Three New Cardenolides from Methanol Extract of Stems and Twigs of Nerium oleander

Liming Bai, Ming Zhao, Asami Toki, Jun-ichi Sakai, Xiao-yang Yang, Yuhua Bai, Mariko Ando, Katsutoshi Hirose, and Masayoshi Ando

College of Chemistry and Chemistry Engineering, Qiqihar University; 30 Wenhuaqai, Qiqihar, Heilongjian Province 161006, China: a Graduate School of Science and Technology, Niigata University: b Department of Chemistry and Chemical Engineering, Graduate School of Engineering, Niigata University; 2–8050 Ikarashi, Nishi-ku, Niigata 950–2181, Japan: c Atmospheric Chemistry & Aerosol Division, Chinese Research Academy of Environmental Science; No. 8 Anwai Beiyan Dayangfang, Chaoyang District, Beijing 100012, China; d Department of Medicinal Chemistry, Pharmaceutical Department, Daqing Campus of Harbin Medical University; Daqing 163319, China: f Technical Division, School of Engineering, Tohoku University; 6–6 Aramaki-aza-Aoba, Aoba-ku, Sendai 980–8579, Japan: and g KNC Laboratories Co., Ltd.: 3–2–34 Takatukadai, Nishi-ku, Kobe, Hyogo 651–2271, Japan.

Received February 21, 2010; accepted May 19, 2010; published online May 25, 2010

Nerium oleander L. is a medium-sized evergreen flowering tree of 2–5 m in height and is planted throughout Japan as a garden and roadside tree. This species was distributed originally in the Mediterranean region, sub-tropical Asia, and the Indo–Pakistan subcontinent. Cardenolides in the leaves, roots, and root bark of this plant were investigated because of the interests in their biological activities. The cardiac glycoside digitoxin and digoxin have been used in treatment of cardiac diseases for many years, but they have a narrow therapeutic window because of arrhythmia and disturbance of atrio-ventricular contraction. Anticancer utilization of digitoxin, digoxin, and related cardenolides has been also investigated. We recently reinvestigated the cardenolide monoglycosides from N. oleander and isolated thirteen kinds of compounds, four of which were new compounds.

Further chemical investigation on N. oleander has led to the isolation of two new cardenolide monoglycoside named cardenolides B-1 (1) and B-2 (2), and oleagenin (3) which is the first isolated compound from natural sources. The structure of compounds 1–3 was established on the basis of their spectroscopic data.

Key words Nerium oleander; cardenolide B-1; cardenolide B-2; oleagenin

Results and Discussion

A methanol extract of air-dried stems and twigs of N. oleander was partitioned successively with hexane, ethyl acetate (EtOAc), and n-BuOH. From the less polar fraction of the extract with EtOAc, we have already reported three cardenolide sarmentosides and eight cardenolid diginosides including four new compounds, the major component of which was odoroside A (17) (0.018%). In this time, we isolated two new cardenolide monoglycosides, cardenolides B-1 (1) and B-2 (2), and oleagenin (3) from more polar fraction of the extract with EtOAc using silica gel column chromatography and reversed-phase HPLC.

Cardenolide B-1 (1) gave the elemental composition, C_{39}H_{44}O_{8}, which was determined by high resolution (HR)-FAB-MS analysis. The IR spectrum of 1 indicated the presence of hydroxyl (3539 cm⁻¹) and \(\alpha,\beta\)-unsaturated-\(\gamma\)-lactone (1786, 1751, 1631 cm⁻¹) groups. The 13C-NMR spectrum displayed 30 carbon signals (Table 1). A carbonyl carbon resonated at \(\delta\) 173.6 and two olefin carbon resonances were located at \(\delta\) 169.5 (qC) and 116.9 (CH). Four resonances for carbons bearing oxygen were observed at \(\delta\) 73.2 (CH2), 73.7 (CH), 70.5 (qC), and 65.3 (qC) in addition to one methoxy methyl and five oxygenated carbon signals of a 6-deoxyhexose sugar. From the distortionless enhancement by polarization transfer (DEPT) and \(\mathrm{H}\)-detected heteronuclear multiple quantum coherence (HMQC) spectra, the remaining carbon resonances were three methyl, nine methylene, three methine, and two quaternary carbons. The 1H-NMR spectra showed two methyl singlets (δ 0.85, 1.01) and one additional methyl doublet from the sugar portion at 1.36 (d, J = 6.3 Hz).

