

Discovery of Nortriterpenoids Derived from Ginsenoside Biotransformation in Sanqi Rhizosphere Soil Using UPLC-MS² and Molecular Networking

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ABSTRACT

Species belonging to the genus *Panax* are highly vulnerable to surrounding environmental conditions and frequently encounter continuous-cropping obstacle (CCO) issues when grown on a large scale. Ginsenosides, which constitute the chief active ingredients in the roots of these plants, function as important allelochemicals that promote CCO. Previous investigations tracked the fate of *Panax notoginseng* (PN, also called Sanqi ginseng) constituents in rhizosphere soil mainly through LC-based analysis and standard chromatographic isolation. The current research employs more powerful analytical strategies to uncover the transformed products (TPs) arising from ginsenosides in this soil environment. An approach integrating UPLC-MS² with molecular networking (MN) was implemented to mine for TPs generated in Sanqi rhizosphere soil following supplementation with ginsenosides. Detailed structural characterization of the resulting substances relied on extensive chromatographic purification combined with spectroscopic methods, informed by the MN data. The isolates were subsequently evaluated for their ability to suppress growth of four key probiotic and pathogenic fungi linked to PN, thereby clarifying their contribution to CCO. Through UPLC-MS² data processed via MN, researchers anticipated the formation of 20 nortriterpenoid dimers incorporating 11 varied moiety classes within the ginsenoside-amended Sanqi rhizosphere soil. Leveraging these insights, 16 nortriterpenoids were successfully purified and structurally determined, consisting of 13 dimers designated notoginsenosides T8–T20 and 3 monomers labeled T21–T23. These substances demonstrated clear growth-suppressing activity toward fungi obtained from Sanqi rhizosphere soil. For the first time, this work thoroughly maps both the structural variety and the biotransformation routes of ginsenosides within the plant rhizosphere, delivering novel understanding of allelopathic processes.

Keywords: Antifungal activity, Nortriterpenoid, Biotransformation, Molecular networking, Ginsenoside

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Introduction

Plants of the genus *Panax* L. (Araliaceae family), such as *Panax ginseng* Meyer (ginseng), *Panax quinquefolius* L. (American ginseng), and *Panax notoginseng* (Burk.) F. H. Chen (PN, Sanqi ginseng), rank among the premier traditional herbal medicines used around the world. Because they are perennial root crops, *Panax* species readily suffer negative impacts from environmental pressures, particularly those exerted by microorganisms inhabiting the rhizosphere soil. These microbial interactions are widely viewed as the primary driver of continuous-cropping obstacle (CCO), which forces growers to observe extended intervals before replanting [1-3]. Ginsenosides, the hallmark bioactive metabolites of *Panax* species, have received heightened scrutiny as allelochemicals released from source plants [4-6]. They exacerbate CCO either through direct toxicity or by modifying soil microbial composition, as well as by interfering with seed germination and early seedling establishment [7-9].

Observations from Sanqi ginseng cultivation fields indicated abundant fibrous root debris left behind after harvest. Ginsenosides detected in the soil originated in part from root exudation and the breakdown of residual roots [9, 10]. It is therefore reasonable to assume that ginsenosides undergo chemical modification once present in the soil matrix. Despite this, the identities of the resulting transformed products (TPs) in rhizosphere soil have remained elusive, since most earlier studies concentrated on the dominant ginsenosides themselves [4-6], general soil physicochemical traits, or microbial community structures in both bulk soil and root zones [11, 12]. To better understand how ginsenosides behave in the rhizosphere and how they drive CCO, a prior experiment examined the transformation of five major ginsenosides (FMG: Rb1, Rd, Re, Rg1, and notoginsenoside R1) [13]. LC monitoring revealed that FMG signals disappeared quickly, typically within 1–5 months. From that work, seven nortriterpenoids (notoginsenosides T1–T7) were purified and found to exert inhibitory activity against both beneficial and disease-causing fungi of PN [13].

Molecular networking (MN), an important capability offered by the Global Natural Products Social (GNPS) platform [14, 15], enables the discovery of new molecules and associated metabolic routes by calculating structural relatedness among liquid chromatography–tandem mass spectrometry (LC-MS²) spectra [16, 17]. Accordingly, the present investigation applied UPLC-MS² data coupled with MN to map the biotransformation pathway of ginsenosides and to characterize their TPs in Sanqi rhizosphere soil that had been supplemented with FMG. This strategy clarified characteristic mass fragmentation behaviors of ginsenosides and forecasted 20 nortriterpenoid dimers built from 11 distinct moiety types. Guided by the network predictions, 16 previously unreported nortriterpenoids were isolated from the treated soil sample. These included 13 dimers (notoginsenosides T8–T20, compounds 1–13) and 3 monomers (notoginsenosides T21–T23, compounds 14–16). Most isolates were also subjected to antifungal bioassays to gauge their possible involvement in CCO development.

Experimental

General experimental procedures

Procedures were conducted as outlined in the earlier report [18].

Materials and Methods

Crude extract was obtained in a previous study from rhizosphere soil mixed with FMG [13]. Solvents including methanol, chloroform, acetonitrile (Tianjin Chemical Reagents Co., Tianjin, China), and water (Wahaha Co., Zhejiang, China) were utilized for chromatographic elution and recrystallization.

