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Supporting Information

Identification and in situ removal of inhibitory intermediate to develop an efficient phytosterol bioconversion process using a cyclodextrin-resting cells system

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Table S1 Detailed parameters of the macroporous adsorbent resin used.

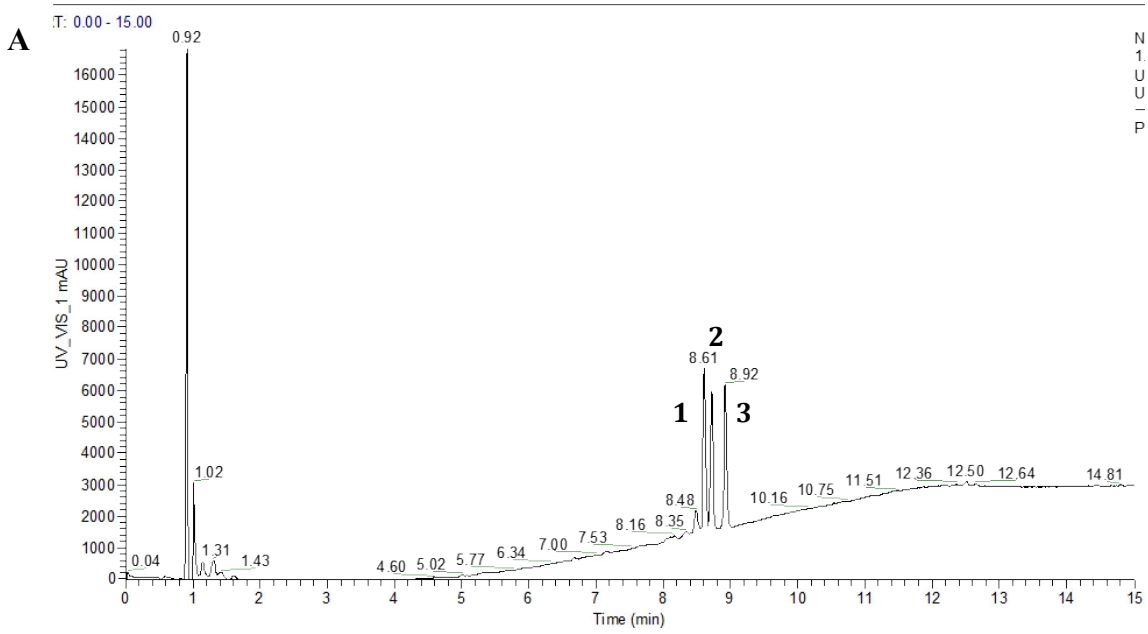
Resin	Manufacturers	Polarity	Specific surface area (m^2/g)	Pore diameter (nm)	Particle size (μm)
D 101	Haiguang Chemical Co., Ltd	Non-polar	480-520	9-10	300-1250
SP 207	Mitsubishi Chemical Industries Limited	Non-polar	900-950	4-5	250-1000
HZ 820	Huazhen Technology Co., Ltd	Non-polar	950-1000	13-15	380-1200
HZ 818	Huazhen Technology Co., Ltd	Non-polar	880-920	7-8	315-1250
HZ 806	Huazhen Technology Co., Ltd	Middle-polar	450-550	6-7	315-1250
HZ 801	Huazhen Technology Co., Ltd	Non-polar	500-550	12-14	315-1250
HPD 826	Baoen Chemical Co., Ltd	Non-polar	485-530	13-14	300-1250
HPD 500	Baoen Chemical Co., Ltd	Polar	500-550	5-6	300-1200
HPD 300	Baoen Chemical Co., Ltd	Non-polar	800-870	5-7	300-1200
HPD 100	Baoen Chemical Co., Ltd	Non-polar	650-700	8-9	300-1250

Note: All adsorbents are polystyrene-divinylbenzene type resins. The parameters depicted in the table were obtained from the manufacturer.

Phytosterols (the main components are campesterol, stigmasterol, β-sitosterol) were used as substrates to obtain steroid intermediates catalyzed by *Mycobacterium neoaurum* NwIB-HK86. The separation and purification of the metabolic intermediates adsorbed in resins was performed by silica gel column chromatography. To prepare the silica gel column, 30 g of silica gel (200-300 mesh) was weighed and mixed in hexane and then packed in a glass column (I.D.21 mm × H200 mm). Subsequently, silica gel with sample were loaded on the column. The intermediates were desorbed by an eluant consisting of hexane and ethyl acetate (hexane: ethyl acetate=5:1, v/v) at a flow rate of 80 mL/h. The eluant was collected per 20 mL. After separation, the samples of the same products identified by TLC were collected together and then dried with a rotary evaporator.

