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Labdanes, Withanolides, and Other Constituents from *Physalis nicandroides*

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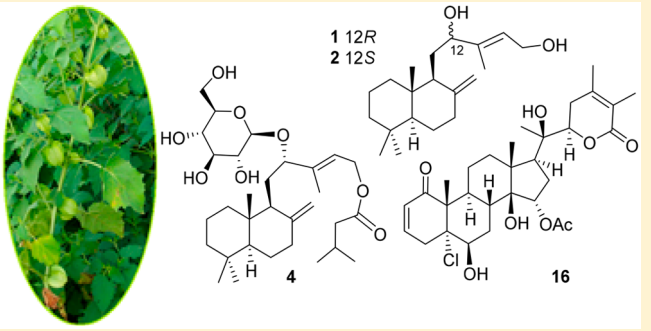
Supporting Information

ABSTRACT: Chemical investigation of the aerial parts (except fruits and calixes) of *Physalis nicandroides* var. *attenuata* led to the isolation of a series of new labdane-type diterpenoids, including the closely related compounds **1**−**3**, the labdane glucosides **4** and **5**, a mixture of the epimeric alcohols **6** and **7**, and one labdanetriol, isolated as its tri-*O*-acetyl derivative **9**. In addition, three new withanolides (**14**−**16**) and six known compounds were isolated. The structures of these compounds were elucidated by analysis of their spectroscopic data and chemical transformations, and those of compounds **1**, **4**, and **16** were confirmed by X-ray diffraction analysis of the natural product (**1**) and of the corresponding acetyl derivatives **4a** and **16a**. Fourteen of these compounds were assayed for their in vitro inhibitory activity against yeast α-glucosidase and acetylcholinesterase enzymes. The results were negative in both cases, except for compound **3a** that marginally inhibited the activity of acetylcholinesterase with an IC_{50} value of 64.4 μM.



A revision of the literature concerning the *Physalis* genus (Solanaceae) showed that the chemistry of these plants is quite complex and diverse, since although withanolides have been the constituents most frequently isolated from these plants,[1−3] there are species like *P. coztomatl* in which the co-occurrence of labdane-type diterpenoids and withanolides was described for the first time[4] or like the well-known producer of withanolides, *P. angulata*,[1,5] from which a series of labdanes were recently isolated.[6] In addition, there are species like *P. sordida*[7] and *P. nicandroides*[8] whose main metabolites are labdanes, and no withanolide was detected. Sucrose esters are another kind of metabolites of *Physalis*; they are the main constituents of fruits and calyxes of these plants,[9,10] which also may contain withanolides.[11,12] Sterols, flavonoids, and ceramides have also been described as constituents of *Physalis*.[1] In parallel to the advance in the knowledge of the chemistry of *Physalis*, different bioassays showed that the isolates from this genus, mainly the withanolides, possess a variety of bioactivities.[1−3] This is not surprising since many of these investigations have been inspired by the traditional use of some of these plants to treat a diversity of conditions, including respiratory and gastrointestinal infections as well as some chronic degenerative disorders like diabetes and cancer, among others.[8,13]

As a part of our studies of the Mexican *Physalis* we described the presence of several sucrose esters in the fruits of *P. nicandroides* var. *attenuata*.[10] In continuation of that work, we now report the isolation and structural elucidation of a series of 11 new labdanes and withanolides and other known compounds, from the aerial parts of this species, as well as the results of the evaluation of the α-glucosidase and acetylcholinesterase inhibitory activities of most of these compounds. These bioassays were carried out by considering the use of *Physalis* species to control the postpandrial blood glucose levels of diabetic patients[14] and the proven capacity of some withanolides to inhibit the activity of acetylcholinesterase.[15]

RESULTS AND DISCUSSION

The MeOH extract of leaves, flowers, and stems of *P. nicandroides* var. *attenuata* was partitioned to afford hexanes and EtOAc extracts. Each of these extracts was subjected to a series of column chromatographies to obtain the new labdane-type diterpenoids **1**−**7** and **9**, together with three new withanolides (**14**−**16**), and several known compounds, including labdanes, sterols, and flavonoids (Chart 1).

Compound **1** was isolated as colorless crystals with a molecular formula $C_{20}H_{34}O_2$, determined by the molecular ion at *m*/*z* 306.2560 in the HRFABMS and the ^{13}C NMR data. Its

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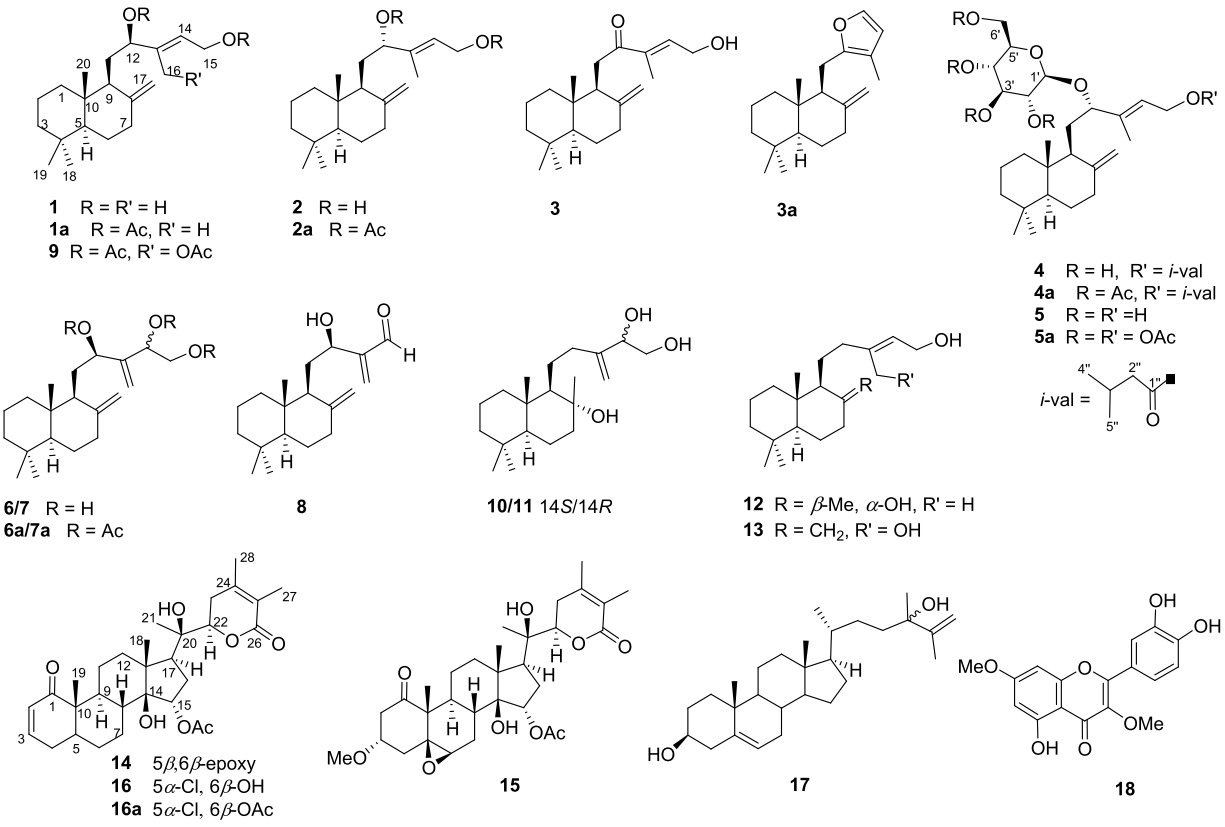
Chart 1



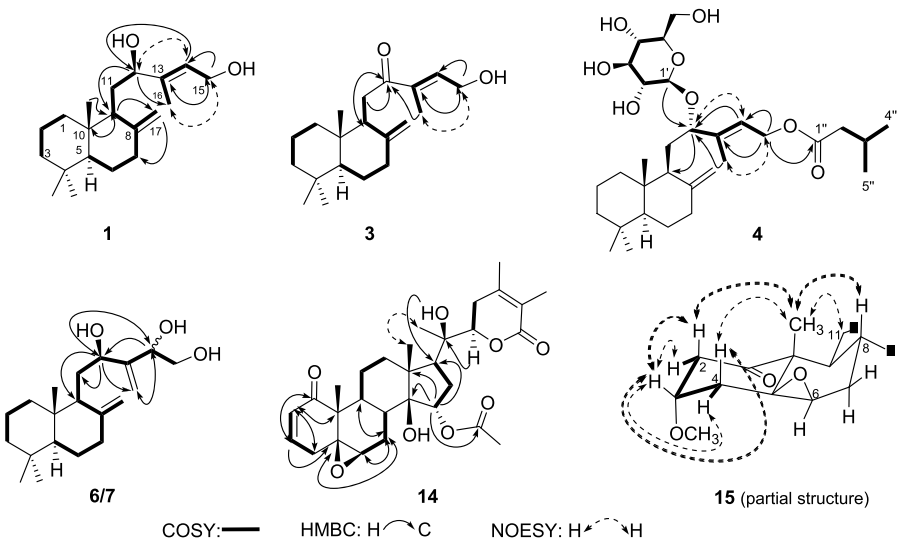


Figure 1. Key COSY, HMBC, and NOESY NMR correlations for compounds **1**, **3**, **4**, **6**/**7**, **14**, and **15**.