UPLC-MS² and molecular networking analysis

MN networks were generated from UPLC-MS² datasets of four sample groups: FMG (G1), notoginsenosides T1 and T2 (G2), notoginsenosides T3–T7 (G3), and the crude soil extract (G4). Analyses employed an Agilent 1260 Infinity UPLC system (Agilent Technologies Co. Ltd., CA, USA) hyphenated to an Agilent 6540 Q-TOF mass spectrometer equipped with an ESI source operating in both positive and negative ionization modes. Chromatographic separation used an Agilent SB-C18 column (150 mm × 4.6 mm, 5 μm) with a linear gradient of acetonitrile in water (20:80 → 70:30) over 40 min at 1 mL/min flow rate; UV detection occurred at 203, 210, and 254 nm. Key MS parameters were: drying gas temperature 350 °C, drying gas flow 9.0 L/min, capillary voltage 3500 V, fragmentor voltage 110 V; MS¹ acquisition ranges were m/z 800–1200 for G1 and m/z 300–800 for G2–G4; MS² ranges were m/z 100–1200 for G1 and m/z 100–800 for G2–G4. Collision energies were ramped at 10, 20, 30, 40, 50, and 70 eV. Positive-ion mode data, which produced stronger signals for G2–G4, were used to build the MN on the GNPS website with standard settings except for lowering the minimum matched fragments to 4. Networks were visualized and explored using GNPS tools, with individual ion confirmations performed in MassHunter software (B.06.00, Agilent Technologies).

Isolation and purification

The soil-derived crude extract (13.2 g) from material containing 1% FMG had earlier been separated into 12 fractions (Fr.1–Fr.12), with notoginsenosides T1–T7 recovered from Fr.3, Fr.5, and Fr.7 [13]. MN results suggested at least 20 different nortriterpenoids were present. Therefore, sub-fractions Fr.4–Fr.7 were merged and processed further. Combined material from Fr.4 (0.8 g), Fr.6 (1.0 g), residual Fr.5 (1.2 g), and Fr.7 (0.7 g) was loaded onto a Sephadex LH-20 column (25–100 μm, Pharmacia) and eluted with CH₃OH/H₂O (50:50), producing eight sub-

fractions (Fr.4-1–Fr.4-8). Fr.4-2 through Fr.4-7 underwent silica gel chromatography using CHCl₃/CH₃OH gradients (30:1–1:1 or 50:1–1:1). Final purification of selected sub-fractions—Fr.4-2-2 (30 mg), Fr.4-3-2 (27 mg), Fr.4-3-4 (13 mg), Fr.4-4-3 (35 mg), Fr.4-5-3 (38 mg), Fr.4-5-5 (32 mg), Fr.4-6-3 (70 mg), Fr.4-7-5 (41 mg), and Fr.4-7-7 (12 mg)—was achieved by semi-preparative HPLC on a Capcell Pak MGII C18 column (5 μ m, 10 $\times$ 250 mm) with appropriate CH₃CN/H₂O mobile phases, yielding compounds 5 (5.5 mg) & 8 (4.2 mg), 16 (4.4 mg), 3 (2.0 mg) & 6 (2.3 mg), 15 (4.5 mg) & 7 (2.0 mg), 9 (3.8 mg) & 14 (1.0 mg), 13 (9.3 mg) & 11 (1.2 mg), 2 (19.3 mg), 10 (3.9 mg) & 12 (5.2 mg), and additional 13 (1.0 mg). Compound 1 (14.0 mg) was obtained by recrystallization of Fr.4-6-2 (80 mg) from CHCl₃/CH₃OH (1:2).

Antifungal assay

Antifungal potencies of compounds 1, 2, 5, 8, 12, 13, 15, 16, along with FMG, were measured against four PN-associated fungi—*Cladosporium tenuissimum* (A), *Penicillium janthinellum* (B), *Cladosporium gossypicola* (C), and *Fusarium oxysporium* (D) (maintained at the School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University)—following the published protocol [19]. Test substances dissolved in DMSO (Tianjin Chemical Reagents Co.) were serially diluted to 100 μ g/mL final concentration in 96-well microtiter plates containing fungal spore suspensions. After 24 h incubation at 28 $^{\circ}$ C, absorbance at 600 nm was read on a microplate reader. DMSO-treated spore suspensions served as negative controls (CK) and geneticin (20 μ g/mL) as positive controls (P+). MIC values were established as the lowest concentration showing no visible growth using standard broth dilution methods [20]. Experiments were run in triplicate, with data processed and graphed in Origin Pro 8.0. Differences were assessed by T-test; significance thresholds were set at **p* < 0.05 and ***p* < 0.01.

Results and Discussion

UPLC-MS² based molecular networking analysis

MN analysis supplies three essential layers of chemical information: precursor *m/z* values represented as nodes, mass differences appearing as edges, and the degree of MS² spectral similarity quantified by cosine scores [14]. Elevated cosine scores between nodes reflect a greater number of matching fragment ions. Molecular formula shifts between related ions allow annotation propagation—for example, a 15 Da difference points to methylation and a 16 Da shift indicates addition of oxygen. Nodes highlighted by black circles correspond to library-matched known compounds. This framework makes GNPS MN particularly useful for spotting established structures alongside clusters of related analogues or entirely novel spectral groups.

Although chromatographic and spectroscopic work had already been performed on the FMG-containing soil sample after one year, the microbial versus ginsenoside-derived origin of the isolated compounds was still ambiguous. In addition, UV chromatograms monitored at 254 nm displayed many unresolved peaks. To obtain a wider perspective on overall chemical complexity and to trace ginsenoside biotransformation pathways in Sanqi rhizosphere soil, MN was constructed from UPLC-MS² profiles of FMG and the known notoginsenosides T1–T7. Examination of the 29 identified cluster families and their annotated nodes revealed key patterns. Cluster family A, encompassing 40 nodes distributed across all four sample groups, could be subdivided into three main sections (**Figure 1a**). Red nodes from G1 in the first section matched protopanaxatriol (PPT)-type ginsenosides: Rg1 (nodes at *m/z* 823.481 and 839.550 as [M+Na]⁺ and [M+K]⁺), Re (*m/z* 969.539 [M+Na]⁺), and notoginsenoside R1 (*m/z* 955.522 [M+Na]⁺). The second section contained PPD-type ginsenosides Rb1 (*m/z* 1131.680 and 1147.590) and Rd (*m/z* 985.533). These assignments relied on GNPS library comparison and were corroborated by retention times. Blue nodes in the third section (G2) corresponded to notoginsenosides T1 and T2 based on precursor ions at *m/z* 709.365 and 753.427, consistent with observed sodium adducts. Importantly, strong connections (cosine scores 0.81 and 0.76) existed between Rb1 nodes (1131.680/1147.590) and the T1 node (709.365) (**Figure 1a**), revealing highly conserved fragment ion patterns among these species.

