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Withanolides from aerial parts of *Nicandra physalodes*

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ABSTRACT

Twenty withanolides, including previously unknown nicanlodes A–M, were isolated from aerial parts of *Nicandra physalodes*. Their structural elucidations were unambiguously achieved through interpretation of extensive spectroscopic data (NMR and HRMS) and by comparison with literature data. Nicanlodes A and B have an unusual aromatic amine moiety. The isolated compounds were evaluated for their cytotoxicity against five human cancer cell lines.

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1. Introduction

Withanolides, a class of naturally occurring C_{28} steroid derivatives, possess a δ- or γ-lactone ring between C-26 and C-22 or C-23 in the side-chain (Chen et al., 2011). This type of compound has been extensively reported from plants of the Solanaceae family in the last 50 years, especially in the genera *Withania*, *Physalis*, *Datura*, *Salpichroa*, *Nicandra*, *Lycium*, *Tubocapsicum*, and *Jaborosa* (Glotter, 1991). Due to their structural diversity, withanolides display a wide spectrum of bioactive properties, such as antimicrobial (Alali et al., 2014), antitumor (Roy et al., 2013; Reyes-Reyes et al., 2013), anti-inflammatory (Yang et al., 2014a,b), immunomodulatory (Yang et al., 2014a,b; Furmanowa et al., 2001), insect-antifeedant (Mareggiani et al., 2000; Vaccarini and Bonetto, 2000), and insecticidal (Nalbandov et al., 1964) activities.

The genus *Nicandra* comprises three species. *N. john-tyleriana* and *N. yacheriana* grow in Peru, while *N. physalodes*, which is a well-known and most widespread species of the genus, occurs in regions from Peru to northern Argentina, as well as being found as a ruderal species in tropical and subtropical areas worldwide (Nicolás et al., 2015). This plant is also distributed widely in Southwest China and its whole plant has been used in folk medicine for the treatment and prevention of sedation, as an expectorant, for fever relief, and for detoxification (Editorial Board of National Herbal Compendium, 1975). Literature reports indicate that *N. physalodes* is an abundant source of withanolides with about twenty withanolides having antifeedant (Andrews-Smith et al., 1991), insecticidal (Nalbandov et al., 1964), and cytotoxic effects (Gunasekera et al., 1981) being identified from this species. Thus, to further discover structurally diverse and biologically significant withanolides from the title plant, a phytochemical investigation on *N. physalodes* was carried out. This resulted in isolation of 13 new withanolides, nicanlodes A–M (**1**–**13**), together with seven known compounds, including nic 1 (**14**) (Begley et al., 1976), salpichrolide A (**15**) (Veleiro et al., 1992), nacaphysalin C (**16**) (Shingu et al., 1994), nic 2 (**17**) (Bates and Morehead, 1974), nicaphysalin A (**18**) (Shingu et al., 1994), withahisolide I (**19**) (Cao et al., 2014), and nic 11 (**20**) (Begley et al., 1973) (Fig. 1). Herein, the isolation, structural

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elucidation, as well as cytotoxicities of these compounds, are reported.

2. Results and discussion

Thirteen new withanolides, nicanlodes A–M (**1**–**13**), together with seven known compounds, were isolated from the ethyl acetate soluble fraction of the aerial parts of *N. physalodes*. Compounds **1**, **3**, **4**, **6**, **8**, **10**, **11**, and **13** were isolated as white amorphous powders, while compounds **2**, **5**, **7**, **9**, and **12** were isolated as light yellow amorphous powders.

