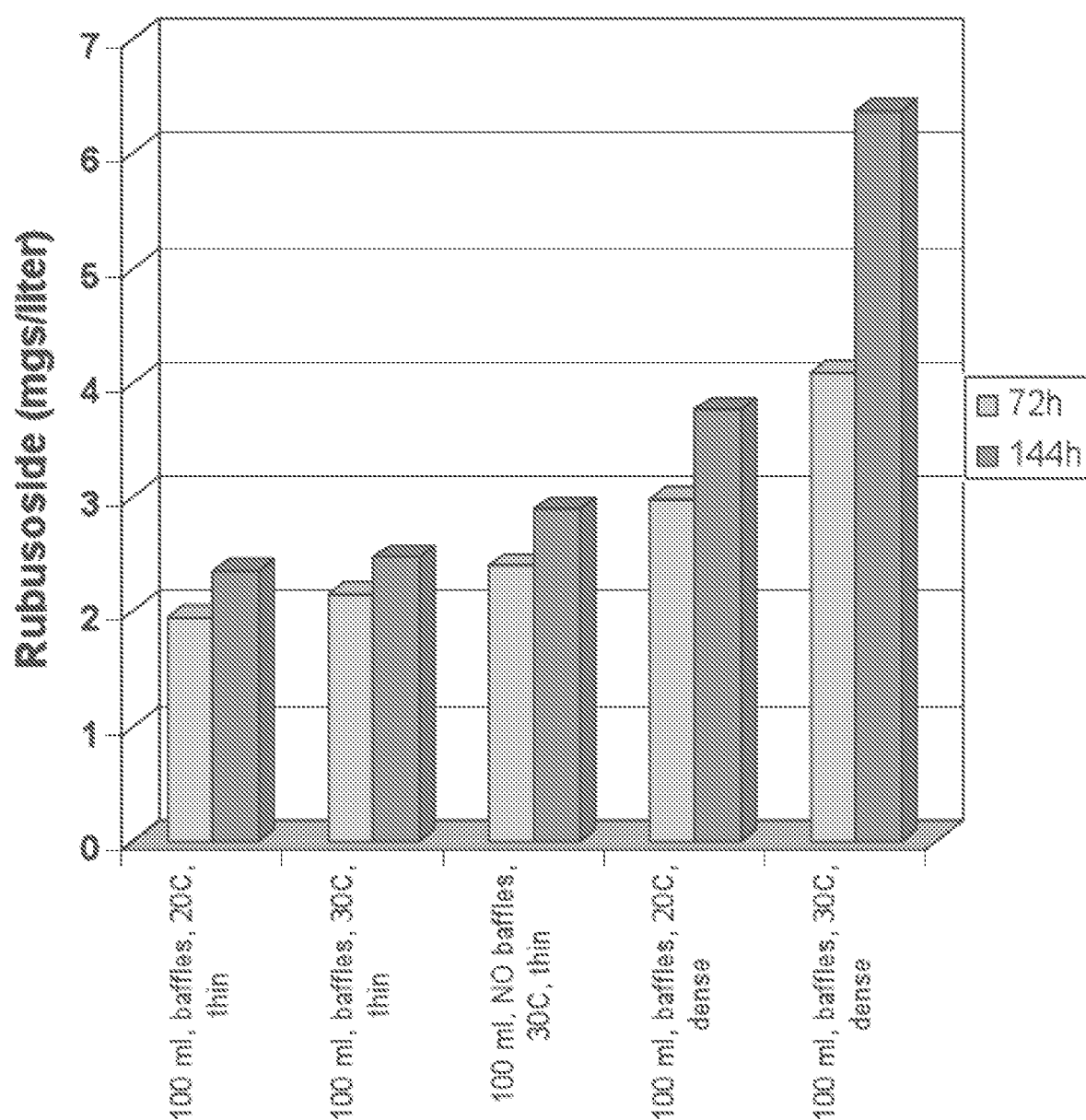
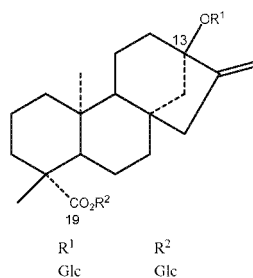