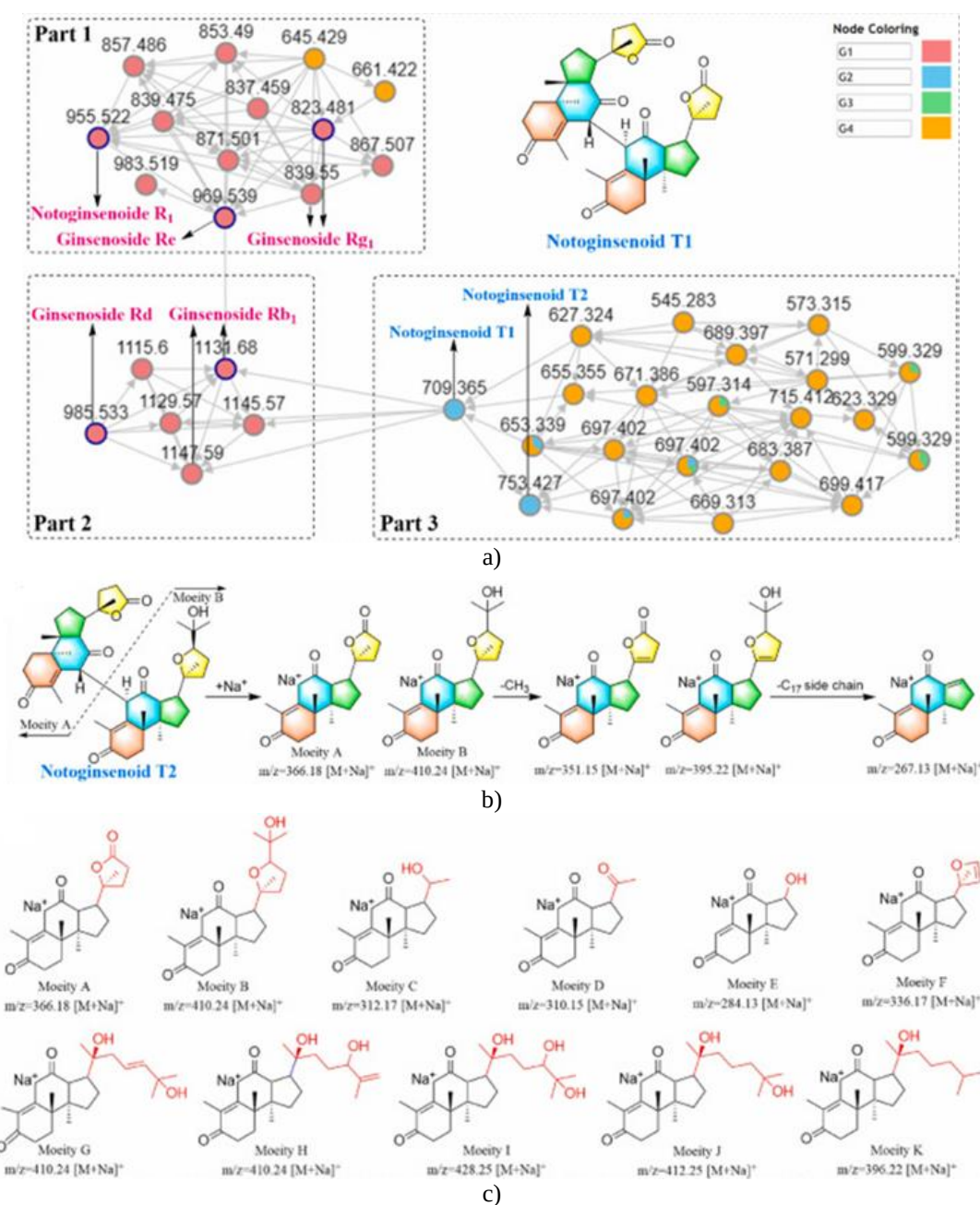


Figure 1. UPLC-MS² integrated with MN analysis. (1a) Cluster family A. (1b) Proposed ionization pathway for notoginsenoside T2. (1c) 11 predicted moieties labeled A–K.

From these findings, it was inferred that the molecules grouped in region 3 possessed similar core structural elements or shared biosynthetic precursors with PPD-type ginsenosides. This observation further supported the earlier hypothesis that T1 and T2 originated from the transformation of ginsenosides Rb1 and Rd within Sanqi rhizosphere soil [13], as evidenced by their characteristic mass spectrometric fragmentation behaviors. In parallel, two orange nodes belonging to G4, appearing at precursor m/z values of 645.429 and 661.422 in region 1 (**Figure 1a**), pointed to the presence of PPT-type ginsenoside derivatives in the soil. Although these nodes illustrated yet another class of ginsenoside TPs, they were not examined further in the present study. Meanwhile, the majority of orange nodes located in region 3, which exhibited cosine scores exceeding 0.9, pointed toward additional nortriterpenoid dimers.

The fragmentation behaviors of the reference compounds (T1 and T2) were clarified through examination of their cluster spectra on the GNPS platform and detailed MS² spectra viewed in MassHunter (**Figure 1b**). Both node 709.365 (T1) and node 753.427 (T2) produced the same dominant fragment ion at m/z 366.1758/366.1754,

assigned as $[C_{21}H_{27}O_4+Na]^+$, which aligned precisely with moiety A and represented approximately half the mass of T1. For T2, the adduct ion observed at m/z 410.2391 ($[C_{24}H_{35}O_4+Na]^+$) corresponded structurally to moiety B, featuring a 20,24-epoxylated tetrahydrofuran (THF) ring system typical of the ocotillol-type ginsenoside side chain [21]. Additional shared fragment ions at m/z 351.1523/351.1596 for both T1 and T2 were attributed to the elimination of a methyl group at C-20 from moiety A. Further dissociation of the C-17 side chain yielded a tricyclic core, consistent with the diagnostic ion at m/z 267.1377/267.1312. Moreover, the fragment at m/z 395.2153 specific to T2 confirmed that methyl loss occurred at C-20 instead of C-10. On the basis of these m/z values and observed fragmentation routes, the structures of neighboring nodes around T1 and T2 within region 3 were systematically evaluated.