2.1. Structural elucidation of new compounds

The molecular formula of nicanlode A (**1**), $C_{35}H_{39}NO_7$, was deduced from its HRESIMS at *m/z* 608.2623 $[M + Na]^+$ (calcd. for $C_{35}H_{39}NO_7Na$, 608.2619) and ^{13}C NMR data. Its 1D (^1H and ^{13}C) NMR spectra (Tables 1 and 3) showed the presence of four methyls [δ_H 1.24 (s, H_3-19), δ_C 14.0; δ_H 1.10 (d, $J = 7.1$ Hz, H_3-21), δ_C 18.2; δ_H 1.44 (s, CH_3-28), δ_C 19.0; and δ_H 1.41 (s, CH_3-27), δ_C 17.4], four methylenes, sixteen methines, nine quaternary carbons and two carbonyl carbons. Among them, the characteristic signals at δ_C 202.7 (C-1), δ_C 128.8 (C-2), and δ_C 140.0 (C-3), along with two methine resonances at δ_C 56.9 (C-6), δ_C 55.7 (C-7), and one oxygenated signal at δ_C 72.8 (C-5) indicated that compound **1** possessed a 1-one-2-ene-5α-hydroxy-6α(7α)-epoxy moiety in rings A and B. Furthermore, resonances for aromatic ring D and a 22,26-epoxy moiety in the side-chain [δ_H 3.81 (m, H-22), 5.11 (d, $J = 7.5$ Hz, H-26), 7.05 (d, $J = 7.9$ Hz, H-15), 6.83 (d, $J = 7.9$ Hz, H-16), 6.77 (s, H-18); δ_C 136.7 (C-13), 134.4 (C-14), 123.7 (C-15), 124.8 (C-16), 142.4 (C-17), 128.0 (C-18), 68.0 (C-22), 79.2 (C-26)] were also observed in its 1D NMR spectrum. In addition, a 24,25-epoxy moiety in the side-chain [63.7 (C-24), 63.3 (C-25)] was indicated by analysis of the NMR spectrum. The above featured fragments were further confirmed by 2D NMR correlations. These data illustrated that compound **1** was a typical withanolide and resembled Nic 1 (**14**), a compound with a 6-membered hemiacetal side-chain.

1D NMR spectra of **1** also showed additional signals for another aromatic moiety [δ_H 6.57 (d, $J = 8.5$ Hz, H-3′), 7.12 (m, H-4′), 6.64 (m, H-5′), and 7.96 (dd, $J = 8.0$, 1.2 Hz, H-6′); δ_C 110.3 (C-1′), 150.0 (C-2′), 113.8 (C-3′), 134.6 (C-4′), 116.2 (C-5′), 132.0 (C-6′), and 171.6 (C-7′)]. The HMBC and ^1H-^1H COSY spectra (Fig. 2), as well as its molecular formula, indicated that the aromatic moiety was 2′-aminobenzeoic acid. The key HMBC correlations of H-26 (δ_H 5.11 d, $J = 7.5$ Hz) with C-2′ established that this substituent was located at C-26, coincident with the downfield shift of C-26 (δ_C 79.2).

The ROESY correlations of H_3-19/H-8/H-7/H-6, of H-20/H-22/H-23b, and H-23a/H_3-27/H_3-28/H-26 established the relative configuration of the 6,7-epoxy group to be β-oriented while H-26 in the α plane (Fig. 2). Therefore, structure **1** was elucidated as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α-hydroxy-26-(2′-amino benzoic acid)-17(13 → 18)-abeo-ergost-2,13,15,17-tetraen-1-one.

A molecular formula of $C_{35}H_{39}NO_8$ was assigned to nicanlode B (**2**) by HRESIMS at *m/z* 624.2566 $[M + Na]^+$ (calcd. for $C_{35}H_{39}NO_8Na$, 624.2568) and ^{13}C NMR data. Its 1D NMR data (Tables 1 and 3) were similar to those of **1** except for the signals of the aromatic substituent. Careful comparison of ^1H NMR spectroscopic data in the downfield region between **2** and **1** showed characteristic signals at H-2′ (δ_H 7.01, d, $J = 1.5$ Hz), H-5′ (δ_H 6.80, d, $J = 8.3$ Hz), and H-6′ (δ_H 7.39, d, $J = 8.1$ Hz) for an ABX system in **2**. The observed HMBC correlations of H-2′ and H-6′ with C-7′ (δ_C 170.0), and those of H-2′ and H-5′ with C-3′ (δ_C 133.3) and C-4′ (δ_C 148.9), further verified the presence of a 3′-hydroxy-4′-aminobenzoic acid substituent. Furthermore, H-26 showed a HMBC cross-peak with C-4′, which indicated that this substituent was connected to C-26. Similar ROESY correlations between **2** and **1** proved they shared the same relative configuration. Consequently, structure **2** was established as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α-hydroxy-26-(3′-hydroxy-4′-aminobenzoic acid)-17(13 → 18)-abeo-ergost-2,13,15,17-tetraen-1-one.

