Electronic Supplementary Material (ESI) for Food & Function.

In vivo absorption and excretion in rats and *in vitro* digestion and fermentation by human intestinal microbiota of 2-*O*-β-D-glucopyranosyl-L-ascorbic acid from the fruits of *Lycium barbarum* L.

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Supplementary materials and methods

The preparation of AA-2βG was carried out according to the reported method with some modifications, and the dried fruits of *Lyciun barbarum* (variety, Ningnonggouqi No.7) were obtained from the National Wolfberry Engineering Research Center (Yinchuan, China). Briefly, the dried fruits were ground into powder by using a grinder and extracted 2 times with 30% (v/v) aqueous ethanol solution in a ratio of material/solvent of 1:10 (w/v) at 70 ℃ for 1 h. The resulting extracts were concentrated, and the residue was dissolved with distilled water and loaded onto a column (5 × 30 cm) of Dowex 1-X8 anion exchange resin (Sigma Chemical Co., St. Louis, MO, USA). The column was washed with distilled water, 0.1 M acetic acid and subsequently eluted with elution of 0.4 M acetic acid solution. The eluent was monitored at 280 nm and collected (10 mL/tube) with an automatic fraction collector (Baixian BS-100A, Shanghai, China). The fractions of tubes 60-95 were combined, concentrated and further purified by semi-preparative chromatography with a YMC-Pack ODS-A C18 column (20 × 250 mm, 5 μm, YMC Co., Ltd., Kyoto, Japan). The purification process was performed on an AKTA purifier system equipped with UV detector and fraction collector. The conditions were as follows: mobile phase, 5% MeOH/0.4 M acetic acid; flow rate, 0.8 mL/min; detector wavelength, 254 nm. The fractions with absorbance at 254 nm were analyzed by HPLC, and the fractions with same chromatographic peak were combined, concentrated under reduced pressure and lyophilized, affording the desired product. The resulting product was analyzed by Agilent 1100 series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with diode array detector

(DAD), matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and nuclear magnetic resonance (NMR) spectrometry (^{1}H- and ^{13}C-NMR).

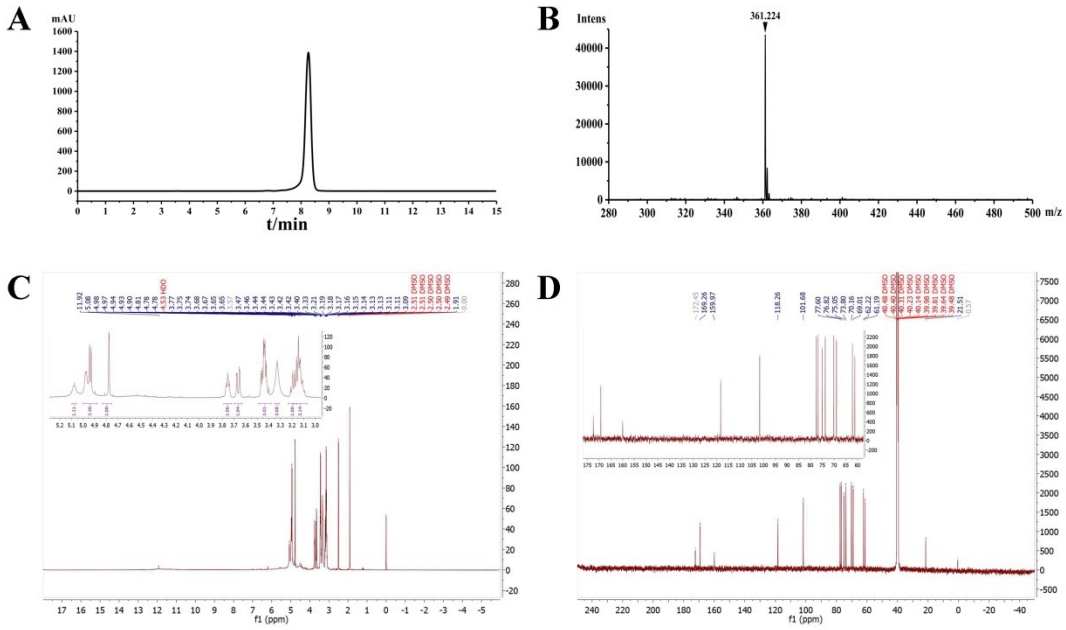

Figure S1. Extraction, purification and structure identification of AA-2βG from the fruits of *Lycium barbarum*. HPLC chromatogram (**A**) Mass spectrum (**B**) ^{1}H NMR (**C**) and ^{13}C NMR (**D**) spectra of the purified fraction (AA-2βG).