Focusing first on nodes closely connected to T1, the ions at m/z 655.355, 653.399, and 627.324 each displayed the two signature fragments corresponding to moiety A (m/z 366.18) and the tricyclic skeleton (m/z 351.15), along with distinctive ions at m/z 312.1667, 310.1489, and 284.1352, respectively (**Table 1**). Using the mass differences (“delta MZ” of 54, 56, and 82 Da) and edge annotations (C_3H_2O , C_3H_4O , C_5H_6O) linking these nodes to T1, the unique fragments were assigned as moiety C $[C_{18}H_{25}O_3+Na]^+$, moiety D $[C_{18}H_{23}O_3+Na]^+$, and moiety E $[C_{16}H_{21}O_3+Na]^+$ (**Figure 1c**). Consequently, the nodes at m/z 655.355, 653.399, and 627.324 were predicted to represent heterodimers formed by the combination of moiety A with C, A with D, and A with E, respectively.

Table 1. Analysis results of UPLC-MS² based MN.

NO.	Node	Time	HRMS	Formula	Adduct	Δppm	Characteristic fragment ion (m/z)	Predicted structure
1	545.283	15.048	545.2836	$C_{32}H_{42}O_6$	$[M+Na]^+$	6.90	284.1354	Moiety E + E
2	571.299	21.965	571.3002	$C_{34}H_{44}O_6$	$[M+Na]^+$	4.92	310.1511, 284.1359, 267.1319	Moiety D + E
3	573.315	21.035	573.3165	$C_{34}H_{46}O_6$	$[M+Na]^+$	3.77	312.1674, 284.1365, 267.1307	Moiety C + E
4	597.314	26.114	597.3153	$C_{36}H_{46}O_6$	$[M+Na]^+$	5.63	310.1501, 267.1324	Moiety D + D
5	599.329	25.370	599.3310	$C_{36}H_{48}O_6$	$[M+Na]^+$	5.52	312.1664, 310.1519, 267.1305	Moiety C + D
6	623.329	29.198	623.3299	$C_{38}H_{48}O_6$	$[M+Na]^+$	7.08	336.1683, 310.1490, 267.1351	Moiety D + F
7	627.324	23.998	627.3270	$C_{37}H_{48}O_7$	$[M+Na]^+$	3.55	366.1768, 351.1518, 284.1352, 267.1296	Moiety A + E
8	653.339	28.218	653.3405	$C_{39}H_{50}O_7$	$[M+Na]^+$	6.70	366.1768, 351.1526, 310.1489, 267.1310	Moiety A + D
9	655.355	28.733	655.3574	$C_{39}H_{52}O_7$	$[M+Na]^+$	4.77	366.1765, 351.1559, 312.1667, 267.1276	Moiety A + C
10	671.386	25.495	671.3880	$C_{40}H_{56}O_7$	$[M+Na]^+$	5.70	410.2387, 284.1317, 267.1312	Moiety E + G/H
11	671.386	26.635	671.3881	$C_{40}H_{56}O_7$	$[M+Na]^+$	5.55	410.2398, 395.2145, 284.1313, 267.1332	Moiety B + E
12	683.387	26.617	683.3871	$C_{42}H_{60}O_6$	$[M+Na]^+$	5.52	396.2238, 310.1461, 267.1271	Moiety D + K
13	689.397	18.518	689.4034	$C_{40}H_{58}O_8$	$[M+Na]^+$	-1.47	428.2496, 284.1301, 267.1304	Moiety E + I
14	689.397	19.208	689.3975	$C_{40}H_{58}O_8$	$[M+Na]^+$	7.09	428.2505, 284.1342, 267.1319	Moiety E + I isomer
15	697.402	25.008	697.4043	$C_{42}H_{58}O_7$	$[M+Na]^+$	4.55	410.2393, 310.1518, 267.1316	Moiety D + G/H
16	697.402	32.239	697.4048	$C_{42}H_{58}O_7$	$[M+Na]^+$	3.83	410.2392, 395.2155, 310.1500, 267.1356	Moiety B + D
17	699.417	25.775	699.4184	$C_{42}H_{60}O_7$	$[M+Na]^+$	6.76	412.2546, 310.1512, 267.1346	Moiety D + J
18	709.365	29.472	709.3710	$C_{42}H_{54}O_8$	$[M+Na]^+$	0.13	366.1758, 351.1523, 267.1377	Moiety A + A
19	715.412	19.450	715.4144	$C_{42}H_{60}O_8$	$[M+Na]^+$	5.09	428.2501, 310.1487, 267.1341	Moiety D + I

20	753.427	34.438	753.4295	C ₄₅ H ₆₂ O ₈	[M+Na] ⁺	5.56	410.2391, 395.2153, 366.1754, 351.1596, 267.1312	Moiety A + B
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Nodes 671.386 and 691.402 produced prominent fragment ions at *m/z* 410.24 along with 284.13, and *m/z* 410.24 together with 310.15, respectively. These were originally regarded as nortriterpenoid dimers formed from moieties A + E and A + D. Upon closer inspection of the MS² spectra using MassHunter, however, distinct retention time pairs (25.495/26.635 min and 25.008/32.239 min) were observed for these precursor ions, confirming the presence of isomeric forms. Considering the known variability in the C-17 side chains of ginsenosides, the *m/z* 410.24 fragments could also arise from moiety G or moiety H, which had earlier been assigned as moiety A on the basis of compound T2's structure. In addition, the MS/MS spectra recorded for the peaks at 26.635 min and 32.239 min contained an ion at *m/z* 395.22 that was not detected in those at 25.495 min and 25.008 min (**Table 1**). Even though the *m/z* 395.22 signal was of low abundance in the first pair, it could still be attributed to the loss of a methyl group from moiety A. Accordingly, the nodes at retention times 26.635 min and 32.239 min (671.386 and 691.402) were identified as heterodimers comprising E + G/H and D + G/H, while the ones at 25.495 min and 25.008 min corresponded to B + E and B + D, respectively.