FIGURE 6

Rubusoside
NMR-Data in d5-Pyridine



No	Literature			Analyte		
	1H		13C	1H		HSQC
	δ [ppm]	M J[Hz]		δ [ppm]	M J[Hz]	
1			40.8			40.7
2			19.4			19.4
3			38.3			38.0
4			44.0			-
5			57.3			57.1
6			22.1			22.0
7			41.1			41.5
8			42.4			-
9			53.9			53.7
10			39.8			-
11			20.7			20.5
12			37.2			36.8
13			85.9			-
14			44.5			44.5
15			47.7			47.6
16			154.5			-
17	4.99 5.33	br s br s	104.4	4.99 5.46	br s br s	104.2
18	1.25	s	28.3	1.25	s	28.3
19			176.9			-
20	1.26	s	15.6	1.21	s	15.6
13-O-Glc						
1	5.13	d 7.7	99.7	5.00	d 8.4	99.0
2	4.07	dd 7.7/9.0	75.2			74.8
2	4.22	dd 9.0/8.9	78.8			78.2
4	4.32	dd 8.9/8.8	72.3			71.8
5	4.00	ddd 8.8/2.4/4.4	78.0			77.2
6	4.45 4.26	dd 2.4/12.0 dd 2.4/12.0	63.0	4.42	dd 2.3/12.5	62.6
19-O-Glc						
1	6.14	d 7.9	95.9	5.99	d 8.1	95.2
2	4.20	dd 7.9/9.0	74.0			73.5
2	4.22	dd 9.0/9.0	79.1			78.4
4	4.09	dd 9.0/8.8	71.1			70.7
5	3.98	ddd 8.8/2.2/4.6	79.3			78.4
6	4.61 4.26	dd 2.2/11.7 dd 4.6/11.7	62.1	4.31 4.22	dd 2.5/12.3 dd 4.0/11.7	61.8

FIGURE 7

Multiple Sequence Alignment

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SEQ ID NO:14  MYNVTYHONSKAMATSDSIVDDRKQLHVATFPWLAFCGHILPELQLSKLIA
SEQ ID NO:16  -----MATSDSIVDDRKQLHVATFPWLAFCGHILPELQLSKLIA
SEQ ID NO:12  -----MATSDSIVDDRKQLHVATFPWLAFCGHILPELQLSKLIA
SEQ ID NO: 5  -----MATSDSIVDDRKQLHVATFPWLAFCGHILPELQLSKLIA
SEQ ID NO:10  -----MATSDSIVDDRKQLHVATFPWLAFCGHILPELQLSKLIA
                *****;*****

SEQ ID NO:14  EKGHKVSFLSTTRNIQRLSSHISFLINVVQLTLPVQELPEDAEATTDVH
SEQ ID NO:16  EKGHKVSFLSTTRNIQRLSSHISFLINVVQLTLPVQELPEDAEATTDVH
SEQ ID NO:12  EKGHKVSFLSTTRNIQRLSSHISFLINVVQLTLPVQELPEDAEATTDVH
SEQ ID NO: 5  EKGHKVSFLSTTRNIQRLSSHISFLINVVQLTLPVQELPEDAEATTDVH
SEQ ID NO:10  EKGHKVSFLSTTRNIQRLSSHISFLINVVQLTLPVQELPEDAEATTDVH
                *****

SEQ ID NO:14  PEDIQYLKXAVDGLQPEVTRFLEQHSFDMIIYDFTHYWLPSPSIAASLGISR
SEQ ID NO:16  PEDIQYLKXAVDGLQPEVTRFLEQHSFDMIIYDFTHYWLPSPSIAASLGISR
SEQ ID NO:12  PEDIQYLKXAVDGLQPEVTRFLEQHSFDMIIYDFTHYWLPSPSIAASLGISR
SEQ ID NO: 5  PEDIQYLKXAVDGLQPEVTRFLEQHSFDMIIYDFTHYWLPSPSIAASLGISR
SEQ ID NO:10  PEDIQYLKXAVDGLQPEVTRFLEQHSFDMIIYDFTHYWLPSPSIAASLGISR
                *****

SEQ ID NO:14  AYFCVITPWTIAYIAPSSDAMINDSGRTTVEDLTTPPKWPEPPTKVCWR
SEQ ID NO:16  AYFCVITPWTIAYIAPSSDAMINDSGRTTVEDLTTPPKWPEPPTKVCWR
SEQ ID NO:12  AYFCVITPWTIAYIAPSSDAMINDSGRTTVEDLTTPPKWPEPPTKVCWR
SEQ ID NO: 5  AHFSVITPWTIAYIAPSSDAMINDSGRTTVEDLTTPPKWPEPPTKVCWR
SEQ ID NO:10  AHFSVITPWTIAYIAPSSDAMINDSGRTTVEDLTTPPKWPEPPTKVCWR
                *****

