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Research Article

STEROIDS AND TRITERPENE FROM THE CHLOROFORM EXTRACTION OF LEAVES AND STEMS VINES OF *STREPTOCAULON JUVENTAS MERR*

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ABSTRACT

From the chloroform extraction of leaves and stems of the vines of Streptocaulon Juventas, by thin layer chromatography and column chromatography, we isolated three organic compounds, including two steroids and one triterpenoid: 3α-acetyl-20,24-epoxy-dammaran-25-ol, acovenosigenin A, and periplogenin. The structures of these compounds were determined by spectra, mainly by nuclear magnetic resonance spectroscopy. This is the first time these structures were found in the leaves and stems of the vines of Streptocaulon Juventas.

Keywords: leaves and stems vines; steroids; *Streptocaulon juventas*; triterpene

1. Introduction

Species *Streptocaulon juventas* Merr., of the Asclepiadaceae family, also known as "day sua bo, cay sung bo, khau nuoc" (Lang Son) or khua mak tang ning (Laos). In Vietnam, *S. juventas* grows abundantly in the mountainous and midland provinces of Bac Giang, Hoa Binh, Thai Nguyen, Quang Ninh, Vinh Phuc, Nghe An, An Giang, and Lam Dong (Do, 2004a). The roots of *S. juventas* species are used to treat colds, hot fever, enteritis, diarrhea, and chronic nephritis. It is also used to treat anemia, kidney, liver weakness, nervous weakness, poor eating and sleep, chronic malaria, rheumatism, numbness, bone tendon pain, irregular menstruation, except snake venom, premature graying of hair, and skin diseases. The leaves of *S. juventas* are also used to boil bath water to treat itchy sores. It is also used as a blood tonic in combination with *Fallopia multiflora* and rarely uses one taste (Do et al., 2004b). Previous studies have shown that the roots of *S. juventas* have potent activity against human cancer cell lines (Ueda et al., 2002). However, the leaves and stems of the vines of *S. juventas* have not been fully studied. This paper reports the isolation of steroid and

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triterpene compounds from leaf chloroform extract residues and vine stems of *S. juventas* and the determination of their structure by nuclear magnetic resonance spectroscopy.

2. Experiments

2.1. Experimental methods

Thin-layer chromatography is performed on DC-Alufolien 60 F_{254} (Merck) pre-coated thin plates. The substances on the thin plate are detected with UV lamps or with an H_2SO_4 solution. The thin plate, after deployment with an appropriate dissociation solvent system, is evenly sprayed with 25% H_2SO_4 solution, dried, and heated until color appears. Column chromatography is usually performed on silica gel columns with a particle size of 0.040–0.063 mm (240–430 mesh, Merck). The NMR spectroscopy was measured on a Bruker AM500 FT-NMR (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer with TMS as the internal standard. Chemical shifts are displayed in units of δ (ppm), and the coupling constants are expressed in hertz.

2.2. Materials

The materials used in this study are the leaves and the roots of *S. juventas* Merr. Raw materials after being collected in An Giang Province in 2018 were identified by traditional medicine physician Nguyen Thien Chung, the chairman of the Oriental Medicine Association of Tinh Bien district, An Giang Province.

2.3. Extraction and Isolation

The leaves and stems of *S. juventas* vines were collected, pooled, then washed, dried, and ground into a powder to obtain 8 kg. This powder was extracted with methanol (30 L, reflux, three h × 3) to obtain 300 g of the methanol extract residue. This residue was suspended in H_2O and successively partitioned with chloroform (12.0 L), and ethyl acetate (15.0 L) to give extract residues of 150 g, and 45 g, respectively, and the rest is water extract. A total of 100 g chloroform residue was chromatographed by gel silica column eluting with gradient solvent of *n*-hexane-EtOAc (100:0, 99:1, 98:2, 95:5, 9:1, 85:5, 8:2, 1:1, EtOAc), to give nine fractions, named S.J.C.1-S.J.C.9. The S.J.C.6 fraction (3.20 g) was chromatographed on a silica gel column eluting with *n*-hexane-EtOAc (85:15, 80:20, 75:25, 60:40, 25:75) to give five subfractions, S.J.C.6.1 - S.J.C.6.5. The S.J.C.6.4 subfraction (720 mg) was repeated by chromatography on a silica gel column eluting with $CHCl_3$-MeOH (49:1, 19:1, 9:1) to obtain compound **1** (5.0 mg).

