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SHORT REPORT

Nicocembranoside A: A new neuroprotective cembrane glycoside from *Nicotiana tabacum* L.

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Abstract: Cembrane-type diterpenoids are recognized for their medicinal significance. The phytochemical investigation of *Nicotiana tabacum* L. led to the isolation of Nicocembranoside A (**1**), a novel cembrane glycoside, represents the ninth naturally occurring cembrane glycoside and the first isolated from *N. tabacum* leaves. The structure of **1** was elucidated through comprehensive spectroscopic analyses, including 1D (^{1}H, ^{13}C NMR) and 2D NMR (COSY, HSQC, HMBC, ROESY), complemented by HRESIMS and biosynthetic precedence. Additionally, seven known compounds were also isolated and characterized. Preliminary evaluation demonstrated **1** exhibits neuroprotective effect against glutamate-induced cytotoxicity in PC12 cells at 10 μM.

Keywords: *Nicotiana tabacum*, cembrane glycoside, neuroprotective

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1 Plant Source

As part of a continuous exploration into structural diversity and the search for aroma precursors with pharmacological properties, the n-butanol fraction of a methanol extract from *Nicotiana tabacum* had been investigated, resulting in the isolation of a new cembrane glycoside (**1**) and seven reported compounds (**2–8**) (Figure 1).

The voucher specimen of *N. tabacum* leaves from Zimbabwe was authenticated in February 2022 by senior engineer Sheng Zhai of China Tobacco Guangdong Industrial Co., Ltd., and archived by the Institute of Chemistry's laboratory (voucher specimen number: ZBW-2022-PY-0562).

2 Previous Studies

Cembrane-type diterpenoids, usually featuring a 14-membered macrocyclic core structure with an isopropyl moiety and three methyl substituents, are a major class of structurally distinct and chemically diverse natural products (Zhang et al., 2023), which are mostly encountered in the marine organisms and *Nicotiana tabacum* (Yang et al., 2022). Due to oxidative modifications, cembrane-type diterpenoids commonly possess varying numbers of hydroxyl groups at different substitution sites (Wu et al., 2020). These hydroxyl functionalities exhibit high susceptibility to glycosylation, readily forming glycosidic bonds with sugar moieties. To date, merely eight cembrane glycosides have been isolated from natural sources (Shi et al., 2001; Todorova et al., 2013; Diao et al., 2020). Notably, none of the 131 cembrane diterpenoids isolated from *N. tabacum* thus far have been characterized as glycosides (Xu et al., 2024).

3 Present Study

Cured leaves of *N. tabacum* L (16 kg) underwent triplicate extraction with MeOH (3 × 40 L, 6 h per extraction). All of the MeOH solution were concentrated *in vacuo* to yield a dark brown residue (5.8 kg), which was suspended in H_2O (5 L) and filtered. The filtrate underwent sequential liquid-liquid partitioning with petroleum ether (5 × 6 L), EtOAc (5 × 6 L), and *n*-BuOH (6 × 6 L). The *n*-BuOH-soluble extract (1.75 kg) was chromatographed over an HP-20 macroporous resin column (200 × 15 cm i.d.) with stepwise elution using ethanol/water mixtures (0%, 15%, 30%, 50%, 75%, and 95% EtOH; 20 L per step), yielding six fractions (A–F). Fraction C (98 g) was fractionated on a Sephadex LH-20 column (120 × 10 cm i.d.) employing a MeOH/H_2O gradient (0–100% MeOH in 10% increments, 6 L per step) guided by HPLC-PDA detection to afford subfractions C1–C12. Subfraction C2 was further resolved on a reverse phase C18 column via stepwise elution with MeOH/H_2O (0%, 10%, 20%, 30%, 40%, 50%, 70%, and 100% v/v; 2 L per step) to give subfractions C2-1–C2-16. Subsequent purification of C2-2 by semi-preparative RP-HPLC (MeCN/H_2O/HOAc = 15:85:0.1 v/v;