IR spectrum showed absorptions for hydroxy groups (3413, 3324 cm^{-1}) and double bonds (1642 cm^{-1}). The ^{1}H and ^{13}C NMR spectra showed signals for three tertiary (δ_H 0.88, 0.81 and 0.67) and one vinylic (δ_H 1.70) methyl group; a terminal (δ_C 149.1, C; δ_H4.85 and 4.47, δ_C 106.5, CH_2) and a trisubstituted (δ_C 142.3, C; δ_H 5.63, δ_C 123.3, CH) double bond; an oxymethine (δ_H 4.05 dd, J = 8.5, 3.0 Hz, δ_C 75.0); and an oxymethylene (δ_H 4.22 and 4.19, δ_C 59.1, CH_2). These data were consistent with a $\Delta^{8(17),13}$-labdadiene bearing a primary and a secondary hydroxy group. The presence of both hydroxy groups was confirmed via the di-*O*-acetyl derivative **1a**, which showed the corresponding low-field shift of the NMR signals (Table S1, Supporting Information) of the oxymethine (δ_H 5.14) and oxymethylene protons (δ_H4.60). The hydroxy groups of compound **1** were located at C-12 and C-15 by the HMBC cross peaks between the oxymethylene protons (CH_2-15) and the olefinic carbons C-13 (δ_C 142.3) and C-14 (δ_C 123.3), between the oxymethine proton (CH-12) and C-9 (δ_C 52.5), C-11 (δ_C 30.1), C-13, C-14, and C-16 (δ_C 12.2), as well as those of H-9 (δ_H 2.01) with C-8, C-10, C-11, C-12, C-17, and C-20, which also confirmed the position of the $\Delta^{8(17)}$-double bond (Figure 1). The *E*-configuration of the

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C-13 double bond was deduced from the NOESY cross peaks between H-12 and H-14 as well as those between CH_2-15 and CH_3-16 (δ_H 1.70) (Figure 1). The (12*R*)-configuration was determined by an X-ray diffraction analysis of **1** (Figure 2), if it

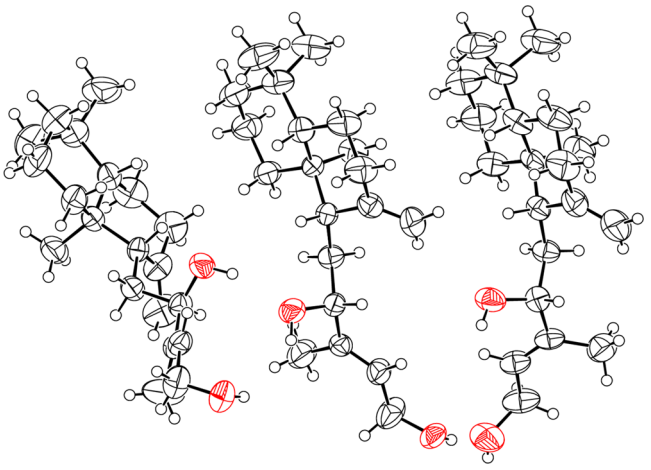

Figure 2. ORTEP projection of nicandrodiol (**1**).

is assumed that compound **1** belongs to the normal labdane series, as will be demonstrated later. Thus, the structure of nicandrodiol (**1**) was assigned as (12*R*,*E*)-labda-8(17),13-diene-12,15-diol.

Compound **2** also showed a molecular formula $C_{20}H_{34}O_2$ (HRFABMS: *m*/*z* 329.2464 $[M + Na]^+$) and IR absorptions for hydroxy groups and double bonds (3330, 1644 cm^{-1}). Analysis of the NMR spectra indicated that it has the same 2D structure as compound **1**. Some differences were observed for the H-9, C-11−C-14, and C-16 signals that in **1** appeared at δ_H 2.01, δ_C 30.1, 75.0, 142.3, 123.3, and 12.2, respectively, while in compound **2**, the signals were observed at δ_H 1.47, δ_C 28.6, 77.2, 140.2, 126.6, and 10.6, respectively. Similar differences were observed between their di-*O*-acetyl derivatives **1a** and **2a** (Tables S1 and S2, Supporting Information), suggesting that compounds **1** and **2** are C-12-epimers. The isolation of the related compound **3** and its correlation with **1** and **2** permitted clarification of this issue. The ion at *m*/*z* 302.2560 $[M]^+$ in the HRFABMS indicated the molecular formula of **3** as $C_{20}H_{32}O_2$. Its 1D and 2D NMR spectra indicated that it is a labdane, which differs from **1** and **2** by the presence of an α,β-unsaturated carbonyl moiety in the side chain. This was evident from the IR band at 1672 cm^{-1}, as well as from the carbon signals at δ_C 201.1 for the ketocarbonyl and δ_C 137.7 and 138.8 for the α and β carbons. These carbon signals were assigned to C-12, C-13, and C-14 by the correlations of H-9, H-11a, H-11b, H-14, and H-16 with C-12, and those of the hydroxymethylene protons (δ_H 4.45, 2H, CH_2−15) with C-13 and C-14, which were observed in the HMBC spectrum. NOE cross peaks between CH_2−15 and CH_3-16 (δ_H 1.76) established the *E*-configuration of the C-13 double bond (Figure 1). Thus, the structure of nicandrone (**3**) was defined as (*E*)-15-hydroxylabda-8(17),13-dien-12-one.

The relationship between compounds **1**, **2**, and **3** was established by reduction of **3** with $NaBH_4$, which afforded the labdanes **1** and **2**, thus confirming the epimeric relationship of these diols and, therefore, a (12*S*)-configuration for compound **2**, which was named 12-*epi*-nicandrodiol and its structure established as (12*S*,*E*)-labda-8(17),13-diene-12,15-diol. Furthermore, this correlation also proved that these diterpenoids belong to the normal labdane series, since compound **3** was transformed into (−)-pumiloxide (**3a**), a compound whose absolute configuration was established by semisynthesis starting from manoyl oxide.[16] The identity of **3a** was confirmed by comparison of its specific rotation and NMR data with those published.[16−18] The transformation of **3** into **3a** occurred during the acquisition of the NMR spectra of **3** ($CDCl_3$ containing traces of DCl). The same result was observed when an acidified $CHCl_3$ solution of **3** was left at room temperature for 2 h.

Compound **4** was isolated as colorless crystals. Its IR spectrum showed bands for double bonds (1642 cm^{-1}), ester (1726 cm^{-1}), and hydroxy groups (3596, 3415 cm^{-1}). The HRFABMS showed a $[M + Na]^+$ peak at *m*/*z* 575.3550, indicative of a molecular formula $C_{31}H_{52}O_8$, which was in accordance with the 31 carbon signals observed in the ^{13}C NMR spectrum. Inspection of the NMR spectra revealed that, like compounds **1** and **3**, compound **4** was an (*E*)-labda-8(17),13-diene with oxygenated functions at C-12 and C-15 (Tables 3 and 4). Moreover, a set of signals between δ_H 3.2 and 4.0 in the ^1H NMR spectrum, together with the signals for an anomeric methine (δ_H 4.16, δ_C 99.3), indicated the presence of a sugar moiety. The sugar was identified as β-D-glucopyranose by the *J* values of its proton signals ($J_{1',2'}$ = 7.5 Hz; $J_{2',3'} = J_{3',4'} = J_{4',5'}$ = 9.0 Hz) and the specific rotation of the sugar[19] obtained by acid hydrolysis of **4**. This reaction also afforded a complex mixture, from which the original aglycone could not be isolated. The sugar was located at C-12 by the HMBC interactions of H-14, H_3-16, and the anomeric proton (H-1′) with C-12 and those of H-12 with C-11, C-14, C-16, and C-1′ (Figure 1). The presence of an isovaleryl group was evident from the NMR signals at δ_C173.0 (C-1″), δ_H 2.19 d, δ_C 43.5 (CH_2−2″), δ_H 2.10 hept, δ_C 25.7 (CH-3″), δ_H 0.96 d, and δ_C 22.5 (CH_3-4″, CH_3-5″). This group was located at C-15 by the chemical shift of the CH_2-15 protons (δ_H 4.67 and 4.60) and their connectivities, in the HMBC spectrum, with C-13, C-14, and C-1″. In order to determine the C-12 configuration, the tetra-*O*-acetyl derivative of **4** was synthesized (Tables 3 and 4). This crystalline derivative (**4a**) was subjected to an X-ray diffraction analysis whose results (Figure 3) confirmed the proposed structure and showed a (12*S*) absolute configuration. Thus, the structure of nicandroside A (**4**) was determined to

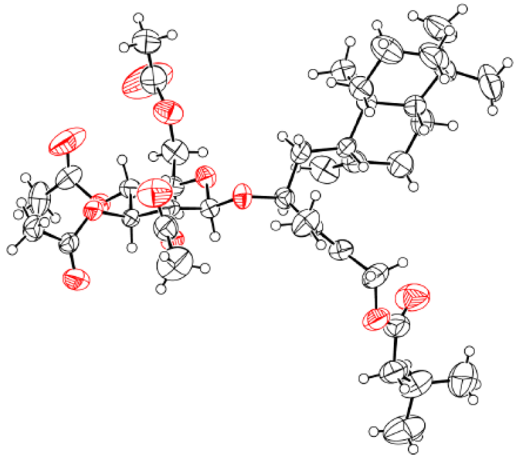

Figure 3. ORTEP projection of tetra-*O*-acetylnicandroside A (**4a**).

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be (12*S*,13*E*)-15-*O*-isovaleryllabda-8(17),13-diene-12,15-diol 12-*O*-*β*-D-glucopyranoside.

Compound **5** was characterized as the deisovaleryl derivative of **4**, since, in addition to the absence of the IR absorption for the ester carbonyl, it showed an $[M + Na]^+$ ion at *m*/*z* 491.2969, which is in accordance with the molecular formula $C_{26}H_{44}O_7$. The NMR data (Tables 3 and 4) of **4** and **5** were quite similar, except those of CH_2-15, which in compound **5** appeared at higher field (δ_H 4.31 and 3.97; δ_C 58.3) compared to those of compound **4**, thus supporting the presence of a hydroxy group connected to CH_2-15 in **5**. The penta-*O*-acetyl derivative **5a** obtained after acetylation proved the presence of this group. Compounds **4** and **5** were chemically correlated through the base hydrolysis[20] of **4** that produced **5**, which was indistinguishable from the natural product (R_f, ^{1}H NMR, and $[\alpha]_D$[20]). Thus, the structure of nicandroside B (**5**) was defined as (12*S*,13*E*)-labda-8(17),13-diene-12,15-diol 12-*O*-*β*-D-glucopyranoside.