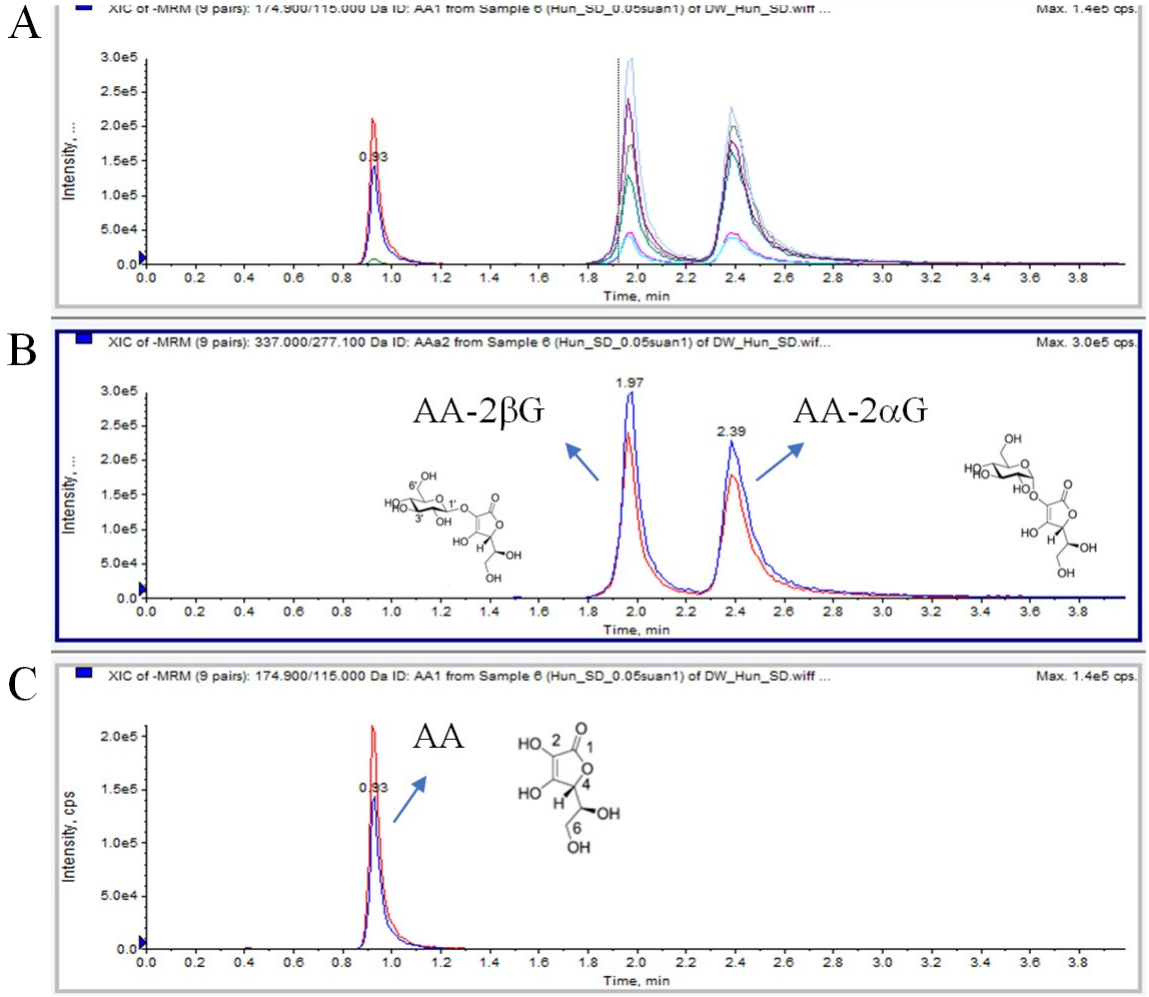

Figure S2. (A) Typical UPLC-MS/MS of AA, AA-2αG, and AA-2βG; (B) UPLC-MS/MS extracted ion chromatograms for m/z 277.100; (C) UPLC-MS/MS extracted ion chromatograms for m/z 174.900.

Table S1. The regression equations, linearity, LOD, and LOQ for the determination of AA-2βG.

Matrix	Linearity	R^2	Range (ng/mL)	LOD (ng/mL)
Plasma	y = 2522.4x-47107	0.9947	14.23-1000	4.70
Urine	y = 2921.7x-55635	0.9948	21.13-1000	6.97
Feces	y = 2745.4x+6541.4	0.9989	27.23-1000	8.98

Table S2. Intra- and inter-day precision and recovery of AA-2βG in rat plasma, urine and feces.

Analyte	Concentration	Recovery (%)	Intra-day Precision (RSD, %)	Inter-day Precision (RSD, %)
Plasma (ng/mL)	50	108.7 ± 5.8	1.8	10.8
	250	93.2 ± 5.9	8.1	4.8
	750	87.8 ± 7.1	9.3	3.6
Urine (ng/mL)	50	92.7 ± 8.8	6.6	11.3
	250	95.8 ± 7.6	6.8	10.2
	750	90.6 ± 1.2	4.7	7.5
Feces (ng/mg)	50	79.3 ± 6.6	10.5	6.0
	250	95.8 ± 3.9	4.9	2.3
	750	88.5 ± 6.3	11.1	5.6

Table S3. The fecal and urine excretion of AA-2βG in rats after oral administration at a dose of 100 mg/kg.

Time (h)	AA-2βG in feces (ng/mg)	AA-2βG in urine (mM)
0-4 h	22.75 ± 15.29	13.44 ± 6.29
4-8 h	30.41 ± 21.84	23.35 ± 13.89
8-12 h	23.51 ± 14.46	6.04 ± 3.58
12-24 h	7.22 ± 3.41	4.08 ± 4.15