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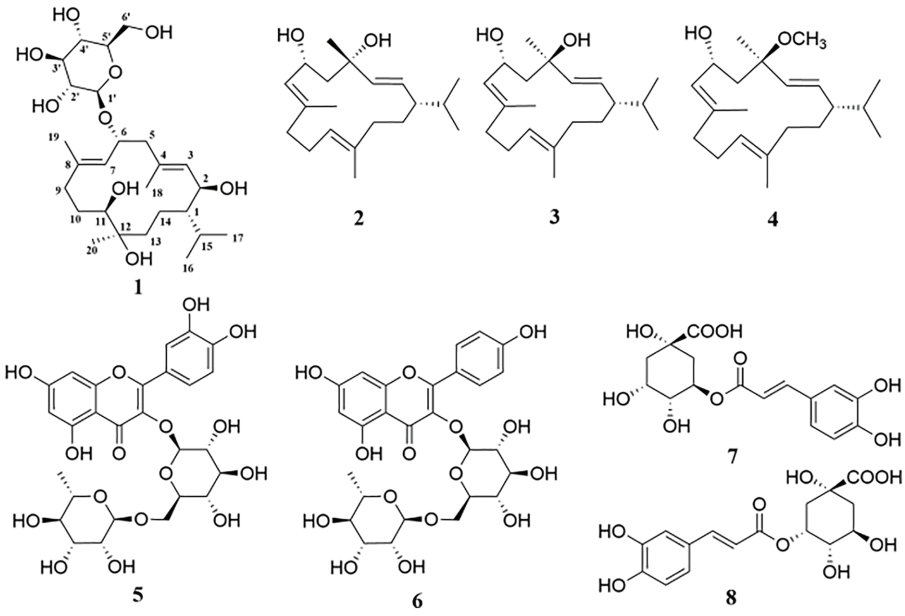

Figure 1. Structure of Nicocembranoside A (**1**) and other compounds isolated from *N. tabacum*

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of Nicocembranoside A in CD_3OD (δ, ppm; *J*, Hz)

No.	δ_H (*J*, Hz)	δ_C	No.	δ_H (*J*, Hz)	δ_C
1	1.26 (1H, m)	48.1	**14**	1.64 (1H, m); 1.50 (1H, m)	19.7
2	4.47 (1H, dd, *J* = 9.6, 4.8 Hz)	72.2	**15**	1.19 (1H, m)	30.2
3	5.46 (1H, d, *J* = 10.8 Hz)	128.5	**16**	0.89 (3H, d, *J* = 6.0 Hz)	21.1
4		138.4	**17**	0.73 (3H, d, *J* = 6.0 Hz)	20.6
5	2.60 (1H, dd, *J* = 12.0, 6.0 Hz) 2.07 (1H, m)	47.2	**18**	1.67 (3H, s)	16.4
6	4.75 (1H, m)	76.9	**19**	1.67 (3H, s)	17.4
7	5.07 (1H, dd, *J* = 10.2, 1.2 Hz)	127.3	**20**	0.98 (3H, s)	24.8
8		139.8	**1′**	4.37 (1H, d, *J* = 7.8 Hz)	102.9
9	2.11 (1H, m); 2.03 (1H, m)	37.6	**2′**	3.16 (1H, m)	75.3
10	1.52(1H, m); 1.34 (1H, m)	31.5	**3′**	3.33 (1H, m)	78.2
11	3.79 (1H, m)	72.2	**4′**	3.28 (1H, m)	71.6
12		77.4	**5′**	3.22 (1H, m)	77.9
13	2.42 (1H, m); 1.23 (1H, m)	34.9	**6′**	3.80 (1H, m); 3.65 (1H, m)	62.8

5 mL/min) afforded compound **1** (8.3 mg, t_R = 30.2 min). **2** (5.6 mg, t_R = 13.4 min), **3** (24.8 mg, t_R = 15.6 min) and **4** (3.3 mg, t_R = 11.1 min) was obtained from C2-14 by semi-preparative RP-HPLC (MeCN/H_2O = 65:35 v/v; 5 mL/min). **5** (149.6 mg, t_R = 4.3 min) and **6** (6.5 mg, t_R = 5.5 min) was collected from C2-4 by semi-preparative RP-HPLC (MeCN/H_2O/HOAc = 25:75:0.1 v/v; 5 mL/min). Purification of C2-3 by semi-preparative RP-HPLC (MeCN/H_2O/HOAc = 20:80:0.1 v/v; 5 mL/min) afforded compound **7** (18.1 mg, t_R = 8.2 min) and 8 (2.3 mg, t_R = 9.4 min).