The connectivity of the protonated carbons (C-1 through C-7; C-9, C-11, and C-12; C-15 through C-17) was determined from the 1H–1H correlation spectroscopy (COSY) spectrum. A heteronuclear multiple bond connectivity (HMBC) experiment was used to determine the carbon–carbon connection through the nonprotonated carbon atoms [HMBC correlations: H-17 (δ 2.57) to C-12, C-13 (δ 41.8, qC), C-15, C-18, C-20 (δ 169.5, qC), C-21 (δ 73.2, CH2), and C-22 (δ 116.9, CH); CH3-18 (δ 0.85) to C-12, C-13 (δ 41.8, qC), C-14 (δ 70.5, qC), and C-17; CH3-19 (δ 1.01) to C-1, C-5, C-9, and C-10 (δ 36.7, qC); H-11 α and β (δ 1.15, 1.26) to C-8 (δ 65.3, qC), C-9, and C-12]. Interpretation of these results suggests that compound 1 has steroid A, B, C, and D rings bearing an 8,14-epoxide ring, and an \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone moiety at C-17. The HMBC correlations [H-3 to C-2, C-5, and C-1′; H-1′ to C-3] were used to place an O-glycosyl bond at C-3. The chemical shift values of C-8 (δ 65.3) and C-14 (δ 70.5) of 1 are in good accordance with those of analogous epoxides \(18^{15}\) [C-8 (δ 65.2) and C-14 (δ 70.1)] and \(19^{15,18,21}\) [C-8 (δ 65.3) and C-14 (δ 70.5)] but different from those of diol \(15^{18}\) [C-8 (δ 77.2) and C-14 (δ 85.9)]. The sugar portion of 1 was assigned to digitalose on the basis of comparisons of the 13C- and 1H-NMR data of 1 (Table 1) with those of an analogous compound such as 4. The 13C-NMR: \(\delta\) 73.9 (C-3), 101.1 (C-1′), 70.8 (C-2′), 82.8 (C-3′), 68.2 (C-4′), 70.3 (C-5′), 16.4 (C-6′), 57.5 (OMe); 1H-NMR:...
Cardenolides B-2 had the composition, C_{20}H_{34}O_{8}, which was determined by HR-FAB-MS analysis. Similar IR data were obtained for compound 2 as compared to compound 1. The $^{13}$C-NMR spectrum displayed 30 carbon signals (Table 1). The $^{3}$β-O-(glycosyl)-5β,14β-card-20(22)-enolide structure of 2 was confirmed by analogous NMR analysis of 2 with that of 1. $^{1}$H- and $^{13}$C-NMR spectra of the sugar portion of 2 are different from those of 1. The coupling constants [H-1 (J_{1,2}β=9.8 Hz and J_{1,2}α=1.7 Hz), H-3 (J_{3,4}α=12.1 Hz, J_{3,4}β=4.8 Hz, and J_{3,5}=3.2 Hz), and CH$_{3}$-18 (J_{dJ}=6.6 Hz)] and NOESY correlations (H-1' with H-2', H-3' and H-5', H-4' with H-3', H-5', 6' and 3'-OMe) of a 2,6-dideoxyhexose sugar of 2 suggested it be diginose. The $^{13}$C- and $^{1}$H-NMR data of the sugar moiety of 2 were actually superimposable with 3β-O-(β-diginosyl)-moiety of 15\(^{(15)}\) \[^{13}\text{C-NMR}: \delta 97.7 (C-1'), 32.0 (C-2'), 78.0 (C-3'), 67.2 (C-4'), 70.4 (C-5'), 16.8 (C-6'), 55.7 (OMe); \] 1$^{1}$H-NMR: \[\delta 4.45 (H-1', dd, J=9.8, 1.7 Hz), 1.93 (H-2'α, m), 1.71 (H-2'β, m), 3.34 (H-3', dd, J=12.9, 4.9, 3.2 Hz), 3.69 (H-4', brs), 3.43 (H-5', q, J=6.6 Hz), 1.32 (H-6', d, J=6.6 Hz), 3.40 (OMe, s)] and 19\(^{(18,21)}\) \[^{13}\text{C-NMR}: \delta 97.7 (C-1'), 32.1 (C-2'), 78.0 (C-3'), 67.1 (C-4'), 70.3 (C-5'), 16.9 (C-6'), 55.7 (OMe); \] 1$^{1}$H-NMR: \[\delta 4.47 (H-1', dd, J=9.8, 2.0 Hz), 1.93 (H-2'α, m), 1.70 (H-2'β, m), 3.34 (H-3', dd, J=12.0, 4.9, 3.2 Hz), 3.69 (H-4', brs), 3.43 (H-5', q, J=6.6 Hz), 1.34 (H-6', d, J=6.6 Hz), 3.40 (OMe, s)]. Since only β-diginose is known in N. oleander, the sugar in 1 is regarded as β-diginose. The conclusion is supported by the coupling constants of \(^{1}$H-NMR spectrum (Table 1) and the nuclear Overhauser effect spectroscopy (NOEY) correlations (H-1' to H-3' and H-5', H-3' to H-1', H-4', and H-5'; H-4' to H-3' and H-5'; H-5' to H-1', H-3', and H-4'; H-6' to H-4' and H-5') of sugar moiety of 1. NOESY correlations [CH$_{19}$ with H-5, H-4c with H-7α and H-9] suggested AB-cis ring junction in 1. The β-configuration of the 8,14-epoxide ring of 1 was strongly suggested by the fact that the chemical shifts of C-8 and C-14 are in good accordance with those of known cardenolides with 8β,14β-epoxide ring as mentioned above. This was also supported by NOE correlation of H-15α with H-7α and H-7β. In \(^{1}$H-NMR-spectra, the small coupling constant of H-3 (W$_{h/2}$=7.5 Hz) was in good agreement with that of α(eq)-H at C-3 of 5β steroids. The above-mentioned spectrosopic analyses and NOEY correlations [CH$_{19}$ with H-6β, and 11β; H-11β with H-12β and CH$_{18}$; CH$_{18}$ with H-21a and H-22; H-12α with H-9 and H-17; H-17 with H-15α and H-16α; H-16β with H-22 and CH$_{18}$] indicated the relative stereochemistry of 1 to be 3β-O-(β-digalactosyl)-8,14-epoxy-5β,14β-card-20(22)-enolide.