5 μL of samples were applied to silica gel plate (MERCK TLC Silica gel 60 F_{254}, Germany), developed in n-hexane/ethyl acetate/chloroform (5:5:2, v/v/v). The visualization was performed under 254 nm UV light and 20% sulfuric acid solution (115 °C for 15 minutes), respectively.

Multiple types of metabolites produced during bioconversion were adsorbed by the resins. The putative inhibitor was identified using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, Thermo Scientific *Q-Exactive plus, USA) to determine the molecular weight and fragmentation pattern of these compounds. Masses were recorded in positive mode in the range of 50-6000 m/z. A Hypersil Gold C18 (2.1 mm× 100 mm) column was used and methanol-water (70% methanol at 0–1 min, linear gradient from 70–100% methanol in 20 min, keep 100% methanol at 20-25 min, and then linear gradient from 100–70% methanol in 3 min, keep for 3 min to equilibrate the column) was used as the mobile phase at a flow rate of 1 mL/min and UV detector at 254 nm.


A
T: 0.00 - 15.00
UV_VIS_1 mAU
Time (min)
0.92
1.02
1.31
1.43
0.04
4.60
5.02
5.77
6.34
7.00
7.53
8.16
8.35
8.48
8.61
8.92
10.16
10.75
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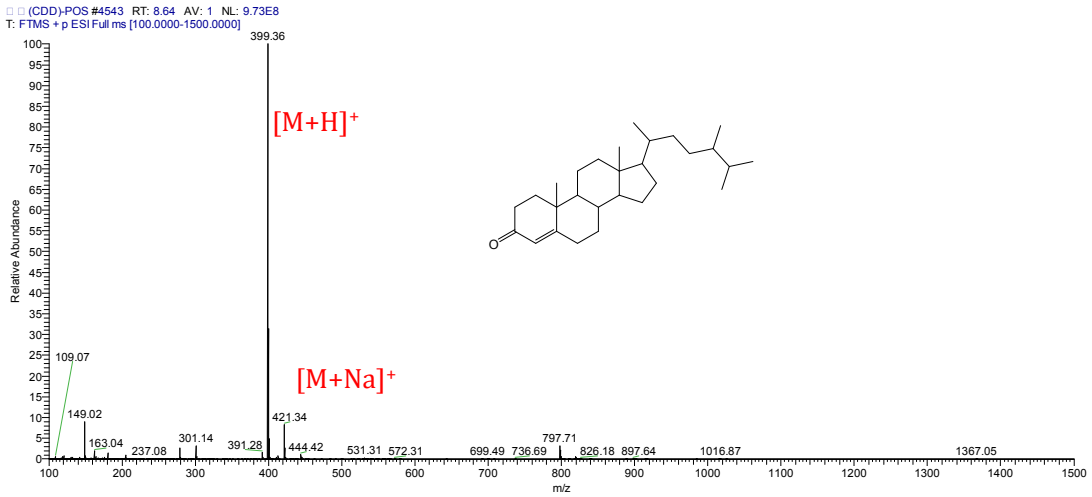
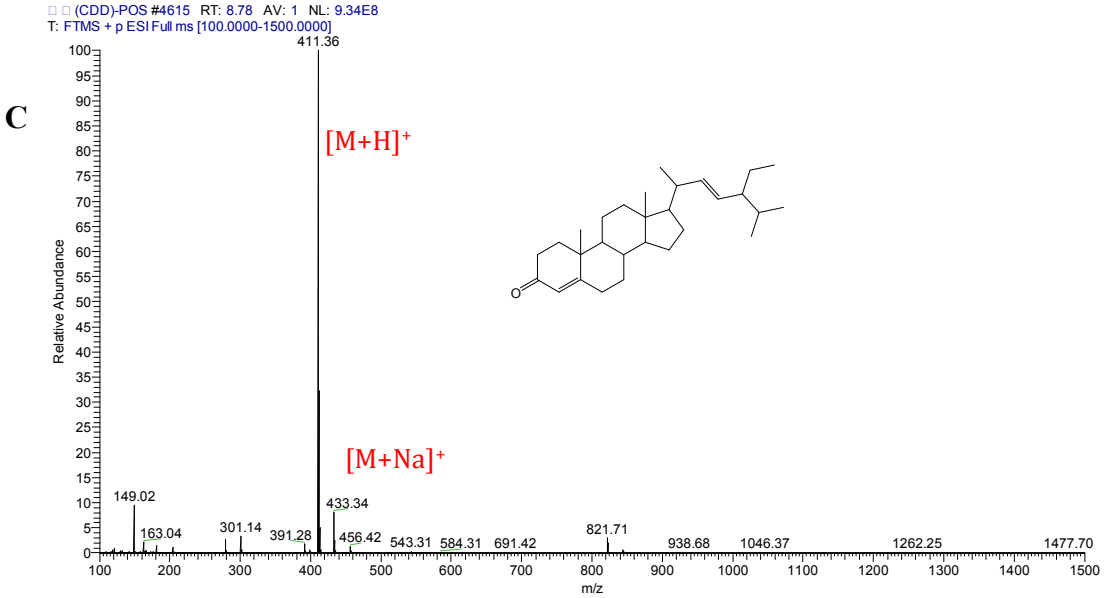
12.36
12.50
12.64
14.81
1
2
3