Nicocembranoside A ***(1)***: white amorphous powder; $[\alpha]_D^{20}$ +23.2 (*c* 0.05, MeOH); ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) see Table 1; HRESIMS: *m/z* 485.3119 $[M - H_2O + H]^+$ (calcd. 485.3109 for $C_{26}H_{45}O_8^+$).

Enzymatic hydrolysis of Nicocembranoside A ***(1)***: Compound **1** (4 mg) and snailase (w/w = 1:3) were combined in H_2O (2 mL) and incubated at 37°C for 30 h. The reaction mixture was extracted with EtOAc (3 × 3 mL). The aqueous phase was lyophilized to afford the sugar residue, whose absolute configuration was determined according to established literature protocols (Yang et al., 2025).

Neuroprotective activity test: Previous studies have documented the neuroprotective properties of cembrane-type diterpenoids (Yang et al., 2022). The neuroprotective activity of the new metabolite (Nicocembranoside A) was evaluated using a glutamate-induced PC12 cell injury model (Hu et al., 2017). Both edaravone (positive control) and Nicocembranoside A were tested at 10 μM. Results were expressed as percentage of cell viability (%).

Compound **1**, obtained as a white amorphous powder, share a molecular formula of $C_{26}H_{46}O_9$ based on the observed positive ion peak at *m/z* 485.3119 $[M - H_2O + H]^+$ (calcd. 485.3109 for $C_{26}H_{45}O_8^+$), implying four indices of

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unsaturation. The ^{1}H NMR data (Table 1) of **1** displayed two olefinic protons at δ_H 5.46 (1H, d, J = 10.8 Hz, H-3) and 5.07 (1H, dd, J = 10.2, 1.2 Hz, H-7), three oxygenated methines at δ_H 4.47 (1H, dd, J = 9.6, 4.8 Hz, H-2), 4.75 (1H, m, H-6) and 3.79 (1H, m, H-11). Moreover, two methyl doublets at δ_H 0.89 (3H, d, J = 6.0 Hz, H-16) and 0.73 (3H, d, J = 6.0 Hz, H-17), two vinyl methyl singlets at δ_H 1.67 (6H, s, H-18, 19), and one methyl singlet at δ_H 0.98 (3H, s, H-20) were also clearly observed in the up-field region. Additionally, the anomeric proton of a glucopyranosyl moiety at δ_H 4.37 (1H, d, J = 7.8 Hz, H-1′) along with resonances corresponding to its six oxymethine protons at δ_H 3.16–3.80 were also presented in the high-field region. The ^{13}C NMR spectrum presented 26 carbon resonances, corroborated by the HSQC spectrum, six of which were attributable to a glucose moiety, the remaining 20 C-atom signals could be assigned to five methyl carbons [δ_C 21.1 (C-16), 20.6 (C-17), 16.4 (C-18), 17.4 (C-19), 24.8 (C-20)], five methylene carbons [δ_C 47.2 (C-5), 37.6 (C-9), 31.5 (C-10), 34.9 (C-13), 19.7 (C-14)], two methine carbons [δ_C 48.1 (C-1), 30.2 (C-15)], three oxygenated methine carbons [δ_C 72.2 (C-2, 11), 76.9 (C-6)] and one oxygenated quaternary carbon [δ_C 77.4 (C-12)]. The resonances at δ_C 128.5 (C-3) and 127.3 (C-7) were attributed to two olefinic methine carbons, and two signals at δ_C 138.4 (C-4) and 139.8 (C-8) were assigned to two olefinic quaternary carbons. A meticulous comparison of the NMR data for compound **1** versus 8 structural analogues (Figure S10 and Table S1), especially compound S1 and S2, revealed two critical structural distinctions. The appearance of two methyl doublets (δ_H 0.89, d, J = 6.0 Hz; δ_H 0.73, d, J = 6.0 Hz), corresponding to H_3-16 and H_3-17 of **1**, confirms C-15 dehydrogenation by eliminating the tertiary hydroxyl present in compound S1 and S2, forming an isopropylidene group. Besides, the disappearance of the characteristic olefinic signal combined with new oxygenated methines [δ_C 72.2 (C-11), 77.4 (C-12)] demonstrated oxidative cleavage of the $\Delta^{11,12}$ double bond, resulting in hydroxylations at C-11 and C-12. Integrating all the information above, **1** is likely to be an isopropyl cembrane glycoside featuring a 3,7-diene-2,6,11,12-tetraol diterpenoid aglycone attached to a glucose unit.