Labdanes **6** and **7** were isolated as a ∼3:2 mixture. The molecular formula of each of these compounds was determined as $C_{20}H_{34}O_3$ by HRFABMS (*m*/*z* 323.2584 $[M + H]^+$). The ^{13}C NMR spectrum showed a single set of signals for the carbons of the decalin (C-1 to C-10) and for C-18−C-20 of both compounds (Table 2). The ^{1}H NMR spectrum also exhibited a single set of signals for the protons associated to these carbons (Table 1). Comparison of these signals with those of **1** showed a close similarity, thus indicating the same substitution in the decalin residue. On the contrary, each of the components of the **6**/**7** mixture generated a set of NMR signals for the side chain that resemble each other. These signals indicated the presence of a terminal $\Delta^{13(16)}$-double bond (δ_C 151.1/151.6, C-13; δ_C 111.9/112.5, δ_H 5.20 and 5.14/5.17, CH_2-16) and three hydroxy groups located at C-12 (δ_C 72.1/71.6, δ_H 4.20/4.24 dd, *J* = 10.0, 1.5 Hz; CH-12), C-14 (δ_C 73.3/73.2, δ_H 4.38/4.40 dd, *J* = 7.0, 4.0 Hz; CH-14), and C-15 (δ_C 66.2/66.7, δ_H 3.73/3.75 dd, *J* = 11.0, 4.0 Hz, δ_H 3.66/3.68 dd, *J* = 11.0, 7.0 Hz; CH_2−15). The position of these groups was determined by the HMBC cross peaks of H-12 with C-9, C-11, C-13, C-14, and C-16, of H-16 with C-12 and C-14, as well as those of H-14 with C-12 and C-16 (Figure 1). The mixture of the tri-*O*-acetyl derivatives **6a**/**7a** (Tables S1 and S2, Supporting Information) obtained via acetylation, confirmed the presence of the hydroxy groups. These data indicated an 8(17),13(16)-labdadiene-12,14,15-triol structure for **6**/**7** and a C-14 epimeric relationship between them, like that exhibited by the mixtures of physanicantriols (**10**/**11**) previously isolated from *P. nicandroides*.[8] The epimeric relationship at C-14 was confirmed when a single product, the aldehyde **8**, was obtained by oxidation of **6**/**7** with $NaIO_4$. Compound **8** showed a molecular formula $C_{19}H_{30}O_2$ (HRESIMS: *m*/*z* 291.23146 $[M + H]^+$), NMR signals for a conjugated formyl moiety (δ_C 194.7, δ_H 9.58, CH-14; δ_C 153.1, C-13; δ_C 133.6, δ_H 6.48 and 6.05, CH_2−16), and a hydroxymethine moiety (δ_C 68.0, δ_H 4.53 br d, *J* = 10.0 Hz; CH-12). Examination of the ^{1}H NMR data of several 12-hydroxylabdanes showed that the chemical shift of H-9 signal is affected by the configuration at C-12. Thus, in the (12*R*) compounds nicandrodiol (**1**) and physacoztomatin,[4] H-9 resonates at ∼δ_H 2.05, while in their 12-*O*-acetyl derivatives it was observed at ∼δ_H 1.70.[7] When the C-12 configuration is *S* as in **2**, H-9 appeared at ∼δ_H 1.5 and at ∼δ_H 1.40 in its acetyl and glucosyl derivatives (**2a**, **4**, **4a**, **5**, and **5a**). In compounds **6**, **7**, and **8**, H-9 resonates at ∼δ_H 2.05 and at ∼δ_H 1.70 in the acetyl derivatives **6a**/**7a**; thus, a (12*R*) configuration was proposed. This was confirmed by chemical correlation between **1** and **6**/**7** through the photo-oxidation of compound **1**, which gave a mixture of **6**/**7**, that upon $NaIO_4$ oxidation afforded compound **8**. The semisynthetic and natural compounds showed the same R_f values (TLC) and the same sets of signals in the ^{1}H and ^{13}C NMR spectra (Figures S43, S44, and S50, Supporting Information). Compounds **6** and **7** were identified as the C-14 epimers of (12*R*)-labda-8(17),13(16)-diene-12,14,15-triol and were named nicantriol and 14-*epi*-nicantriol.

Compound **9** was isolated after acetylating a complex mixture. The spectroscopic data of this tri-*O*-acetyl derivative indicated the structure of an 8(17),13-labdadiene with acetoxy groups at C-12, C-15, and C-16, as deduced from the ^{1}H NMR signals at δ_H 5.23 (dd, *J* = 11.0, 2.0 Hz; H-12), 4.72 (dd, *J* = 14.0, 6.5 Hz; H-15a), 4.70 (dd, *J* = 14.0, 6.5 Hz; H-15b), 4.70 (d, *J* = 13.0 Hz; H-16a), and 4.66 (d, *J* = 13.0 Hz; H-16b). This was confirmed by the long-range HMBC connectivities of H-14 (δ_H 5.82 td, *J* = 6.5, 0.5 Hz) with C-12 (δ_C 74.5), C-15 (δ_C 60.3), and C-16 (δ_C 59.5). The (13*E*)-configuration was determined via the NOESY interactions of H-12 with H-14 and H_2-16, while the (12*R*)-configuration was defined based on the chemical shift of H-9 at δ_H1.68. Compound **9** was named triacetylisonicantriol and its structure determined as the tri-*O*-acetyl derivative of (12*R*,13*E*)-labda-8(17),13-diene-12,15,16-triol.

The molecular formula of compound **14** was determined as $C_{30}H_{40}O_8$ by the $[M + Na]^+$ ion at *m*/*z* 551.2628 in the HRESIMS. It was identified as a withanolide by the characteristic NMR signals for a 2-en-1-one moiety in ring A (δ_C 203.8, C-1; δ_C 128.8, δ_H 6.00, CH-2; δ_C 145.0, δ_H 6.86, CH-3; and δ_C 33.0, δ_H 2.98 and 1.94, CH_2−4); an *α*,*β*-unsaturated-*δ*-lactone (δ_C 80.94, δ_H 4.24, CH-22; δ_C 31.6, δ_H 2.37 and 2.05, CH_2−23; δ_C 148.5, C-24; δ_C 122.2, C-25 and δ_C 165.5, C-26); and for two tertiary, one secondary, and two vinylic methyl groups (Tables 5 and 6). The presence of a 5*β*,6*β*-epoxy group was deduced from the chemical shift of the C-5 (δ_C 61.3) and CH-6 (δ_C 63.8, δ_H 3.21 d, *J* = 2.5 Hz) signals.[3,21,22] One acetoxy (δ_C 169.7 and 21.6, δ_H 1.99 s) and two hydroxy groups were present in **14** (IR 1730, 3413 cm^{-1}). The chemical shifts of C-20 (δ_C 75.3) and CH_3-21 (δ_C 21.2, δ_H 1.44), the multiplicity of the H-22 signal (dd), and the HMBC correlation of the OH proton with C-17 (δ_C 54.1) permitted location of the first OH-group (δ_H 3.06 br s) at C-20. The second hydroxy group was connected to C-14 as indicated by the chemical shift of C-14 (δ_C 82.8). The chemical shift of H-15 (δ_H 4.99 d, *J* = 6.5 Hz) and its HMBC correlations with C-13 (δ_C 48.3), C-14, C-17, and with the acetoxy carbonyl (Figure 1), located this ester group at C-15. The NOESY correlation between CH_3-18 and CH_3-21 and a W-coupling between the HO-20 proton and H-17, observed in the COSY spectrum, established the *β*-orientation of the side chain. NOESY correlations of **14** were not conclusive to determine the orientation of HO-14 and AcO-15 groups. They were proposed as *β*- and *α*-oriented, respectively, by the close similarity of the NMR signals of ring D to those of withaneomexolides B-D.[21] Configurations of C-20 and C-22 were assumed as *R*, but this will be confirmed later. Thus, the structure of physanicandrolide A (**14**) was defined as (20*R*,22*R*)-15*α*-acetoxy-5*β*,6*β*-epoxy-14*β*,20-dihydroxy-1-oxo-witha-2,24-dienolide.