Since all known cardenolides isolated from N. oleander possess the same absolute configuration in genin moiety as shown in structures 4, 15, 18 and 19, the absolute configuration of 1 is regarded as (3$^{S}$, 5$^{R}$, 8$^{S}$, 9$^{R}$, 10$^{S}$, 13$^{R}$, 14$^{R}$, 17$^{R}$). This conclusion was also supported by the fact that [α]$_{D}$ sign of 1 ([α]$_{D}^{20}$+28.57 ($c$=0.392, CHCl$_{3}$)] is the same as those of 3β-O-(β-digalactosyl)- and 3β-O-(β-diginosyl)-cardenolides with analogous structures such as 4 ([α]$_{D}^{20}$+5.57 ($c$=0.56, MeOH)] and 19 ([α]$_{D}^{20}$+13.4 ($c$=0.55, CHCl$_{3}$)].
Two olefin carbon resonances were located at δ 171.9 (qC) and 116.4 (CH). Two resonances for carbons bearing oxygen were observed at δ 73.4 (CH₃) and 65.8 (CH). From the DEPT and HMOC spectra, the remaining carbon resonances were two methyl, nine methylene, three quaternary carbons. The ¹H-NMR spectra showed two methinyl singlets (δ 0.91, 0.81). The connectivity of the protonated carbons (C-1 through C-7; C-9, C-11, and C-12; C-15 through C-17) was determined from the ¹H-¹H COSY spectrum. An HMBC experiment was used to determine the carbon–carbon connection through the nonprotonated carbon atoms [HMBC correlations: H-17 with H-5, H-6, and H-12β; H-2α with H-9α; H-4α with H-7α; H-9 with H-15α; H-11α with H-16α; CH₂-18 with H-12β and H-22; H-22 with H-15β] indicated full stereochemistry of 3 as (8R)-3β-hydroxy-14-oxo-15(14)→8β)abeo-5β-card-20(22)-enolide. Although the structure 3 is identical with that of aglycone of oleasone A that was obtained by acid hydrolysis, this is the first isolation of 3 from natural sources.