The S.J.C.8 fraction (1.50 g) was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (99:1, 98:2, 95:5, 90:10) to give four subfractions, S.J.C.8.1 - S.J.C.8.4. The S.J.C.6.3 subfraction (125 mg) was repeated by chromatography on an RP-18 column eluting with H_2O-MeCN (3:1, 2:1, 1:1) to obtain compound **2** (15.0 mg) and **3** (25.0 mg).

2.4. Spectroscopic data

3*β*-Acetyl-20,24-epoxy-dammaran-25-ol (**1**): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, *δ* ppm): 4.48 (1H, dd, *J*=10.5, 5.5 Hz, H-3); 0.80 (1H, m, H-5); 1.28 (1H, m, H-7a); 1.53 (1H, m, H-7b); 1.35 (1H, m, H-9); 1.56 (1H, m, H-13); 1.45 (1H, m, H-15a);

1.07 (1H, m, H-15b); 1.47 (1H, m, H-16a); 1.78 (1H, m, H-16b); 1.80 (1H, m, H-17); 0.96 (3H, s, H-18); 0.86 (3H, s, H-19); 1.10 (3H, s, H-21); 1.50 (1H, m, H-23a); 1.20 (1H, m, H-23b); 3.72 (1H, dd; *J*=14.5; 7.5 Hz, H-24); 1.13 (3H, s, H-26); 1.20 (3H, s, H-27); 0.85 (3H, s, H-28); 0.87 (3H, s, H-29); 0.88 (3H, s, H-30); 2.06 (3H, s, OCOMe); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 38.8 (C-1); 26.1 (C-2); 80.9 (C-3); 37.9 (C-4); 56.0 (C-5); 18.2 (C-6); 35.2 (C-7); 40.4 (C-8); 50.7 (C-9); 37.1 (C-10); 23.5 (C-11); 27.3 (C-12); 43.0 (C-13); 49.5 (C-14); 31.5 (C-15); 25.7 (C-16); 50.0 (C-17); 15.5 (C-18); 16.3 (C-19); 86.4 (C-20); 24.3 (C-21); 35.7 (C-22); 21.6 (C-23); 83.3 (C-24); 71.4 (C-25); 23.7 (C-26); 27.4 (C-27); 28.0 (C-28); 16.4 (C-29); 16.5 (C-30); 21.3 (OCOMe); 171.0 (OCOMe)

Acovenosigenin A (**2**): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.84 (1H, brs, H-1); 4.20 (1H, m, H-3); 2.77 (1H, dd, *J*=9.0, 5.5 Hz, H-17); 0.89 (1H, s, H-18); 1.11 (1H, s, H-19); 4,97 (dd, *J*=18.0, 1.0 Hz, H-21a); 4.79 (dd, *J*=18.0, 1.0 Hz, H-21b); 5.88 (1H, s, H-22); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 73.4 (C-1); 32.4 (C-2); 68.2 (C-3); 33.6 (C-4); 30.1 (C-5); 26.0 (C-6); 21.2 (C-7); 41.9 (C-8); 37.4 (C-9); 40.0 (C-10); 21.0 (C-11); 39.9 (C-12); 49.4 (C-13); 85.4 (C-14); 33.2 (C-15); 26.9 (C-16); 50.8 (C-17); 15.8 (C-18); 18.8 (C-19); 174.4 (C-20); 73.4 (C-21); 117.9 (C-22); 174.2 (C-23).