B
(CDD)-POS #4543 RT: 8.64 AV: 1 NL: 9.73E8
T: FTMS + p ESI Full ms [100.0000-1500.0000]
Relative Abundance
m/z
399.36
[M+H]+
[M+Na]+
109.07
149.02
163.04
237.08
301.14
391.28
421.34
444.42
531.31
572.31
699.49
736.69
797.71
826.18
897.64
1016.87
1367.05


C
(CDD)-POS #4615 RT: 8.78 AV: 1 NL: 9.34E8
T: FTMS + p ESI Full ms [100.0000-1500.0000]
Relative Abundance
m/z
411.36
[M+H]+
[M+Na]+
149.02
163.04
301.14
391.28
433.34
456.42
543.31
584.31
691.42
821.71
938.68
1046.37
1262.25
1477.70

D

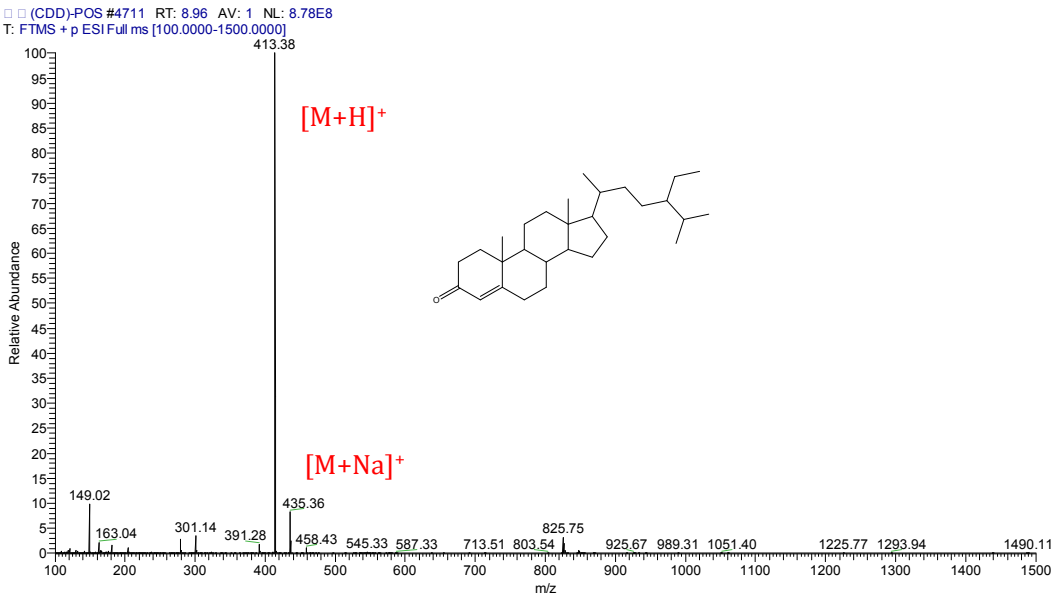


Figure S1. Identification of putative inhibitory intermediates by UPLC-MS/MS. A, UPLC profiles of 4-ene-3-keto steroids; B, MS of 4-ene-3-keto steroids produced from campesterol, corresponding to peak 1 in the UPLC profile; C, MS of 4-ene-3-keto steroids produced from stigmasterol, corresponding to peak 2 in the UPLC profile; D, MS of 4-ene-3-keto steroids produced from β-sitosterol, corresponding to peak 3 in the UPLC profile.