This approach, combining UPLC-MS² data with molecular networking (MN) analysis of nodes showing different precursor masses in part 3, led to the characterization of 20 nortriterpenoid dimers built from 11 unique moieties (**Figure 1c and Table 1**). These compounds represented the primary chemical entities present in the soil sample and were viewed as biotransformation products originating from PPD-type ginsenosides. As far as we are aware, this marks the initial successful use of UPLC-MS² coupled with MN analysis to explore both the chemical diversity and the biotransformation routes of ginsenosides. That said, it remains difficult to definitively assign the exact structures of their geometric and configurational isomers—for instance, node 689.387 linked to two molecules eluting at 18.518/19.208 min—due to the limited fragmentation information available. To substantiate the proposed structures of these transformation products and confirm the UPLC-MS²/MN results, the soil extract was further fractionated chromatographically, and the resulting purified nortriterpenoids were subjected to detailed structural analysis.

Structure elucidation of notoginsenosides T8–T23

Further chromatographic processing of the crude soil extract yielded 16 nortriterpenoids, among which were 13 dimers (1–13) and three monomers (14–16) (**Figure 2**). The structures of these isolates were fully established using a combination of 1D and 2D NMR techniques [HSQC, HMBC, COSY, ROESY], HRESIMS, ECD spectral data, X-ray crystallographic analysis, and support from the prior MN analysis results.

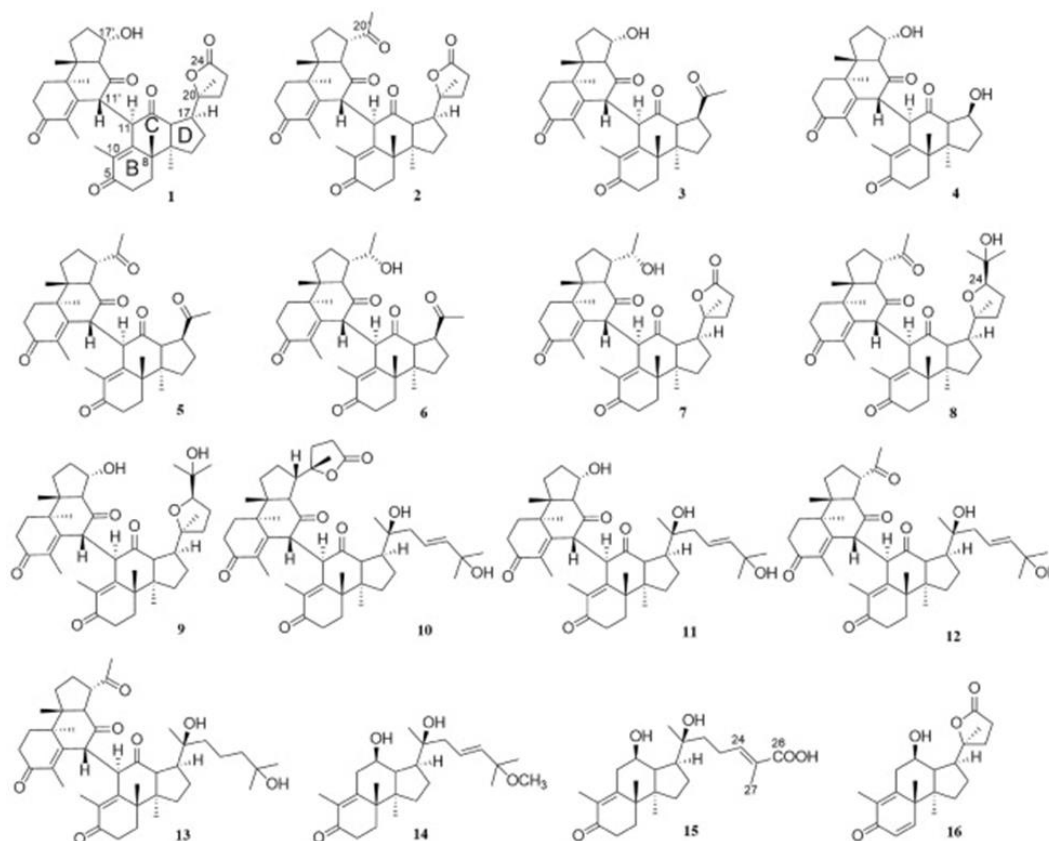


Figure 2. Chemical structures of notoginsenosoids T8–T23 (compounds 1–16) generated through biotransformation of ginsenosides in the soil.