The proposed cembrane diterpenoid skeleton assignments were confirmed through heteronuclear multiple bond correlation (HMBC) and ^{1}H–^{1}H correlation spectroscopy (COSY) experiments (Figure 2). Key ^{1}H–^{1}H COSY cross-peaks of H_2-13–H_2-14–H-1–H-2–H-3, H-1–H-15, H_3-16–H-15–H_3-17 confirmed the presence of structural fragment I (C-3–C-2–C-1–(C-15)–C-14–C-13) and II (C-16–C-15–C-17). Likewise, fragment III (C-5–C-6–C-7) and IV (C-8–C-9–C-10) were also determined by the spin-coupling sequences of H_2-5–H-6–H-7 and H_2-9–H_2-10–H-11. Furthermore, in the HMBC spectrum, correlations from H-3 to C-5 and H-5 to C-4 demonstrated that fragments I and III were linked via C-4, cross-peaks from H-7 to C-8/C-9, and H_2-9 to C-8 suggested the linkage of fragments III and IV via C-8, while correlations from H_2-10, H-11 and H_2-13 to C-12 permitted the linkage of fragments IV and I via C-12. The connectivity of the glucopyranosyl moiety at C-6 was determined by the HMBC correlation from the anomeric proton H-1′ to C-6. Thus, the planar structure of compound **1** was tentatively elucidated.

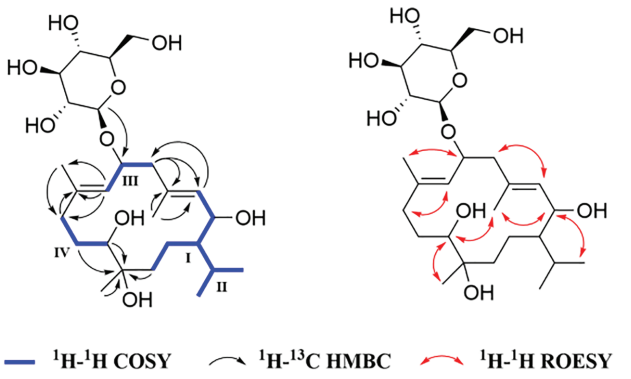

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and ROESY correlations for Nicocembranoside A (**1**)