SEQ ID NO:14  KHDLARMEPYEAPGISDGYRMGMVFKGSDCLLFCYHEFGTQWLPLETL
SEQ ID NO:16  KHDLARMEPYEAPGISDGYRMGMVFKGSDCLLFCYHEFGTQWLPLETL
SEQ ID NO:12  KHDLARMEPYEAPGISDGYRMGMVFKGSDCLLFCYHEFGTQWLPLETL
SEQ ID NO: 5  KHDLARIVFKAPGISDGYRMGLVFKGSDCLLFCYHEFGTQWLPLETL
SEQ ID NO:10  KHDLARIVFKAPGISDGYRMGLVFKGSDCLLFCYHEFGTQWLPLETL
                *****;*****;*****

SEQ ID NO:14  HQVPVVVVGLLPELPGDEKDETWVSLKNWLDGKQKGVVYVALGSSEALV

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FIGURE 8

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SEQ ID NO:16 HQVVPVVGILLPPEIFGDEKDETWVS IKKWL D GKSGSVVYVVGSEALV
SEQ ID NO:12 HQVVPVVGILLPPEIFGDEKDETWVS IKKWL D GKSGSVVYVVGSEALV
SEQ ID NO: 5 HQVVPVVGILLPPEIFGDEKDETWVS IKKWL D GKSGSVVYVVGSEALV
SEQ ID NO:10 HQVVPVVGILLPPEIFGDEKDETWVS IKKWL D GKSGSVVYVVGSEALV
*****;*****
SEQ ID NO:14 SQTEVVELALGIELSGLPFVWAYRKP KPAKSDSVL PDGFVERTDRGL
SEQ ID NO:16 SQTEVVELALGIELSGLPFVWAYRKP KPAKSDSVL PDGFVERTDRGL
SEQ ID NO:12 SQTEVVELALGIELSGLPFVWAYRKP KPAKSDSVL PDGFVERTDRGL
SEQ ID NO: 5 SQTEVVELALGIELSGLPFVWAYRKP KPAKSDSVL PDGFVERTDRGL
SEQ ID NO:10 SQTEVVELALGIELSGLPFVWAYRKP KPAKSDSVL PDGFVERTDRGL
*****
SEQ ID NO:14 VWTSWAPQLRIILSHESVCGFLTHCGSGSIVEGLMFHGLIMLPITCDQPL
SEQ ID NO:16 VWTSWAPQLRIILSHESVCGFLTHCGSGSIVEGLMFHGLIMLPITCDQPL
SEQ ID NO:12 VWTSWAPQLRIILSHESVCGFLTHCGSGSIVEGLMFHGLIMLPITCDQPL
SEQ ID NO: 5 VWTSWAPQLRIILSHESVCGFLTHCGSGSIVEGLMFHGLIMLPITCDQPL
SEQ ID NO:10 VWTSWAPQLRIILSHESVCGFLTHCGSGSIVEGLMFHGLIMLPITCDQPL
*****;*****
SEQ ID NO:14 NARLLEDKQVGIETIPRNEEDGCLTKESVARSLRSVVVNEGEIYKANARA
SEQ ID NO:16 NARLLEDKQVGIETIPRNEEDGCLTKESVARSLRSVVVNEGEIYKANARA
SEQ ID NO:12 NARLLEDKQVGIETIPRNEEDGCLTKESVARSLRSVVVNEGEIYKANARE
SEQ ID NO: 5 NARLLEDKQVGIETIPRNEEDGCLTKESVARSLRSVVVNEGEIYKANARE
SEQ ID NO:10 NARLLEDKQVGIETIPRNEEDGCLTKESVARSLRSVVVNEGEIYKANARE
*****;*****
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SEQ ID NO:16 LSKIIYNDTKVEKEYVSQFVDYLEKNARAVAI D HES
SEQ ID NO:12 LSKIIYNDTKVEKEYVSQFVDYLEKNARAVAI D HES
SEQ ID NO: 5 LSKIIYNDTKVEKEYVSQFVDYLEKNARAVAI D HES
SEQ ID NO:10 LSKIIYNDTKVEKEYVSQFVDYLEKNARAVAI D HES
*****;*****
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CLUSTAL W (1.82) multiple sequence alignment

FIGURE 8 (CONT.)