Periplogenin (**3**): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 4.19 (1H, m, H-3); 2.80 (1H, dd, *J*=9.5, 5.5 Hz, H-17); 0.90 (1H, s H-18); 0.96 (1H, s H-19); 5.00 (1H, dd, *J*=18.0 1.5 Hz, H-21a); 4.98 (1H, dd, *J*=18.0; 2.0 Hz, H-21b); 5.88 (1H, dd, *J*=1.0; 1.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 24.9 (C-1); 28.0 (C-2); 68.0 (C-3); 36.9 (C-4); 74.7 (C-5); 35.3 (C-6); 23.8 (C-7); 40.8 (C-8); 39.0 (C-9); 40.7 (C-10); 21.5 (C-11); 40.1 (C-12); 49.5 (C-13); 85.5 (C-14); 33.0 (C-15); 26.9 (C-16); 50.7 (C-17); 15.8 (C-18); 16.7 (C-19); 174.6 (C-20); 73.5 (C-21); 117.7 (C-22); 174.5 (C-23).

3. Results and Discussion

Using the column chromatography method together with the thin chromatography layer method on the $CHCl_3$ residues of the leaves of *S. Juventas*, we isolated three compounds (**1**–**3**). The chemical structures of these compounds were determined by spectroscopic methods.

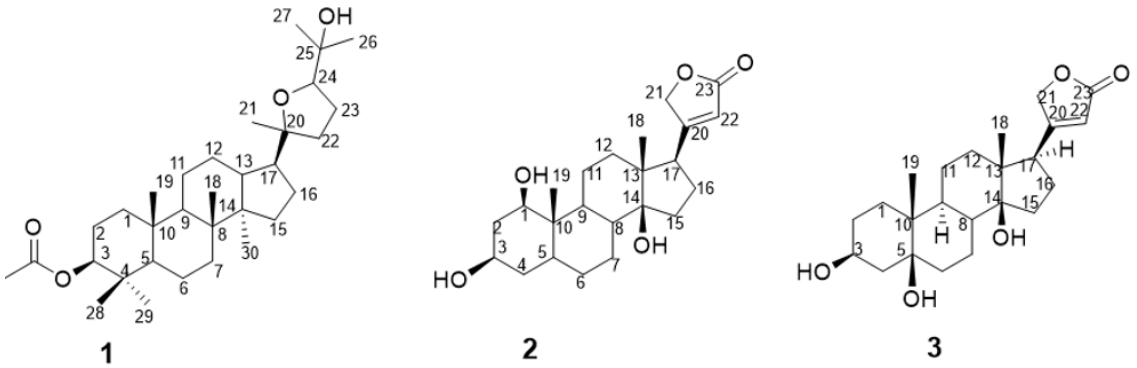

Figure 1. *Chemical structure of isolated compounds*

The ^{1}H-NMR spectrum of compound **1** shows the resonance signal of two oxygenated methine groups at δ_H 4.48 (1H, m) and δ_H 3.72 (1H, t, J = 7.5; 14.5 Hz). On the ^{1}H-NMR spectrum, it also shows the resonance signal of protons of eight methyl groups at δ_H 0.85 (3H, s); 0.86 (3H, s); 0.87 (3H, s); 0.88 (3H, s); 0.96 (3H, s), 1.11 (3H, s); 1.13 (3H, s); 1.20 (3H, s) ppm, and protons of acetoxy group at δ_H 2.03 (3H, s). In addition, the ^{1}H-NMR spectrum has the presence of methylene and methine proton resonance signals in the range δ_H 1.20 to δ_H 1.90 ppm. The ^{13}C-NMR spectrum of compound **1** shows the presence of 32 carbons in the range δ_C 15.5 to 171 ppm, including nine resonance signals of methyl carbon, ten methylene carbons, six methine carbons including two oxygenated methine carbons at δ_C 80.9 (C–3) and 83.3 (C–24), seven quaternary carbons including one carbonyl at δ_C 171 (C-32), two oxygenated carbons at δ_C 83.3 (C–24) and 86.4 (C–20). From ^{1}H-NMR spectroscopic data and ^{13}C-NMR spectroscopy together with HQC spectroscopy, it