Compound **1** underwent hydrolysis to afford D-glucose and the aglycone. The anomeric proton signal at δ_H 4.37 (1H, d, J = 7.8 Hz) indicated a β configuration for the glucopyranosyl moiety. An *E*-configuration for the $\Delta^{3,4}$ double bond was assigned based on the rotational Overhauser effect spectroscopy (ROESY) correlations between H-3/H_2-5, and H-2/H_3-18, and the characteristic upfield chemical shift of C-18 (δ_C 10.54) (Diao et al., 2020; Zou et al., 2010; Mulholland et al., 2010). Similarly, the ROESY spectrum showed cross-peaks from H-6 to H_3-19, and from H-7 to H_2-9, suggesting an *E*-configuration of the $\Delta^{7,8}$ double bond, and this configuration was also evidenced by the upfield-shielded C-19 signal (δ_C 17.4). Additionally, the relative configurations of the five chiral centers at C-1, C-2, C-6, C-11, and C-12 in **1** were elucidated through detailed ROESY analysis. Key correlations from H-2 to H_3-17, H-2 to H_3-18, H_3-18 to H-11, H-11 to H_3-20 revealed that H-2, H-11, H_3-20 and the isopropyl group at C-1 all reside on the same face of the molecule. Given that all characterized *Nicotiana* cembranoids possess a (1*S*,6*R*) configuration, the absolute configuration of compound **1** was assigned tentatively as (1*S*,2*S*,3*E*,6*R*,7*E*,11*R*,12*R*)-6-*O*-β-D-glucopyranosyl-3,7-cembradiene-2,11,12-triol based on biosynthetic precedence, and named as Nicocembranoside A.

To the best of our knowledge, Nicocembranoside A represents the ninth naturally occurring cembrane glycoside and the first isolated from *N. tabacum* leaves. This compound enriches the structural diversity of this class of natural products, thereby providing guiding significance for the isolation and characterization of analogous constituents in tobacco. In addition, despite the extensive characterization of over 2,500 secondary metabolites, including terpenoids, flavonoids, lignans, and steroids, from *N. tabacum* (Feng et al., 2017), these compounds were also frequently encountered in other genera of the Solanaceae family and across various species of the genus *Nicotiana*. However, Nicocembranoside A, the first cembrane glycoside identified exclusively from *N. tabacum* leaves, demonstrates its significant potential as a chemotaxonomic marker for distinguishing *N. tabacum* from other

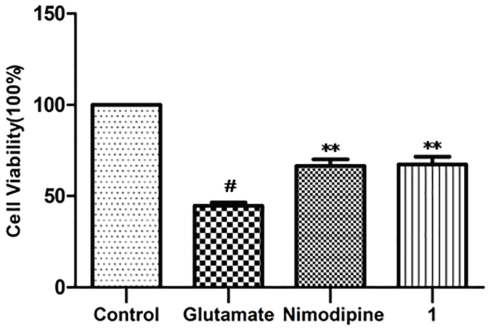

Figure 3. Neuroprotective activity of **1** (10 μM) against glutamate-induced injury of PC12 cells. Nimodipine served as a positive control. Results were expressed as the mean ± SD (n = 3). ($^{\#}p < 0.05$ compared with control, $^{**}p < 0.01$ compared with glutamate group)

species in the genus *Nicotiana* based on secondary metabolite profiles.

Through comparison of experimental and reported spectroscopic data, seven known compounds were identified as α-CBT-diol (**2**) (Yang et al., 2022), β-CBT-diol (**3**) (Yang et al., 2022), 4-*O*-methyl-β-CBT-diol (**4**) (Yang et al., 2022), rutin (**5**) (Jassbi et al., 2017), kaempferol-3-*O*-rutinoside (**6**) (Jassbi et al., 2017), chlorogenic acid (**7**) (Tseng et al., 2025), neochlorogenic acid (**8**) (Tseng et al., 2025).

Nicocembranoside A (**1**) was evaluated for neuroprotectiove activity in a glutamate-induced PC12 cell injury model. Both 1 and positive control Nimodipine were used at concentrations of 10 μM. Clearly, **1** showed strong neuroprotective activity via cell viabilities of 67.36% (Figure 3).

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Author Contributions

Jun-Zhang Wu: Methodology, Formal analysis, Writing original manuscript; Bao-Min Lin: Methodology, Investigation; Data Curation; Ping-Zhang Yao: Methodology, Investigation; Cui-Ling Chen: Formal analysis; Miao Liang: Methodology, Formal analysis; Ya-Ke Luo: Methodology, Formal analysis; Peng-Fei Yang: Writing-review & editing, Conceptualization; Jun-Ping Gu: Writing-review & editing, Project administration, Conceptualization, Supervision.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Ethics Approval

This study only involved in vitro biochemical assays. No human participants or live animals were involved.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

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