The molecular formula of nicanlode C (**3**) was deduced as $C_{28}H_{32}O_7$ by HRESIMS at *m/z* 503.2041 $[M + Na]^+$ (calcd. for $C_{28}H_{32}O_7Na$, 503.2040) and ^{13}C NMR data, requiring 13 degrees of unsaturation. Comparison of 1D NMR data (Tables 1 and 3) of **3** and **14** showed that they were similar withanolides with a 6-membered hemiacetal side-chain, differing only in the presence of a carbonyl group in **3** instead of a methylene at C-12 observed in **14**. This was further supported by HMBC correlations of H-18 (δ_H 8.23), H-9 (δ_H 2.96), and H-11 (δ_H 2.57, 4.25) with C-12 (δ_C 197.9), together with ^1H-^1H COSY correlations of H-6/H-7/H-8/H-9/H-11. The ROESY spectrum showed that compounds **3** and **14** possessed similar configuration. Therefore, structure **3** was assigned as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,26-dihydroxy-17(13→ 18)-abeo-ergost-2,13,15,17-tetraen-1,12-dione.

Nicanlode D (**4**) was assigned a molecular formula of $C_{28}H_{36}O_6$ based on its HRESIMS (*m/z* 491.2404 $[M + Na]^+$) and NMR data with two more hydrogen atoms than Nic 1 (**14**). Detailed comparison of its 1D NMR data (Tables 1 and 3) with **14** showed an oxymethine rather than an unsaturated carbonyl in ring A of **4**, which was further supported by HMBC correlations of H_3-19 (δ_H 0.84) with C-1 (δ_C 70.6), of H-1 (δ_H 3.71) with C-2 (δ_C 129.5), C-3 (δ_C 124.0), and C-19 (δ_C 14.5), of H-2 (δ_H 5.99) and H-3 (δ_H 5.78) with C-1 and C-4 (δ_C 35.7), respectively. Furthermore, ROESY correlations between H_3-19β and H-1 proved that the configuration of OH-1 was α-oriented, and that correlations of H-22β with H-26 were absent in the ROESY spectrum, which also further confirmed the configuration of the side-chain. Thus, structure **4** was elucidated as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-1α,5α,26-trihydroxy-17(13→ 18)-abeo-ergost-2,13,15,17-tetraene.

The molecular formula of nicanlode E (**5**) was established to be $C_{28}H_{34}O_5$ by analysis of the HRESIMS (*m/z* 473.2298 $[M + Na]^+$) and ^{13}C NMR data. Its 1D NMR data (Tables 1 and 3) was similar to Nic 1 (**14**), except for the replacement of 6α,7α-epoxide moiety in ring B in **14** by a double bond [δ_H 5.80 (1H, dd, $J = 10.0$, 2.7 Hz, H-6), 6.48 (1H, dd, $J = 10.0$, 1.8 Hz, H-7); δ_C 130.1 (C-6), 130.8 (C-7)] in **5**. HMBC correlations from H_3-19 (δ_H 1.27) to C-5 (δ_C 74.4), from H-4β (δ_H 2.99) to C-5/C-6 (δ_C130.1), and from H-6 (δ_H 5.80) to C-4/C-10, confirmed the above deduction. Furthermore, similar ROESY correlations between **5** and **4** implied identical relative configurations. Accordingly, structure **5** was identified as 20*S*,22*R*,24*S*,25*S*,26*R*) 22(26),24(25)-diepoxy-5α,26-dihydroxy-17(13→ 18)-abeo-ergost-2,6,13,15,17-pentaen-1-one.

A molecular formula $C_{28}H_{34}O_6$ of nicanlode F (**6**) was determined on the basis of its HRESIMS (*m/z* 489.2254 $[M + Na]^+$) and ^{13}C NMR data. Inspection of the 1D and 2D NMR spectra suggested that it was also a withanolide similar to salpichrolide A (**15**), except for the presence of an extra 7-hydroxy group [4.70 (1H, m, H-7); δ_C 64.5 (C-7)] in **6**. HMBC correlations of CH_3-19 (δ_H 1.36)/C-5, H-4β (δ_H 2.01) with C-5/C-6, and H-6 with C-4/C-10 suggested this inference. Furthermore, the 7-hydroxy group and the 5,6-epoxide in **6** were both determined to be in an α-orientation based on ROESY correlations of H-8/H-7/H-6/H_3-19. Consequently, structure **6** was elucidated as 20*S*,22*R*,24*S*,25*S*,26*R*)5α(6α),22(26),24(25)-triepoxy-7α,26-dihydroxy-17(13→ 18)-abeo-ergost-2,13,15,17-tetraen-1-one.

The HRESIMS (*m/z* 511.2669 $[M + Na]^+$) and NMR data of nicanlode G (**7**) established the molecular formula $C_{28}H_{40}O_7$. Its 1D NMR spectra (Tables 1 and 3) exhibited the presence of five methyls [δ_H 0.75 (s, H_3-18), δ_C 8.0; δ_H 1.16 (s, H_3-19), δ_C 14.6; δ_H 1.06 (d,

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