The HRESIMS $[M + Na]^+$ ion at *m*/*z* 583.2855 and the ^{13}C NMR data of compound **15** indicated a molecular formula of

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Table 1. ^{1}H NMR Data of Compounds 1−3 and 6−9 ($CDCl_3$, 500 MHz)

position	1	2	3	6/7	8	9[a]
1	1.09 ddd (12.5, 12.5, 2.5)	0.92 ddd (12.5, 12.5, 4.0)	1.09 ddd (13.0, 13.0, 3.5)	1.09 br dd (13.0,13.0)	1.06 ddd (13.0,13.0, 4.0)	1.01 ddd (13.0, 13.0, 4.0)
	1.72 m	1.73 m	1.54 m	1.71 m	1.68 m	1.71 m
2	1.51 m	1.49 m	1.46 m	1.52 m	1.50 m	1.52 m
	1.56 m	1.57 m	1.58 m	1.59 m	1.56 m	1.59 m
3	1.20 ddd (13.0, 13.0, 4.5)	1.17 ddd (13.0, 13.0, 4.0)	1.20 ddd (13.0, 13.0, 4.5)	1.21 m	1.19 ddd (13.5, 13.5, 4.5)	1.19 ddd (13.5, 13.5, 4.0)
	1.39 dddd (13.0, 3.0, 3.0, 1.5)	1.39 dddd (13.0, 3.5, 3.5, 1.0)	1.40 dddd (13.0, 3.5, 3.5, 1.5)	1.40 br d (14.0)	1.39 br d (13.0)	1.41 br d (13.5)
5	1.18 dd (12.5, 2.5)	1.06 dd (12.5, 3.0)	1.24 dd (13.0, 2.5)	1.18 dd (12.5, 2.5)	1.18 dd (12.5, 2.5)	1.09 dd (13.0, 2.5)
6	1.75 m	1.73 m	1.73 m	1.75 m	1.74 m	1.73 m
	1.34 dddd (13.0, 13.0, 13.0, 4.0)	1.31 dddd (13.0, 13.0, 13.0, 4.0)	1.32 dddd (13.0, 13.0, 13.0, 4.0)	1.34 dddd (13.0, 13.0, 13.0, 4.5)	1.35 dddd (13.0, 13.0, 13.0, 4.5)	1.31 dddd (13.0, 13.0, 13.0, 4.5)
7	2.41 ddd (12.5, 4.0, 2.5)	2.38 ddd (12.5, 4.0, 2.5)	2.37 ddd (13.0, 4.0, 2.5)	2.41 ddd (13.0, 4.0, 2.0)	2.41 ddd (13.0, 4.0, 2.5)	2.39 ddd (13.0, 4.0, 2.5)
	2.04 ddd (12.5, 12.5, 5.0)	1.93 ddd (12.5, 12.5, 5.0)	2.13 ddd (13.0, 13.0, 5.0)	2.05 m	2.04 ddd (13.0, 13.0, 5.0)	1.95 ddd (13.0, 13.0, 5.5)
9	2.01 m	1.47 dd (10.0, 3.0)	2.55 br d (10.0)	2.02 m	2.07 br d (10.0)	1.68 m
11	1.58 m	1.70 m	2.98 dd (17.0, 10.0)	1.76 m	1.71 m	1.82 br dd (13.5, 11.0)
	1.58 m	1.67 m	2.59 dd (17.0, 3.5)	1.65 m	1.63 m	1.64 dd (13.5, 2.0)
12	4.05 dd (8.5, 3.0)	4.13 dd (9.0, 6.0)		4.20/4.24 dd (10.0, 1.5)	4.53 br d (10.0)	5.23 dd (11.0, 2.0)
14	5.63 tdq (6.5, 1.5, 1.0)	5.52 br t (7.0)	6.73 br tq (5.5, 1.5)	4.38/4.40 br dd (7.0, 4.0)	9.58 s	5.82 td (6.5, 0.5)
15	4.22 br dd (13.5, 6.5)	4.24 br dd (12.5, 7.0)	4.45 br dt (5.5, 1.0)	3.73/3.75 dd (11.0, 4.0)		4.72 dd (14.0, 6.5)
	4.19 br dd (13.5, 6.5)	4.19 br dd (12.5, 6.5)		3.66/3.68 dd (11.0, 7.0)		4.70 dd (14.0, 6.5)
16	1.70 dt (1.5, 1.0)	1.67 br s	1.76 dt (1.5, 1.0)	5.20 s	6.48 s	4.70 d (13.0)
				5.14/5.17 s	6.05 s	4.66 d (13.0)
17	4.85 m	4.87 m	4.69 br dd (3.0, 1.5)	4.85 br d (1.5)	4.89 br d (1.5)	4.86 br d (1.5)
	4.47 br d (1.5)	4.68 br d (1.5)	4.24 br dd (3.0, 1.5)	4.42/4.45 br d (1.5)	4.71 br d (1.0)	4.49 br d (1.5)
18	0.88 s	0.87 s	0.89 s	0.88 s	0.88 s	0.88 s
19	0.81 s	0.80 s	0.82 s	0.81 s	0.80 s	0.80 s
20	0.67 s	0.69 s	0.74 s	0.69/0.68 s	0.66 s	0.67 s

[a]Acetyl: δ 2.06 (6H); δ 2.05 (3H).

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Table 2. ^{13}C NMR Data of Compounds 1−3 and 6−9 ($CDCl_3$, 125 MHz)

position	1	2	3	6/7	8	9[c]
1 CH_2	39.0	39.0	39.2	39.1	39.0	39.1
2 CH_2	19.3	19.4	19.3	19.3	19.3	19.3
3 CH_2	42.1	42.1	42.0	42.1	42.1	42.1
4 C	33.6	33.6	33.5	33.6	33.6	33.6
5 CH	55.5	55.6	55.1	55.5	55.5	55.6
6 CH_2	24.4	24.4	24.0	24.4	24.4	24.3
7 CH_2	38.3	38.3	38.3	38.3	38.3	38.2
8 C	149.1	149.1	149.6	149.1/149.0	148.5	148.2
9 CH	52.5	53.2	51.6	52.5/52.6	52.1	52.6
10 C	39.3	39.5	39.0	39.4	39.3	39.3
11 CH_2	30.1	28.6	32.9	31.0/30.6	30.9	28.9
12 CH	75.0	77.2	201.1[a]	72.1/71.6	68.0	74.5
13 C	142.3	140.2	137.7	151.1/151.6	153.1	138.4
14 CH	123.3	126.6	138.8	73.3/73.2	194.7	126.7
15 CH_2	59.1	59.2	60.2	66.2/66.7		60.3
16 CH_3	12.2	10.6	11.9	111.9/112.5[b]	133.6[b]	59.5[b]
17 CH_2	106.5	106.7	106.1	106.4/106.6	107.1	106.6
18 CH_3	33.6	33.5	33.6	33.6	33.6	33.5
19 CH_3	21.7	21.7	21.8	21.7	21.7	21.7
20 CH_3	14.6	14.6	14.8	14.6	14.6	14.6

[a]C. [b]CH_2. [c]Ac: δ_C170.7, 170.3, 170.5, 21.2, 20.9, 20.8.

$C_{31}H_{44}O_9$. The NMR data were similar to those of **14**, except for the signals concerning the A-ring, which were in accordance with the presence of a 3-methoxycyclohexan-1-one moiety, as deduced from the signals for a ketocarbonyl at δ_C 210.9 (C-1), an α-methylene (δ_C 42.7, δ_H 2.77 dd, J = 14.0, 6.0 Hz and δ_H 2.67 ddd, J = 14.0, 4.5, 1.0 Hz; CH_2-2), an oxymethine (δ_C 72.9, δ_H 3.75 br s; CH-3), another methylene (δ_C 36.2, δ_H 2.22 dd, J = 14.5, 4.0 Hz and δ_H 1.59 m; CH_2-4), and a methoxy group (δ_C 55.9, δ_H 3.27). The NOESY interactions of CH_3-19 with H-2a (δ_H 2.77), H-4a (δ_H 2.22), and H-8 (δ_H 1.83) established the β-axial orientation of these protons, which was confirmed by the W-coupling (J = 1.0 Hz) between the equatorial H-2b (δ_H 2.67) and H-4b (δ_H 1.59) protons, observed in the COSY spectrum (Figure 1). The magnitude of the coupling constants of H-3 was consistent with an eq−ax relationship with H-2a (J = 6.0 Hz), an eq−eq relationship with 2b (J = 4.5 Hz), and an eq−ax relationship with H-4a (J = 4.0 Hz), indicating an equatorial disposition of H-3. The observed strong NOESY cross-peaks of H-3 with the vicinal β-axial protons and its weak interactions with the α-equatorial H-2b and H-4b protons established the α-orientation of the CH_3O-3 group (Figure 1). The H−H distances measured in the energy-minimized 3D structure of **15** (Figure S76 and Table S3, Supporting Information) together with the similarity of the NOESY correlations and J values of the ring A protons of **15** and those of the 3α-methoxywithanolide, physangulatin I, whose structure was determined by X-ray analysis, gave further support to this assumption.[23] Therefore, the structure of physanicandrolide B (**15**) was determined as (20*R*,22*R*)-15α-acetoxy-5β,6β-epoxy-14β,20-dihydroxy-3α-methoxy-1-oxowith-24-enolide. This compound could be an artifact produced by Michael addition of MeOH to compound **14**, however, this must be proven since the natural or artificial origin of 3-OH and 3-alkoxy withanolides is controversial.[11,24]

Compound **16** was isolated as colorless crystals with a molecular formula $C_{30}H_{41}ClO_8$, determined by the $[M + H]^+$ ion at m/z 565.2562 in the HRFABMS. The ^1H and ^{13}C NMR signals of **16** were closely related to those of **14**, except those concerning to ring B, which indicated the presence of a 5α-chloro-6β-hydroxywithanolide. This was deduced from the chemical shifts of H-4β (δ_H 3.56 dt, J = 20.5, 2.5 Hz), C-5 (δ_C 80.9), and CH-6 (δ_H 4.10 br s; δ_C 74.5), as well as from the small coupling constants of H-6, which were in accordance with its equatorial orientation.[22] This was confirmed by the NOESY cross peak between the α-equatorial H-4 (δ_H 2.54 dd, J = 20.5, 5.0 Hz) and H-6. Finally, the structure and the absolute configuration of **16**, especially those of D-ring and side chain were confirmed as (14*S*,15*S*,17*S*,20*R*,22*R*) by X-ray diffraction analysis of its *O*-acetyl derivative **16a** (Figure 4), using the anomalous dispersion of the chlorine atom [Flack parameter 0.00 (4)]. These results can be extended to **14** and **15** by the similarity of their NMR data. Thus, the structure of physanicandrolide C (**16**) was defined as (20*R*,22*R*)-15α-acetoxy-5α-chloro-6β,14β,20-trihydroxy-1-oxowitha-2,24-dienolide.

In addition to the new compounds, the investigation of *P. nicandroides* led to the isolation of the known labdanes: (−)-(13*E*)-labd-13-ene-8α,15-diol (**12**),(+)-(*Z*)-labda-8-(17),13-diene-15,16-diol (**13**), and the mixture of physanicantriol and 14-*epi*-physanicantriol (**10**/**11**), previously isolated from *P. nicandroides*.[8] The flavonol 3,7-di-*O*-methylquercetin (**18**)[25,26] and the known phytosterols β-sitosterol/stigmasterol and physalindicanol A (**17**)[27] were also isolated.