Quantitative analysis of 4-ene-3-keto steroids was performed by HPLC equipped with an Agilent ZORBAX 300SB-C8 (4.6 mm× 250 mm) column. Methanol-water (80:20, v/v) was used as the mobile phase at a flow rate of 1 mL/min and UV detector at 254 nm. 4-ene-3-keto steroids content in 12 h samples of the control group, which total peak areas was 298.48, was set to a relative concentration of 1 in the manuscript (the relative content of 4-ene-3-keto steroids can be calculated according to the following formula: $Relative\ content\ of\ 4\text{-}ene\text{-}3\text{-}keto\ steroids = {Area(4\text{-}ene\text{-}3\text{-}keto\ steroids)}/{298.48}$.

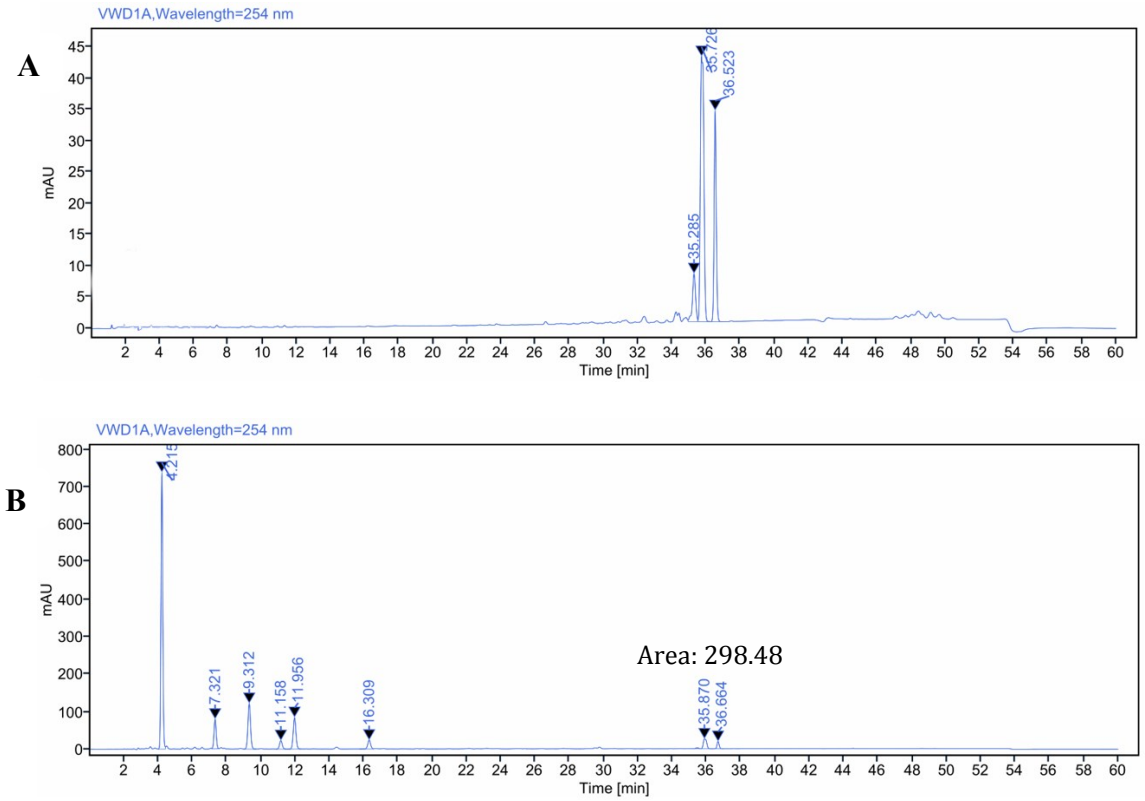


Figure S2. HPLC profiles of 4-ene-3-keto steroids. A, 4-ene-3-keto steroids obtained by silica gel column chromatography; B, 4-ene-3-keto steroids content in 12 h samples of the control group.