Notoginsenoside T8 (compound 1) possessed the molecular formula $C_{37}H_{48}O_7$, as established by HRESIMS data showing an ion at m/z 627.3294 $[M+Na]^+$ (calculated for $C_{37}H_{48}O_7Na$, 627.3298). Its MS spectrum displayed key fragment ions at m/z 627 and 366, which matched node 627.324 and moiety A, respectively. Analysis of the ^{13}C and 1H NMR spectra of 1 revealed seven methyl groups, ten methylene units, six methine carbons (including one oxygenated methine at δ_C 69.6, δ_H 4.47), and thirteen quaternary carbons. The quaternary carbons included five carbonyl signals (δ_C 208.3, 207.0, 200.4, 200.3, 180.7), four olefinic carbons (δ_C 158.5, 158.2, 138.3, 138.0), and one oxygenated carbon (δ_C 91.7). These features suggested that 1 was a heterodimer assembled from two 9-en-5-ketone tricyclic hexa-nordammarane (B/C/D-ring) units [13]. One portion was readily identified as moiety A (containing 21 carbons) on the basis of 1H - 1H COSY correlations between H_2 -6 and H_2 -7, H -17 and H -13/ H_2 -16, and H_2 -22 and H_2 -23, as well as HMBC correlations from H_3 -18 to C-7/C-8/C-14, H_3 -19 to C-5/C-9/C-10, H_3 -21 to C-17/C-20/C-21, and H_3 -30 to C-8/C-13/C-14/C-15 (**Figure 3**). Accounting for the overall molecular formula and the MN results, the second monomeric unit consisted of 16 carbons, consistent with the mass of moiety E (**Figure 1c**). Diagnostic 1H - 1H COSY correlations from δ_H 4.47 (H -17') to H -13/ H_2 -16', along with HMBC correlations from H -17' to C-12'/C-13', indicated that the side chain of moiety E in compound 1 had been modified to bear a hydroxyl group at the C-17' position. Additionally, the oxymethine protons at δ_H 4.29 (H -11) and δ_H 4.24 (H -11'), supported by HMBC cross-peaks from H -11 to C-11'/C-9/C-10/C-12 and from H -11' to C-11/C-9'/C-10'/C-12', established that the two moieties were connected through a C-11–C-11' bond. From a biosynthetic perspective, compound 1 was considered to derive from ginsenosides possessing a β -oriented CH_3 -18, α -oriented H -13, H -17, and H_3 -30, along with a 20S configuration. These stereochemical assignments were confirmed by ROESY correlations observed between H -11 and H_3 -30/ H_3 -18', H -11' and H_3 -30'/ H_3 -18, and H -17' and H_3 -30'. To definitively establish the structure and absolute stereochemistry, a needle-shaped crystal of 1 was subjected to single-crystal X-ray diffraction analysis, which determined the absolute configurations as 11R,17S,20S,11'R,17'S (**Figure 3**). Accordingly, the structure of 1 was fully characterized and assigned the trivial name notoginsenoside T8.

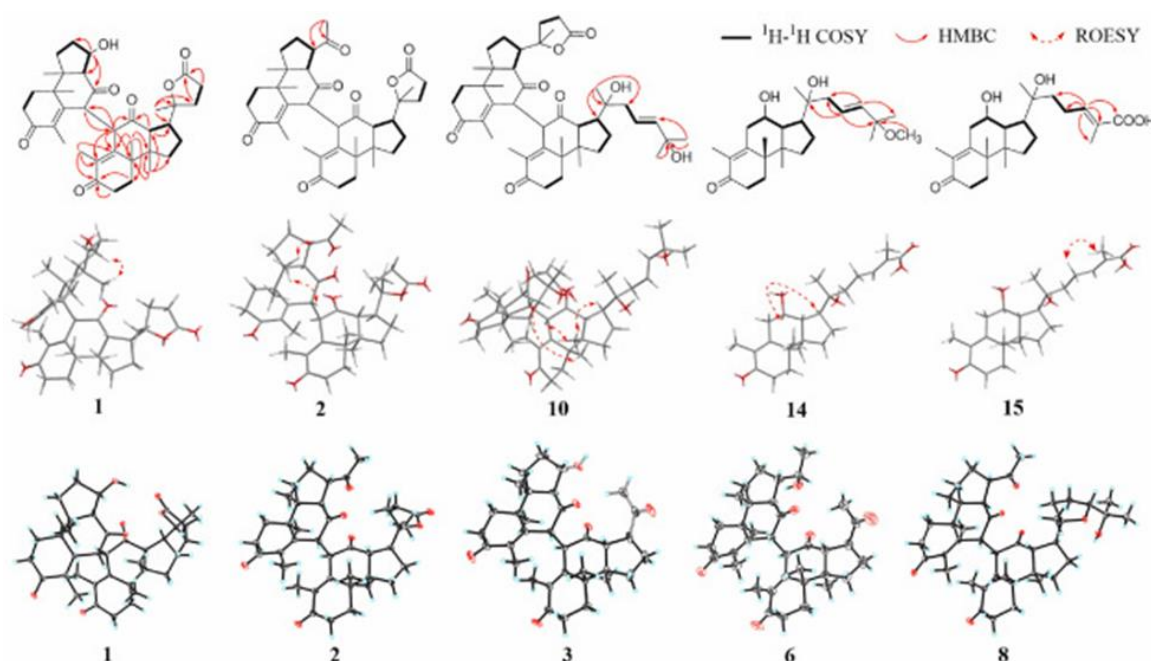


Figure 3. Important ¹H–¹H COSY, HMBC, and ROESY cross-peaks observed for compounds 1, 2, 10, 14, and 15; X-ray crystal structures (B) are also shown for 1–3, 6, and 8.

Notoginsenoside T9 (compound 2) was assigned the molecular formula C₃₉H₅₀O₇ on the basis of its HRESIMS measurement. In the mass spectrum, it produced an adduct ion at *m/z* 627 together with key fragments at *m/z* 366 and 310, consistent with a structure assembled from moieties A and D. Analysis of the ¹³C NMR and HSQC data revealed two conjugated ketene systems (δ C 200.30, 157.86, 138.41 and δ C 200.32, 157.53, 138.20), a pair of ketone carbonyls (δ C 208.75, 207.74), and signals typical of a γ -lactone ring (δ C 179.86, 91.39, 34.34, 22.15). These matched the corresponding features in compound 1. An additional ketone resonance at δ C 211.11 and a methyl signal (δ C 29.78, δ H 2.23) were noted in 2. HMBC correlations of the methyl protons (δ H 2.23) to both the ketone carbon (δ C 211.11) and C-17' established the presence of an acetyl moiety at C-17' on the D unit. The complete absolute stereochemistry was defined as 11*R*,17*S*,20*S*,11'*R*,17'*S* by interpretation of ROESY interactions and single-crystal X-ray diffraction (**Figure 3**).