The acetylcholinesterase inhibitory activity of compounds **1**−**5**, **3a**, **4a**, **10**−**17**, and **16a** was evaluated. At the highest used concentration (100 μM) most of these compounds increased the enzymatic activity in a range of 6.7 to 96% except compounds **3a**, **13**, and **17** that moderately inhibited the activity in 57.5, 33.9, and 24%, respectively (Figure S77 and Table S4, Supporting Information). The IC_{50} of the most active compound **3a** was determined, but it resulted 4 orders of magnitude higher (IC_{50} = 64.4 μM) than that of the AChE inhibitor, physostigmine (IC_{50} = 3.0 nM). The ability of compounds **1**-**5**, **4a**, **10**-**17**, and **16a** to inhibit the activity of yeast α-glucosidase was also evaluated. In this case, a slight activation of the enzyme by the labdanes (from 1.1 to 29.4%,

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Table 3. ^{1}H NMR Data of Compounds 4, 4a, 5, and 5a (500 MHz, $CDCl_3$)

position	4	4a	5	5a
1	1.70 m	1.69 m	1.72 m	1.69 m
	0.86 m	0.85 m	0.86 m	0.85 m
2	1.55 br dd (13.5, 13.5)	1.52 br dd (13.5, 13.5)	1.56 br dd (13.5, 13.5)	1.55 br dd (13.5, 13.5)
	1.48 m	1.45 m	1.49 m	1.45 m
3	1.39 m	1.37 br t (13.0)	1.37 m	1.37 m
	1.15 m	1.14 ddd (13.5, 13.5, 4.0)	1.17 m	1.14 ddd (13.0, 13.0, 4.0)
5	1.01 dd (12.0, 2.5)	0.99 m	1.04 dd (12.0, 2.5)	0.99 m
6	1.70 m	1.69 m	1.70 m	1.69 m
	1.29 dddd (13.0, 13.0, 13.0, 4.0)	1.29 dddd (13.0, 13.0, 13.0, 4.0)	1.28 m	1.29 dddd (13.0,13.0,13.0, 4.0)
7	2.35 br d (12.5)	2.34 ddd (13.0, 4.0, 2.5)	2.36 br d (12.0)	2.34 ddd (13.0, 4.0, 2.5)
	1.85 ddd (13.0,12.5, 4.5)	1.84 ddd (13.0,13.0, 4.5)	1.87 m	1.84 ddd (13.0,13.0, 4.5)
9	1.37 m	1.32 m	1.37 m	1.32 m
11	1.76 m	1.70 m	1.79 m	1.70 m
	1.65 m	1.70 m	1.70 m	1.70 m
12	4.24 dd (11.0, 4.0)	4.19 dd (9.5, 7.5)	4.17 dd (10.5, 3.0)	4.19 dd (9.5, 7.5)
14	5.38 br t (7.0)	5.38 br t (7.0)	5.50 br t (7.0)	5.38 br t (7.0)
15	4.67 dd (13.0, 7.0)	4.65 dd (13.0, 7.0)	4.31 dd (12.0, 8.0)	4.65 dd (13.0, 7.0)
	4.60 dd (13.0, 7.0)	4.61 dd (13.0, 7.0)	3.97 dd (12.0, 4.0)	4.61 dd (13.0, 7.0)
16	1.66 s	1.58 s	1.64 s	1.57 s
17	4.83 br s	4.82 br s	4.85 br s	4.82 br s
	4.65 br s	4.63 br s	4.68 br s	4.63 br s
18	0.86 s	0.85 s	0.87 s	0.85 s
19	0.79 s	0.78 s	0.80 s	0.78 s
20	0.68 s	0.67 s	0.70 s	0.67 s
1′	4.16 d (7.5)	4.35 d (8.0)	4.24 d (8.0)	4.35 d (8.0)
2′	3.39 dd (9.0, 7.5)	4.98 dd (9.5, 8.0)	3.32 br t (8.5)	4.98 dd (9.5, 8.0)
3′	3.48 dd (9.0, 9.0)	5.15 dd (9.5, 9.5)	3.47 t (9.0)	5.15 dd (9.5, 9.5)
4′	3.61 dd (9.0, 9.0)	5.05 dd (9.5, 9.5)	3.50 t (9.0)	5.05 dd (9.5, 9.5)
5′	3.21 br d (9.0)	3.61 ddd (9.5, 5.5, 2.5)	3.29 m	3.61 ddd (9.5, 5.5, 2.5)
6′	3.83 dd (12.5, 3.0)	4.23 dd (12.0, 5.5)	3.79 m	4.23 dd (12.0, 5.5)
	3.80 dd (12.5, 3.0)	4.10 dd (12.0, 2.5)	3.79 m	4.10 dd (12.0, 2.5)
2′′	2.19 d (7.0)	2.21 d (7.0)		
3′′	2.10 br hept[a] (7.0)	2.11 br hept[a] (7.0)		
4′′, 5′′	0.96 d (7.0, 6H)	0.98 d (7.0, 6H)		
Ac		2.08, 2.01 (6H), 1.99		2.08, 2.01 (6H), 1.99

[a]hept = heptuplet.

100 μM) was observed, except for **4a** and **13** which, like the steroidal compounds **14**-**17**, and **16a** showed poor inhibition.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points (uncorrected) were determined on a Fisher-Johns melting point apparatus (Fisher Scientific, U.S.A.). Optical rotations were measured on a PerkinElmer 343 polarimeter. IR spectra were measured on a Nicolet FTIR-Magna 750 or Bruker Tensor 27 spectrophotometers. NMR spectra were recorded on a Varian 500 spectrometer (^{1}H at 500 MHz; ^{13}C at 125 MHz), with TMS as internal standard. HRMS were measured on a JEOL JMS-SX102A, JEOL MStation JMS-700, Bruker micrOTOF II or Jeol AccuTOF JMS-T100LC mass spectrometers. Vacuum column chromatography (VCC) was performed on silica gel 60 G (Macherey-Nagel). Analytical TLC was carried out on precoated Alugram Sil G/UV_{254} plates. Preparative TLC was performed on precoated Sil G-200 UV254 plates (Macherey-Nagel). Reversed-phase HPLC analysis was performed on a Waters 600 apparatus (Macrosphere C_{18}, 250 × 2.1 mm, 5 μm) equipped with a photodiode array detector (λ = 200−650 nm). X-ray crystallographic analysis was carried out on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ 0.71073 Å). The structures were solved by direct methods using the SHELXS program.[28] Non-hydrogen atoms were refined with anisotropic displacement parameters using the SHELXL program.[28] Hydrogen atoms, except those bonded to oxygen atoms, were included at calculated positions and were not refined.

Plant Material. *Physalis nicandroides* var. *attenuata* Waterf. was collected in Tlayacapan, State of Morelos, Mexico, in August 2004. A voucher specimen of the plant (M. Martinez s/n) was identified by Dr. Mahinda Martínez and deposited at the Herbarium of the Universidad Autónoma de Querétaro.

Extraction and Isolation. Dried and ground aerial parts of the plant, without fruits and calices (3.3 kg), were extracted with MeOH. The extract (578 g) was dissolved in MeOH/H_2O (4:1, 1 L) and partitioned with hexanes to obtain a hexanes-soluble extract (197.2 g). After removal of MeOH from the aq MeOH solution and addition of 800 mL of H_2O, this fraction was extracted with EtOAc to obtain an EtOAc-soluble (81.47 g) and a H_2O-soluble (297.0 g) extract. The hexanes-soluble extract was fractioned by silica gel VCC, eluted with hexanes-acetone mixtures of increasing polarity to obtain fractions: A1-A6 (1:0), A7-A22 (19:1), A23-A37 (9:1), A38-A45 (17:3), A46-A51 (4:1), A52-A60 (7:3), A61-A63 (3:2), A64-A74 (2:3), A75-A76 (1:4), and A77 (0:1). Fractions A8−A25 (63.0 g) were discolored with activated charcoal and fractioned by silica gel VCC with a gradient of hexanes−EtOAc to give fractions B1−B11 (39:1), B12−B17 (95:5), B18−22 (9:1), B23−B29 (17:3), B30−B34 (4:1), and B35−B37 (3:1). A mixture of β-sitosterol and stigmasterol (3.51g) was obtained from fractions B5−B10. Fractions B11−B37 (13.64 g) were purified by VCC (hexanes−EtOAc 19:1 to 4:1) to obtain fractions B′1−B′44. Repeated VCC of fractions B′10−B′17 (7.73 g)

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Table 4. ^{13}C NMR Data of Compounds 4, 4a, 5, and 5a (125 MHz, $CDCl_3$)

position	4	4a[a]	5	5a[b]
1 CH_2	39.0	39.0	39.1	39.0
2 CH_2	19.4	19.4	19.5	19.3
3 CH_2	42.1	42.1	42.1	42.1
4 C	33.6	33.6	33.5	33.5
5 CH	55.6	55.6	55.5	55.7
6 CH_2	24.3	24.3	24.4	24.3
7 CH_2	38.2	38.2	38.3	38.2
8 C	148.2	148.0	148.2	148.0
9 CH	52.4	52.4	52.4	52.4
10 C	39.3	39.3	39.4	39.3
11 CH_2	26.8	26.9	26.9	26.9
12 CH	82.2	82.9	83.0	82.8
13 C	138.6	138.6	136.5	138.5
14 CH	125.1	124.9	130.0	124.8
15 CH_2	60.4	60.2	58.3	60.6
16 CH_3	10.4	10.1	10.5	10.1
17 CH_3	106.9	106.9	107.0	106.9
18 CH_3	33.5	33.5	33.6	33.5
19 CH_3	21.7	21.7	21.8	21.7
20 CH_3	14.7	14.5	14.7	14.5
1′ CH	99.3	97.8	99.2	97.8
2′ CH	73.3	71.3	73.3	71.3
3′ CH	76.6	73.1	76.5	73.0
4′ CH	69.8	68.8	70.1	68.8
5′ CH	75.3	71.7	75.3	71.6
6′ CH_2	61.8	62.3	61.9	62.3
1″ C	173.0	172.8		
2″ CH_2	43.5	43.5		
3″ CH	25.7	25.7		
4″, 5″ CH_3	22.5	22.5		