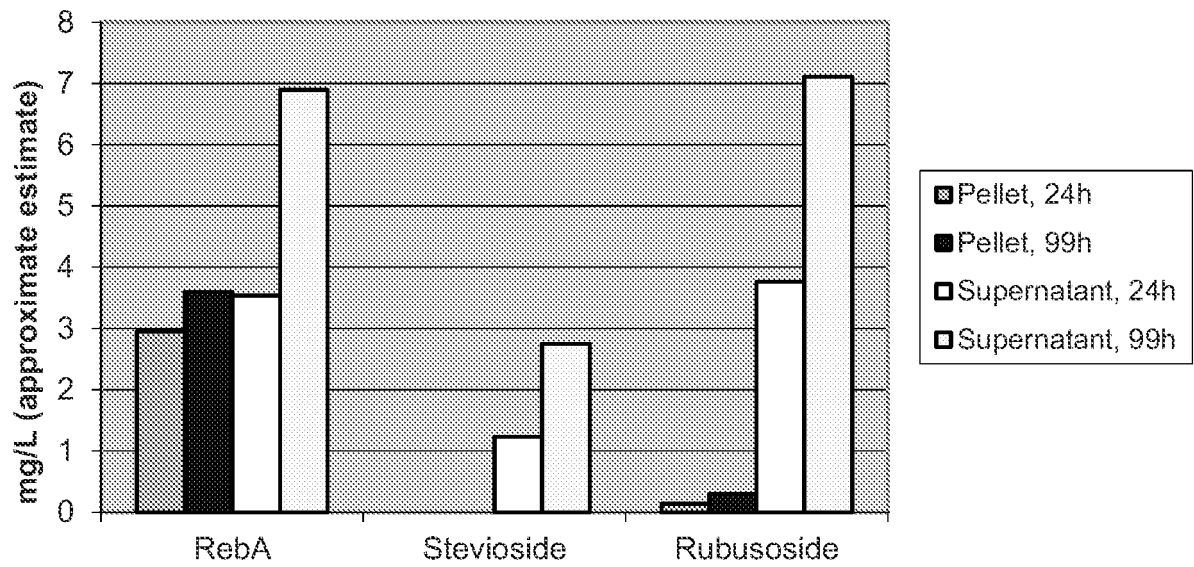


FIGURE 9

FIGURE 10A

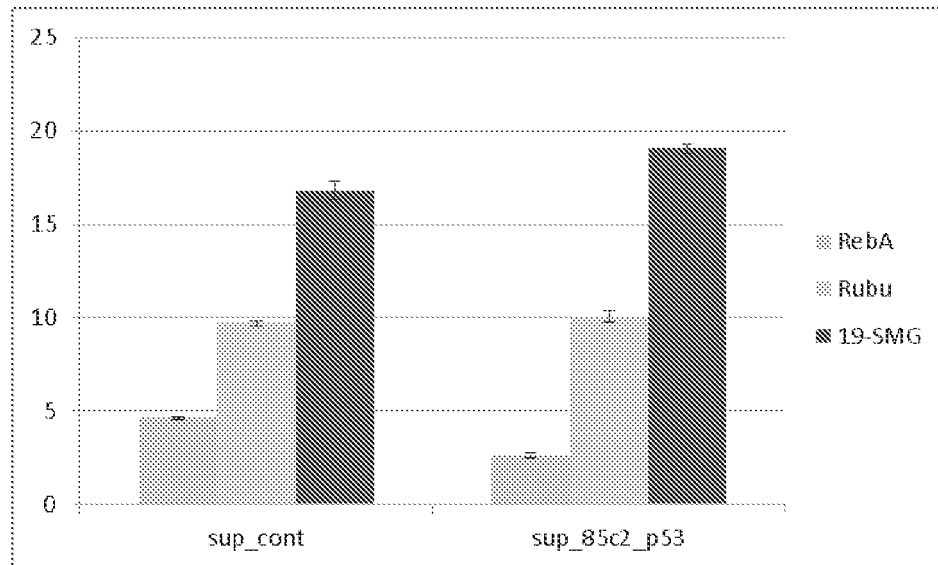


FIGURE 10B