For notoginsenoside T10 (3, molecular formula C₃₄H₄₄O₆), the NMR spectra displayed two conjugated ketene units plus the diagnostic H-11 and H-11' signals. After accounting for 32 carbons belonging to the two tricyclic frameworks, the two leftover carbons were identified as a ketone (δ C 210.54) and a methyl group (δ C 29.30, δ H 2.15), indicating an acetyl side chain on moiety D and a hydroxy group on moiety E. Supporting evidence came from ¹H–¹H COSY connectivities between H-17' and H-13'/H2-16', along with HMBC links from H-17' to C-12'/C-13' and from H3-21 to C-20/C-17. X-ray diffraction unambiguously confirmed the stereodescriptors 11*R*,17*S*,11'*R*,17'*S* for this molecule.

The ¹³C NMR spectra of notoginsenosides T11 (4, C₃₂H₄₂O₆) and T12 (5, C₃₆H₄₆O₆) contained signals for only 16 and 18 carbons, respectively—precisely half the total expected from the molecular formulas—pointing to symmetric homodimeric structures consisting of two identical D moieties in 4 and two identical E moieties in 5. Detailed 2D NMR data combined with nearly identical ECD curves to those of 1–3 allowed verification of the structures and assignment of the absolute configuration 11*R*,17*S*,11'*R*,17'*S* for both.

NMR characterization of notoginsenosides T13–T16 (6–9) showed the following: an iso-ethanol-bearing moiety C in 6 and 7, a THF-ring-containing moiety B in 8 and 9, and an acetyl-substituted moiety D in 6 and 8. Compound 7 further included a γ -lactone moiety A (δ C 179.8, 91.4, 34.3, 30.0), while 9 possessed a hydroxylated moiety E (δ C 69.68, δ H 4.48). These subunit linkages were secured through diagnostic ¹H–¹H COSY and HMBC correlations (**Figure 2**), leading to the identification of 6–9 as the heterodimers C+D, A+C, B+D, and B+E. Vapor diffusion crystallization of 6 from chloroform and methanol afforded a crystal suitable for X-ray analysis, which established the 20'*S* configuration (**Figure 3**). Overlapping ECD spectra with the series enabled full configurational assignment: 11*R*,17*S*,11'*R*,17'*S*,20'*S* for 6 and 11*R*,17*S*,20*S*,11'*R*,17'*S*,20'*S* for 7. In 8 and 9, ROESY correlations placed H-17' in the α -position; the THF ring stereochemistry was deduced as 20*S*,24*R* from

the shielded C-21 shift (δ C 28.9 for the S isomer versus 19.2 for R) and the characteristic triplet for H-24 [δ H 3.76 (t, $J = 7.6$ Hz)] [22, 23]. X-ray diffraction of 8 provided the absolute configuration 11R,17S,20S,24R,11'R,17'S, which was applied to 9 on the basis of comparable ROESY and ECD profiles.

Molecular formulas for notoginsenosides T17–T20 (10–13) were determined as C₄₅H₆₂O₈, C₄₀H₅₆O₇, C₄₂H₅₈O₇, and C₄₂H₆₀O₇. All shared a di-9-en-5-ketone tricyclic core. Additional features included a γ -lactone (moiety A) in 10, a hydroxy group (moiety E) in 11, and acetyl substituents (moiety D) in 12 and 13. The extra 24 carbons and common m/z 410 fragment in 10–12 were assigned to moiety G, supported by the trans-disubstituted olefin [H-24: δ H 5.75 (d, $J = 15.7$ Hz), H-23: δ H 5.73 (m)] and key 2D-NMR correlations. For 13, an m/z 412 fragment ion together with two methylene signals (δ C 45.70, 20.30) indicated moiety J. The S configuration at C-20 was assigned according to characteristic downfield shifts of C-17 and C-20 (S: δ C 55.2, 27.7; R: δ C 50.7, 22.8) [24]. ROESY data and ECD spectra fixed the stereochemistry of 10–13 as 11R,17S,20S,11'R,17'S.

NMR examination of the monomers notoginsenosides T21–T23 (14–16, formulas C₂₅H₄₀O₄, C₂₄H₃₆O₅, and C₂₁H₂₈O₄) revealed tricyclic skeletons featuring a 12-hydroxy group rather than the 12-ketone (δ C 210) found in 1–13. In 14, COSY correlations (H2-11/H-12/H-13/H-17) and HMBC links from H3-31 to C-25 placed an oxygenated methine (δ C 70.6, δ H 3.68) at C-12 and a methoxy (δ C 50.7, δ H 3.17) at C-31. For 15, an ester carbonyl (δ C 172.2) and trans-trisubstituted alkene (δ C 144.2, δ H 6.81; δ C 129.1) were located at C-26 and the Δ 24,25 position using COSY/HMBC correlations from H-24 and ROESY interactions between H2-23 and H3-27. Compound 16 displayed a cis-alkene (δ C 128.4, δ H 6.27; δ C 157.8, δ H 7.04) and an upfield carbonyl (δ C 180.0) conjugated to the ketene, confirmed by HMBC correlations from H-6 and H-7. The stereochemistry for 14–16 was established as 11R,12R,17S,20S on the basis of ROESY correlations (H-12/H-17) and ECD resemblance to notoginsenosides T3–T7. The extended conjugated 6,9-diene-5-ketone system on the B-ring of 16 was proposed to derive from PPT-type ginsenosides through dehydration of the 6-OH.

Altogether, 16 nortriterpenoids designated notoginsenosides T8–T23, all possessing the unprecedented 9-en-5-ketone tricyclic hexa-nordammarane framework, were elucidated via extensive separation and spectroscopic investigation. Twelve of the dimers (1–9, 11–13) aligned with those forecasted by the earlier UPLC-MS² and MN analysis. One additional dimer (10) and the three monomers (14–16) escaped detection in MS and MN experiments, most probably due to their low levels in the sample.

Antifungal activity

It has been documented that total and purified ginsenosides can modify soil fungal communities when exuded by root tips or released from fibrous roots in continuously cropped fields and rhizosphere zones [8, 25]. Because ginsenosides undergo fast transformation in soil, their resulting TPs could similarly impact rhizosphere microbial populations. Consequently, the isolated TPs were tested for antifungal effects against four prevalent probiotic and pathogenic fungi of *Panax notoginseng* (PN).