[a]Ac signals of **4a**: δ_C 170.6, 170.3, 169.4, 169.2, 20.74, 20.69, 20.64, 20.62. [b]Ac signals of **5a**: δ_C170.7, 170.6, 170.3, 169.4, 169.2, 20.9, 20.7, 20.63, 20.59, 20.57.

using hexanes−*i*PrOH 98:2 as eluent, afforded compound **3** (3.33 g). Fractions B′18−B′26 (1.83 g) were combined and purified by repeated VCC eluted with hexanes−EtOAc 9:1 to obtain 36.7 mg of **17**. Fractions A26−A52 were combined (24.26 g), discolored with activated charcoal, and fractioned by VCC eluted with hexanes−acetone gradient (9:1 to 7:3) to give fractions C1−C23 (9:1), C24−C29 (17:3), C30−C32 (4:1), and C33−C37 (7:3). Fractions C4−C26 (18.30 g) were subjected to a VCC using mixtures of hexanes−EtOAc 3:1 as eluent to afford fractions D1−D39. Compound **1** was obtained from fractions D3−D17 by crystallization from EtOAc−hexanes. Mother liquors of **1** were combined with fractions D18−D33 (11.37 g) and subjected to repeated VCC (benzene−EtOAc 4:1) and crystallization (EtOAc−hexanes) to obtain **1** (total yield 4.17 g). Two successive VCCs of the mother liquors of **1** (hexanes−acetone 4:1 and hexanes−EtOAc 3:1) gave compound **12** (898 mg). The combined fractions A53−A77 (74.5 g) were discolored with activated charcoal to afford 53.7 g of residue which was fractioned by VCC using a gradient of hexanes−EtOAc−MeOH to give fractions E1−E47 (60:40:0.6), E48−E52 (55:45:0.6), E53−E55 (50:50:0.6), and E56−E58 (40:60:0.6). Fractions E17−E56 were repeatedly subjected to VCC (benzene−EtOAc 3:2) and crystallization (hexanes) to obtain **4** (15.95 g).

The EtOAc-soluble extract (81.47 g) was fractioned by VCC and eluted with hexanes−acetone mixtures of increasing polarity to obtain fractions: F1−F6 (19:1), F7−F18 (9:1), F19−F26 (17:3), F27−F32 (4:1), F33−F47 (7:3), F48−F65 (6:4), F66−F81 (1:1), F82−F95 (2:3), F96−F108 (1:4), and F109−F113 (0:1). Fractions F4−F8 (2.84 g) were subjected to repeated VCC (hexanes−EtOAc 9:1) and preparative TLC (hexanes−$CHCl_3$1:3) to afford **3** (43 mg). Fractions F9−F32 (12.2 g) were discolored with activated charcoal and fractioned by VCC eluted with hexanes−acetone to obtain fractions G1−G44 (9:1) and G45−G80 (17:3). Compound **1** was obtained from fractions G26−G40. Mother liquors of **1** and fractions G14−G25 and G41−G68 were mixed and subjected to VCC eluted with hexanes−EtOAc 7:3 to obtain fractions H1−H88. Fractions H18−H26 gave **1** (1.43 g). Fractions H41−H43 gave 93.4 mg of **2**, and fractions H51−H54 afforded 121.2 mg of **13**. Fractions F33−F39 (6.92 g) were subjected to VCC eluted with mixtures of hexanes−EtOAc 7:3 to 1:1 to afford fractions I1−I98. Crystallization of fraction I9−I11 (EtOAc-hexanes) gave **18** (17.4 g). Compound **14** was obtained from fractions I15−I31. Mother liquors of **14** and **18** were mixed with fractions I12−I14 and purified by VCC using a gradient of hexanes−EtOAc (7:3 to 1:1) to give fractions J1−J25 (7:3), J26−J33 (3:2), J34−J38 (1:1). Fractions J31−J38 (3.94 g) were purified by VCC eluted with hexanes−EtOAc 7:3 to afford fractions K1−K60. Fractions K30−K40 gave **14** (2.84 g). Fractions J10−J30 and K10−K24 were combined and purified by two successive VCCs (hexanes−EtOAc 7:3; $CHCl_3$−acetone 9:1) to give 246 mg of a mixture of **6**/**7**. Fractions I32−I65 were subjected to VCC ($CHCl_3$−acetone 9:1) to give fractions L1−L54. Compound **15** (47 mg) was obtained from fractions L9−L18 after repeated VCC ($CHCl_3$−acetone 9:1). Fractions L19−L30 were purified by VCC ($CHCl_3$−acetone 17:3) to obtain compound **16** (63 mg). Compounds contained in fractions I88−I93 could not be purified; therefore, a portion (48.9 mg) was acetylated, and the reaction mixture was purified by preparative TLC (hexanes−EtOAc 17:3) to obtain **9** (11.9 mg). Fractions F40−F68 and I66−I94 contained compound **4**; therefore, they were combined (42.60 g), discolored with activated charcoal and purified by three consecutive silica gel VCC's (hexanes−acetone 7:3; hexanes−acetone 4:1; benzene−EtOAc 4:1) to give two fractions enriched in compound **4**: M1 (25.07 g, 32%) and M2 (14.15 g, 48%), indicating a yield of 14.95 g of **4** in these fractions. The percentages were determined by reversed-phase HPLC eluted with MeOH−H_2O 7:3 to 19:1. Fractions F69−F91 (13.82 g) were discolored with activated charcoal and purified by VCC eluted with EtOAc to obtain fractions N1−N11. Fractions N3−N7 were subjected to VCC using mixtures of hexanes−EtOAc 17:3 to obtain fractions O1−O80. Fractions O55−O76 (2.27 g) contained crude **5**, and 71.2 mg of these fractions were purified by preparative TLC (benzene−MeOH 9:1, × 3) to obtain pure **5** (29.6 mg). Fractions of columns I to O containing **10**/**11** were combined (4.87 g) and purified by VCC eluted with $CHCl_3$−acetone 17:3 to afford 126.7 mg of **16** and a mixture of **10**/**11** which was subjected to VCC eluted with hexanes−acetone 3:1 to obtain 3.24 g of **10**/**11**.

*Nicandrodiol (**1**):* colorless crystals (EtOAc-hexanes); mp 130−132 °C; $[\alpha]^{20}_D$ +52 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3413, 3324, 1642 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; HRFABMS *m*/*z* 306.2560 $[M]^+$ (calcd for $C_{20}H_{34}O_2$, 306.2559).

*X-ray Crystallographic Data of Nicandrodiol (**1**).*[29] The data were collected from a colorless prism (0.452 × 0.182 × 0.072 mm) 298(2) K: $C_{20}H_{34}O_2$, M_r 306.47; monoclinic, space group $P2_1$; a = 19.3184(15) Å, b = 7.2915(6) Å, c = 22.0305(17) Å; α = 90°, β = 113.7375 (17)°, γ = 90°, V = 2840.7(4) $Å^3$; Z = 6; D_{calc}= 1.075 g/cm^3; $F(000)$ = 1020.0. Reflections collected 9948; independent reflections 5673 for $I \geq 2\sigma$ (I).

*12-epi-Nicandrodiol (**2**):* colorless crystals (EtOAc−hexanes); mp 136−138 °C; $[\alpha]^{20}_D$ −9 (*c* 0.10, $CHCl_3$); IR (film) ν_{max} 3330, 1644 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; HRFABMS *m*/*z* 329.2464 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_2Na$, 306.2457).

*Nicandrone (**3**):* pale yellow oil; $[\alpha]^{20}_D$ −8 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (3.88) nm; IR (film) ν_{max} 3443, 1672, 1645 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; HRFABMS *m*/*z* 304.2560 $[M]^+$ (calcd for $C_{20}H_{32}O_2$, 304.2559).

*Nicandroside A (**4**):* colorless crystals; mp 90−94 °C; $[\alpha]^{20}_D$ −24 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3596, 3415, 1726, 1642 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Tables 3 and 4; HRFABMS *m*/*z* 575.3550 $[M + Na]^+$ (calcd for $C_{31}H_{52}O_8Na$, 575. 3560).