italosyl)-8,14-epoxy-5β,14β-card-16,20(22)-enolide (10), 12,30 3β-O-([β-D-digiosinyl])-14-hydroxy-5β,14β-card-16,20(22)-enolide (11), 29,30 oleaside A ([8β,9β]-3β-O-([β-D-digiosinyl])-14-oxo-15(14-9)-abeo-5β-card-20(22)-enolide) (12), 3,10 neeraside 3β-O-([β-D-digiosinyl])-8,14-seco-14α-hydroxy-8,14-oxo-5β-card-20(22)-enolide (13). 5,6 The most abundant component of this fraction is odoroside H (2) (0.008%).

Experimental

Melting points are uncorrected. Optical rotation values were measured using a Horiba SePA-200 polarimeter. IR spectra were recorded on a Shimadzu FTIR-4200 infrared spectrometer. 1H- and 13C-NMR spectra were measured at 300 and 75 MHz, respectively, with TMS as an internal standard on a Varian Unity-Inova 300 spectrometer. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10×250 mm stainless steel column and an Inertsil Prep-tocadecyl functionalized silica gel column (ODS) GL 10×250 mm stainless steel column and monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.

Plant Material

The stems and twigs of N. oleander were collected in Niigata City, Niigata Province, Japan, in November 2001. The plant was identified by Dr. K. Yonekura, Department of Biology, Faculty of Science, Tohoku University, Sendai, Japan. A voucher specimen (2001-11-10) was deposited at the Department of Chemistry and Chemical Engineering, Niigata University.

Extraction and Isolation of Compounds 1—13

The air-dried stems and twigs (19.5 kg) were combined and extracted with MeOH (851) for 20 d. The MeOH extract was concentrated to 41 and extracted with hexane (8×1000 ml). Water (1.31) was added to the MeOH layer, extracted with EtOAc (3×3000 ml), dried (Na2SO4), and concentrated to a dry oil material (96.5 g). The water layer was further extracted with n-BuOH (3×500 ml), dried (Na2SO4), and concentrated to give an oil residue (53.7 g).

The EtOAc extract (96.5 g) was separated by column chromatography [silica gel (1.1 kg), a gradient of hexane, EtOAc, and MeOH] into five fractions, B71—B75. B73 (1.31 g) afforded compound 5 [3.51 mg (0.00027%)] by separation using HPLC [ODS, MeOH–MeCN–H2O (1 : 1 : 2)]. B8 was subjected to column chromatography [silica gel (230—400 mesh) for flash column chromatography. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10×250 mm stainless steel column and an Inertsil Prep-tocadecyl functionalized silica gel column (ODS) GL 10×250 mm stainless steel column and monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.]

Acknowledgements

The Project sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2009-36-1341) and QiQi har science and technology bureau, China. We thank Ms. Seiko Oka and Ms. Hiroko Tsushima of Center for Instrumental Analysis, Hokkaido University for HR-FAB-MS.

References and Notes


23) The [α]D value of cardenolide B-2 (2) was evaluated as following. The observed [α]D values of digitoxigenin (16)18 and odoroside A (17)18 are +40.3 and +1.5, respectively. From these values, the contribution of diginosyl moiety to [α]D values of 17 is estimated to be −38.8. The [α]D value of 2 was calculated to be −24.7 based on the observed and calculated [α]D values of tannigenin (14)24,25 and diginosyl moiety, +14.1 and −38.8, respectively.