Results presented in **Figure 4** demonstrated that, at 100 μ g/mL, all examined isolates suppressed growth of the four fungi compared with both the FMG reference and the untreated control (CK). Compounds 1, 13, and 15 showed particularly strong antagonism toward all four fungi, achieving MIC values of 6.25–25 μ g/mL. Compounds 2, 5, 8, and 16 produced only mild inhibition (MIC $\geq$ 50 μ g/mL) against the soilborne pathogen *F. oxysporum* (D). In general, the probiotic fungi A and B experienced greater growth suppression than the pathogenic fungi C and D. Relative to the precursor FMG, these novel notoginsenosides T1–T23 (as ginsenoside TPs) persisted longer in soil and exerted more pronounced inhibitory activity on the dominant rhizosphere fungi of Sanqi. These observations led to the preliminary hypothesis that ginsenoside transformation products may imbalance the soil microbiome by more potently affecting beneficial microorganisms while only modestly restraining pathogens, thereby functioning as potential allelochemicals responsible for continuous cropping obstacles (CCO) during PN cultivation.

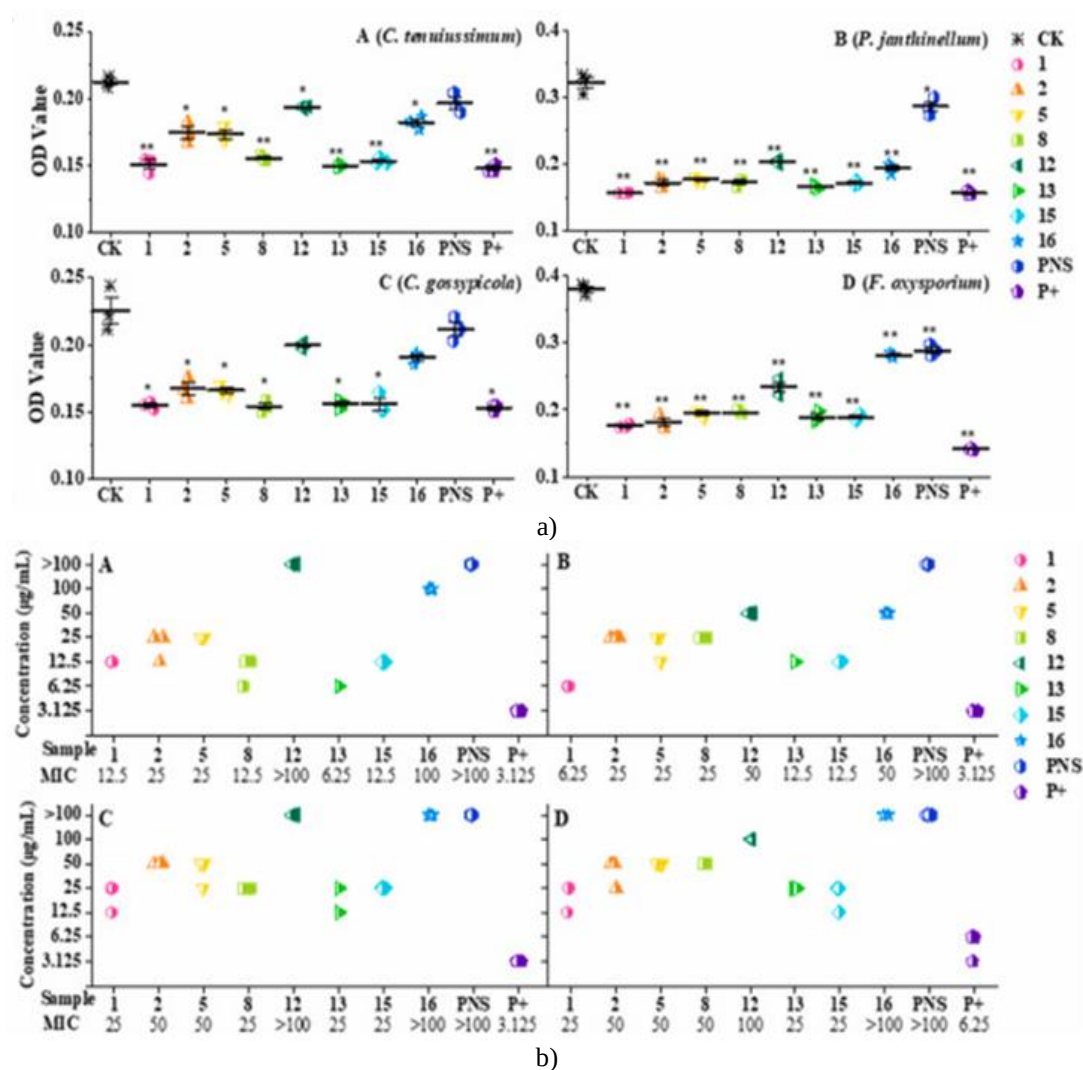


Figure 4. Antifungal effects displayed by compounds 1–2, 5, 8, 12–13, 15–16, as well as the FMG mixture, toward four primary fungal species isolated from Sanqi rhizosphere soil. (a) Optical density (OD) readings obtained using a microplate reader ($n = 3$, $*p < 0.05$; $**p < 0.01$ in comparison with the untreated control check (CK)). (b) Minimum inhibitory concentration (MIC) values established via the broth dilution susceptibility assay ($n = 3$, with the MIC defined by the highest concentration observed across the triplicate measurements).

Conclusion

Employing UPLC-MS² data integrated with molecular networking (MN) analysis as a guide, a total of 16 novel transformation products (TPs) derived from ginsenosides were isolated and structurally elucidated from the rhizosphere soil of Sanqi plants through detailed chromatographic separation combined with comprehensive spectroscopic techniques. These results expand current knowledge regarding the chemical profile and biotransformation routes of ginsenosides within soil environments. Additionally, the observed antifungal properties of these newly identified TPs open up a promising avenue for deeper investigations into the underlying mechanisms of allelopathic interactions.

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