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Table 5. ^{1}H NMR Data of Compounds 14−16 and 16a (500 MHz, $CDCl_3$)

position	14	15[a]	16	16a[b]
2	6.00 dd (10.0, 2.5)	2.77 dd (14.0, 6.0)	5.91 dd (10.0, 2.5)	5.94 dd (10.0, 2.5)
		2.67 ddd (14.0, 4.5, 1.0)		
3	6.86 ddd (10.0, 6.0, 2.5)	3.75 br s	6.67 ddd (10.0, 5.0, 2.0)	6.62 ddd (10.0, 5.0, 2.0)
4	2.98 dt (19.0, 2.5)	2.22 dd (14.5, 4.0)	3.56 dt (20.5, 2.5)	3.02 dt (20.0, 2.5)
	1.94 dd (19.0, 6.0)	1.59 m	2.54 dd (20.5, 5.0)	2.56 dd (20.0, 5.0)
6	3.21 d (2.5)	3.26 br s	4.10 br s	5.28 t (3.0)
7	2.50 br dt (14.0, 2.5)	2.53 dt (14.5, 3.0)	2.10 m	2.12 m
	1.44 m	1.50 m	2.10 m	2.12 m
8	1.97 m	1.83 td (12.0, 3.5)	2.12 m	2.00 m
9	1.97 m	2.01 m	2.77 td (12.0, 3.5)	2.80 td (12.5, 3.5)
11	1.98 m	1.38 m	2.29 ddd (13.5, 7.0, 3.5)	2.31 ddd (13.5, 7.0, 3.5)
	1.52 m	1.35 m	1.34 m	1.33 m
12	1.98 m	1.92 m	2.09 m	2.09 m
	1.52 m	1.48 m	1.53 dt (10.0, 3.0)	1.59 m
15	4.99 d (6.5)	5.01 d (6.5)	5.07 d (6.5)	5.05 d (6.5)
16	2.37 ddd (15.0, 8.5, 6.5)	2.39 m	2.42 ddd (15.0, 7.0, 7.0)	2.41 ddd (15.0, 7.5, 3.5)
	1.58 dd (15.0, 9.5)	1.62 m	1.64 dd (15.0, 9.5)	1.64 dd (15.0, 9.0)
17	1.82 br t (9.0)	1.81 br t (9.0)	1.84 br t (8.5)	1.86 m
18	1.35 s	1.31 s	1.392 s	1.40 s
19	1.26 s	1.18 s	1.385 s	1.34 s
21	1.44 s	1.43 s	1.44 s	1.46 s
22	4.24 dd (13.5, 3.5)	4.25 dd (13.5, 3.5)	4.28 dd (13.5, 3.5)	4.27 dd (13.5, 3.5)
23	2.37 m	2.39 m	2.36 br t (13.5)	2.37 m
	2.05 br dd (13.5, 3.5)	2.02 m	2.07 m	2.09 m
27	1.89 s	1.89 s	1.88 s	1.89 s
28	1.96 s	1.96 s	1.97 s	1.97 s
14-OH	3.59 br s	3.69 br s	4.35 br s	3.65 br s
20-OH	3.06 br s	3.11 br s	4.00 br s	3.10 d (2.5)
15-OAc	1.99 s	2.05 s	2.07 s	2.07 s

[a]3-OMe: δ_H 3.27 s. [b]6-OAc: δ_H 2.13 s.

*Nicandroside B (**5**):* colorless crystals; mp 121−122 °C; $[\alpha]^{20}_D$ −39 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3360, 1643 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) see Tables 3 and 4; HRESIMS *m*/*z* 491.2969 [M + Na]$^+$ (calcd for $C_{26}H_{44}O_7Na$, 491.2979).

*Nicantriol/14-epi-nicantriol (**6**/**7**):* colorless oil; IR (film) ν_{max} 3379, 1642 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; HRFABMS *m*/*z* 323.2584 [M + H]$^+$ (calcd for $C_{20}H_{35}O_3$, 323.2586).

*Tri-O-acetylisonicantriol (**9**):* colorless oil; $[\alpha]^{20}_D$ +43 (*c* 0.3, $CHCl_3$); IR (film) ν_{max} 1744, 1643 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; FABMS *m*/*z* 471 [M + Na]$^+$ ($C_{26}H_{40}O_6Na$), 449 [M + H]$^+$, 389 [449 − AcOH]$^+$, 329 [449 − 2AcOH]$^+$, 269 [449 − 3AcOH]$^+$ (38).

*Physanicandrolide A (**14**):* colorless crystals (acetone−hexanes); mp 167−168 °C; $[\alpha]^{20}_D$ +58 (*c* 0. 1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (4.18) nm; IR (film) ν_{max} 3413, 1730 sh, 1708, 1670 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 5 and 6; HRFABMS 551.2628 [M + Na]$^+$ (calcd for $C_{30}H_{40}O_8Na$, 551.2621).

*Physanicandrolide B (**15**):* colorless crystals; mp 166−169 °C; $[\alpha]^{20}_D$ +14 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3409, 1710 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 5 and 6; HRESIMS 583.2855 [M + Na]$^+$ (calcd for $C_{31}H_{44}O_9Na$, 583.2878).

*Physanicandrolide C (**16**):* colorless crystals; mp 208−210 °C; $[\alpha]^{20}_D$ +68 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (3.91) nm; IR (film) ν_{max} 3413, 1725 sh, 1689 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 5 and 6; HRFABMS 565.2562 [M + H]$^+$ (calcd for $C_{30}H_{42}ClO_8$, 565.2568).

*Reduction of Nicandrone (**3**).* Sodium borohydride (85.3 mg) was stepwise added to a stirred solution of **3** (45.3 mg) in EtOH (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4 h, neutralized with HOAc, dried with an air stream, and partitioned between EtOAc and water. The organic layer was washed with water, dried with Na_2SO_4, and purified by preparative TLC [(hexanes−EtOAc 7:3)−MeOH 98:2, × 3] to afford 3.9 mg of **1** and 5.4 mg of **2**.

*Treatment of Nicandrenone (**3**) with Acid.* Concentrated HCl (0.1 mL) was added to a solution of **3** in $CHCl_3$ (5 mL). The mixture was stirred by 2 h at room temperature, washed with $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and purified by crystallization from EtOAc−hexanes to afford 16 mg of pumiloxide (**3a**): colorless crystals; mp 86−90 °C; $[\alpha]^{20}_D$ −21 (*c* 0.2, $CHCl_3$) [lit.[13] mp 87−89 °C; $[\alpha]^{20}_D$ −20 (*c* 0.8, $CHCl_3$)].

*Acetylation of Compounds **1**, **2**, **4**−**7**, and **16**.* Compounds **1** (48.3 mg), **2** (16.8 mg), **4** (433.5 mg), **5** (114.1 mg), **6**/**7** (41.8 mg), and **16** (32.2 mg) were acetylated with pyridine−acetic anhydride at room temperature (**16** at 45 °C). After completion, the reaction mixtures were treated in the usual way to obtain crudes **1a**, **2a**, **4a**−**7a**, and **16a**, respectively. These derivatives were purified by different techniques (**1a**:VCC hexanes−EtOAc 19:1; **2a**: preparative TLC hexanes−EtOAc 98:2; **4a**: VCC hexanes−EtOAc 88:12 and crystallization from EtOAc−hexanes; **5a**: VCC hexanes−EtOAc 4:1 and preparative TLC hexanes−EtOAc 7:3; **6a**/**7a**: VCC hexanes−EtOAc 95:5; **16**: crystallization from EtOAc−hexanes). Yield: 52.4 mg of **1a**, 13.2 mg of **2a**, 487 mg of **4a**, 88 mg of **5a**, 53.5 mg of **6a**/**7a**, and 30.7 mg of **16**.

*12,15-Di-O-acetylnicandrodiol (**1a**):* colorless oil; $[\alpha]^{20}_D$ +43 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1731, 1642 cm^{-1}; ^{1}H and ^{13}C NMR see Tables S1 and S2 (Supporting Information); HRFABMS *m*/*z* 391.2839 [M + H]$^+$ (calcd for $C_{24}H_{39}O_4$, 391.2848).

*12,15-Di-O-acetyl-12-epi-nicandrodiol (**2a**):* colorless oil; $[\alpha]^{20}_D$ +2 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 1741, 1644 cm^{-1}; ^{1}H and ^{13}C NMR see Tables S1 and S2 (Supporting Information); HRFABMS *m*/*z* 391.2843 [M + H]$^+$ (calcd for $C_{24}H_{39}O_4$, 391.2848).

*Acid Hydrolysis of Compound **4**.* Compound **4** in dioxane (3 mL) was hydrolyzed with 2 N HCl at 78 °C for 2 h. The reaction mixture

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Table 6. ^{13}C NMR Data of Compounds 14−16 and 16a (125 MHz, $CDCl_3$)

position	14	15[a]	16	16a[c]
1 C	203.8	210.9	201.5	200.6
2 CH	128.8	42.7[b]	128.5	128.7
3 CH	145.0	72.9	141.8	140.7
4 CH_2	33.0	36.2	37.3	36.7
5 C	61.3	61.4	80.9	78.6
6 CH	63.8	62.1	74.5	74.8
7 CH_2	26.3	26.2	28.7	25.7
8 CH	33.5	32.9	33.6	34.6
9 CH	38.9	37.1	36.1	35.9
10 C	48.6	52.1	53.1	53.0
11 CH_2	22.7	21.2	21.9	22.2
12 CH_2	40.1	39.6	40.2	40.7
13 C	48.3	48.4	49.0	48.7
14 C	82.8	82.9	83.2	83.0
15 CH	80.88	81.1	80.4	80.2
16 CH_2	31.2	31.1	31.3	31.4
17 CH	54.1	54.1	54.1	54.1
18 CH_3	19.1	19.0	19.7	19.6
19 CH_3	14.9	13.8	15.6	15.0
20 C	75.3	75.3	75.1	75.4
21 CH_3	21.2	21.2	21.2	21.3
22 CH	80.94	80.9	81.4	81.0
23 CH_2	31.6	31.6	31.6	31.7
24 C	148.5	148.4	149.0	148.6
25 C	122.2	122.2	122.1	122.2
26 C	165.5	165.5	166.4	165.5
27 CH_3	12.4	12.4	12.4	12.5
28 CH_3	20.6	20.5	20.6	20.6
15-OAc	169.7, 21.6	169.7, 21.5	170.2, 21.7	170.0, 21.7

[a]OMe signal: δ_C 55.9. [b]CH_2. [c]6-OAc signals: δ_C 169.4, 21.3.

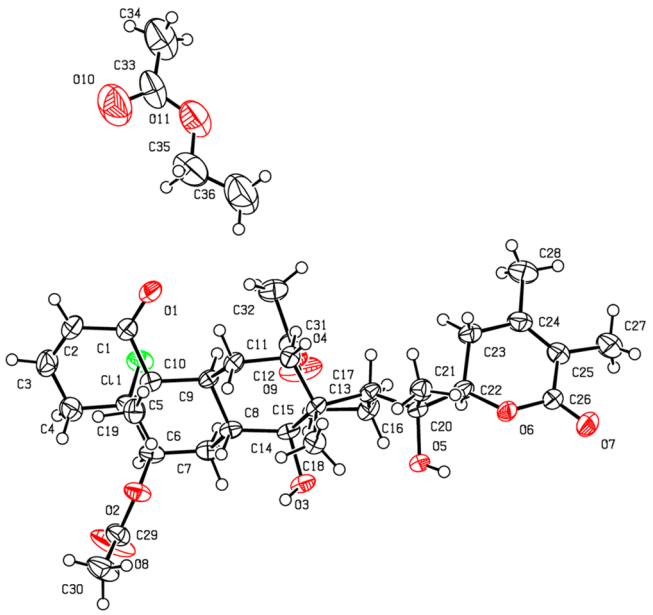

Figure 4. ORTEP projection of 6-*O*-acetylphysanicandrolide C (**16a**) (crystallographic numbering).

was extracted with EtOAc. The aqueous phase was concentrated and purified by flash CC eluted with EtOAc−MeOH 3:1 to afford 35.3 mg of D-glucose: $[\alpha]^{20}_D$ +36 (*c* 0.2, H_2O).

Basic Hydrolysis of Compound **4**.[20] Aqueous NaOH (5% w/v, 3.2 mL) was added dropwise (0.4 mL/each h) to a stirred solution of **4** (89.4 mg) in MeOH−CH_2Cl_2 3:2 (5 mL). The reaction mixture was allowed to stand overnight (16 h). TLC ($CHCl_3$−MeOH 9:1) showed partial hydrolysis of **4**. Then a NaOH solution (0.5 mL) was added. After 4 h, no progress of the reaction was observed; therefore, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with a satd solution of NH_4Cl. The CH_2Cl_2 layer was separated and the aqueous layer re-extracted with CH_2Cl_2. The combined CH_2Cl_2 layers were washed with H_2O, dried with Na_2SO_4, and purified by VCC ($CHCl_3$−MeOH 12:1) to obtain 53 mg of the starting compound **4** and 27.1 mg of **5**.

*Tetra-O-acetylnicandroside A (**4a**):* colorless crystals; mp 110−113 °C; $[\alpha]^{20}_D$ −31 (*c* 0.3, $CHCl_3$); IR (film) ν_{max} 1755, 1643 cm^{-1}; ^1H and ^{13}C NMR see Tables S1 and S2, Supporting Information; HRFABMS *m/z* 743.3979 $[M + Na]^+$ (calcd for $C_{39}H_{60}O_{12}Na$, 743.3982).

*X-ray Crystallographic Data of Tetra-O-acetylnicandroside A (**4a**).*[29] The data were collected from a colorless needle (0.446 × 0.136 × 0.032 mm) 298(2) K: $C_{39}H_{60}O_{12}$, M_r 720.87; monoclinic, space group $P2_1$; a = 10.8266(14) Å, b = 7.7886(11) Å, c = 23.976(3) Å; α = 90°, β = 90.913°, γ = 90°, V = 2021.5(5) $Å^3$; Z = 2; D_{calc} = 1.184 g/cm^3; $F(000)$ = 780. Reflections collected 6812; independent reflections 4555 for $I \geq 2\sigma(I)$.

*Penta-O-acetylnicandroside B (**5a**):* colorless oil; $[\alpha]^{20}_D$ −20 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 1756, 1643 cm^{-1}; ^1H and ^{13}C NMR see Tables S1 and S2 (Supporting Information); FABMS *m/z* 701 $[M + Na]^+$ ($C_{36}H_{54}O_{12}Na$), 679 $[M + H]^+$ ($C_{36}H_{55}O_{12}$).

*Tri-O-acetylnicantriol/tri-O-acetyl-14-epi-nicantriol (**6a/7a**):* colorless oil; IR (film) ν_{max} 1746, 1646 cm^{-1}; ^1H and ^{13}C NMR see Tables S1 and S2 (Supporting Information); HRFABMS *m/z* 449.2906 $[M + H]^+$ (calcd for $C_{26}H_{41}O_6$ 449.2903).

*6-O-Acetylphysanicandrolide C (**16a**):* colorless crystals; mp 263−264 °C; $[\alpha]^{20}_D$ +98 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (4.47) nm; IR (film) ν_{max}: 3526, 1742, 1714, 1691 cm^{-1}; ^1H and ^{13}C NMR, see Tables 5 and 6; EIMS *m/z* 607 $[M + H]^+$ (2), 564 (3), 511 (4), 265 (61); 169 (63); 125 (94), 43 (100).

*X-ray Crystallographic Data of 6-O-Acetylphysanicandrolide C (**16a**).*[29] The data were collected from a colorless prism (0.278 × 0.098 × 0.058 mm) at 173(2) K: $C_{32}H_{43}ClO_9$, $C_4H_8O_2$ M_r 695.21; monoclinic, space group $P2_1$; a = 14.5187(14) Å, b = 7.7890(8) Å, c = 15.6480(15) Å; α = 90°, β = 91.811 (2)°, γ = 90°, V = 1768.7(3) $Å^3$; Z = 2; D_{calc}= 1.305 g/cm^3; $F(000)$ = 7.44. Reflections collected 6442; independent reflections 5139 for $I \geq 2\sigma(I)$.

Photooxidation of Compound 1. Methylene blue (5.2 mg) was added to a solution of compound **1** (96.4 mg) in acetone (20 mL) at 0 °C. The mixture was stirred under a 100 W lamp for 22 h (temperature was allowed to rise to 40 °C). Acetone was removed with an air stream, and then the residue was dissolved in MeOH (10 mL) and $NaBH_4$ (52 mg) was added. The mixture was stirred for 0.5 h, quenched with glacial HOAc (0.5 mL), concentrated, diluted with H_2O (~15 mL), and extracted with EtOAc (4 × 5 mL). The organic fraction was washed with H_2O, dried with anhyd Na_2SO_4, concentrated, and purified by VCC (hexanes−EtOAc gradient 4:1−0:1) to give 17 mg of a mixture of semisynthetic compounds **6**/**7** and 22 mg of the starting material (**1**).

Oxidation of Compounds 6/7. Si gel supported $NaIO_4$ reagent[30] (170 mg) was added to a solution of the mixture of **6**/**7** (74.7 mg) in CH_2Cl_2 (3 mL). The suspension was stirred at room temperature for 3 h, and the solids were filtered off. The filtrate was purified by preparative TLC eluted with hexanes−EtOAc 4:1 to give the aldehyde **8** (19.7 mg) as a pale yellow gum: $[\alpha]^{20}_D$ +32 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3442, 1690, 1642 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 291.23146 $[M + H]^+$ (calcd for $C_{19}H_{31}O_2$, 291.23240).

Oxidation of Semisynthetic Compounds 6/7. Si gel supported $NaIO_4$ reagent[30] (36.4 mg) was added to a solution of the mixture of **6**/**7** (13.1 mg) in CH_2Cl_2 (3 mL). The suspension was stirred at room temperature for 2.5 h, and the solids were filtered off. The

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filtrate was purified by flash CC hexanes−acetone 19:1 to give the aldehyde **8** (2.4 mg): $[\alpha]^{20}_{D}$ +30 (*c* 0.1, $CHCl_3$).

Enzyme Inhibition Assays. *Yeast α-Glucosidase Assay.* The evaluation of the α-glucosidase inhibitory activity was performed using an adapted method previously described.[31] A solution (25 μL) of samples in DMSO−H_2O 1:1 was added to 150 μL of phosphate buffer solution (PBS, 67 mM, pH 6.8) and incubated at 37 °C for 10 min with glutathione (25 μL, 3 mM in PBS) and α-glucosidase type I (25 μL, 0.2 U mL^{-1} in PBS). *p*-Nitrophenyl α-D-glucopyranoside in PBS (25 μL, 23.2 mM) was added and incubated with agitation (15 min at 37 °C). The reaction was quenched with 50 μL of Na_2CO_3 (1 M), and the absorbance was determined at 405 nm after 5 min of agitation in a BioTek microplate reader Synergy HT. Quercetin was used as positive control. The percentage of inhibition was calculated by the equation: inhibition (%) = [(AC − AS)/AC] × 100, where AC is the absorbance of the negative control and AS is the absorbance of the tested sample. All samples were tested in triplicated. Data are presented as the mean ± standard deviation.

Acetylcholinesterase Assay. Inhibition of acetylcholinesterase (AChE from *Electrophorus electricus*) activity was determined using Ellman's colorimetric method[32] with some modifications.[33] Briefly, a solution (50 μL) of test compound in 2% MeCN in PBS (11.3 mM, pH 7.4) was added to an enzyme solution (50 μL, 0.195 U mL^{-1}) and incubated for 30 min at 25 °C. The reaction was started by addition of 100 μL of substrate solution containing 5,5′-dithiobis(2-nitrobenzoic acid) (0.20 mM, DTNB) plus 0.24 mM acetylthiocholine iodide (ATChI) in PBS (15.85 mM, pH 7.5). The formation of the colored product was measured in a Synergy HT Bio Tek microplate reader at 412 nm after 5 min. All experiments were carried out in triplicate. Physostigmine was used as a positive control. The percentages of inhibition and the statistical analysis were calculated using the same method as described for the glucosidase assay. Data were presented as the mean ± standard deviation. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by a Dunnett's test. Significant differences between groups were determined at $p \leq 0.05$ and $p \leq 0.01$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00233.

Tables S1 and S2 containing the ^1H and ^{13}C NMR data of derivatives **1a**, **2a**, and **6a**/**7a**; 1D and 2D NMR spectra of compounds **1**−**7**, **9**, **14**−**16**, and **5a**, and 1D NMR spectra of their derivatives as well as the results of the AChE inhibition assay (PDF)

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Notes

The authors declare no competing financial interest.

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