can be predicted that compound **1** is a triterpene with a dammarane skeleton structure (Hidayat et al., 2018). The HMBC spectroscopy of compound **1** shows a correlation of protons at δ_H 4.48 (H–3) with carbon carbonyl at δ_C 171 (COO) and protons at δ_H 2.03 (CH_3COO) correlating with carbon carbonyl (COO) confirming the presence of an acetoxy group at the C-3 position. The HMBC correlation of protons H-3 to carbon at δ_C 16.4 (C–28); 23.5 (C–2); 37.9 (C–4) and HMBC correlation of protons at δ_H 0.85 (H–29) to carbon at δ_C 80.9 (C–3); 37.9 (C-4); 56 (C-5) confirms two methyl groups of C-28 and C-29 attached at C-4. Besides, the positions of methyl groups δ_C 15.5 (C-18), 16.3 (C-19), 24.3 (C-21), 23.7 (C-26), 27.4 (C-27), and 16.5 (C-30) were determined by the HMBC correlation of the corresponding proton to neighboring carbon via HMBC correlation (shown in Figure 2). In addition, protons at δ_H 1.47 (H–16) correlated with carbon δ_C 86.4 (C-20) and δ_C 50.0 (C-17), confirming branch wires attached at C-17. The HMBC correlation of protons at δ_H 1.10 (H–21) to carbon at δ_C 83.3 (C-24) confirms the ring closure of C-20 and C-24 via tetrahydrofuran ring-forming ether joining. Additionally, HMBC spectroscopy shows the correlation of protons at δ_H 1.20 (H–23) to carbon δ_C 23.7 (C-26), protons at δ_H 1.20 (H–27) correlated with carbon at δ_C 83.3 (C-24), 71.4 (C25). These correlations confirm the location of the three-carbon branch wire attached at C-24.

From the above spectral figures combined with reference comparisons, the recommended structure of compound **1** is 3β-acetyl-20,24-epoxy-dammaran-25-ol (Tanaka et al., 1990; R.Hidayat et al., 2018). The stereochemistry of C-20 and C-24 has not been identified.

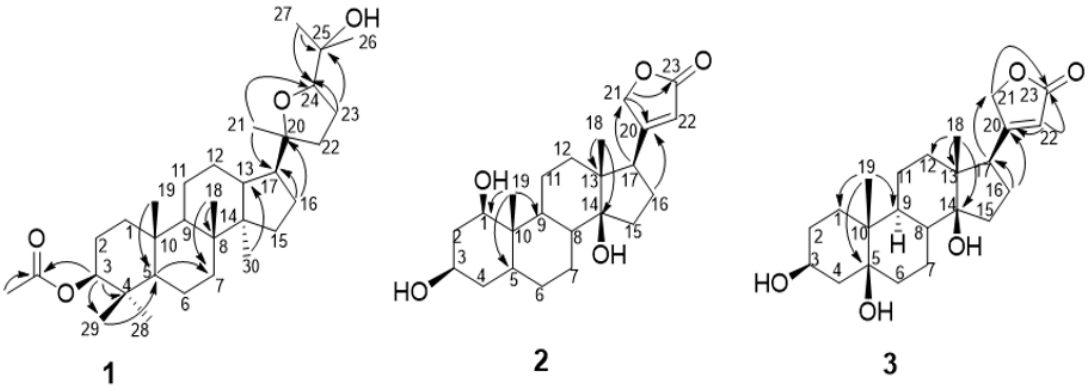

Figure 2. *The HMBC correlations of* ***1-3***

The ^{1}H-NMR spectrum of compound **2** shows the resonance signals of two singlet methyl at δ_H 0.89 (3H, s, H-18) and 1.11 (3H, s, H-19), the two singlet signals of two carbinol protons resonate at δ_H 4.20 (1H, m, H-3) and 3.84 (1H, m, H-1). There is also the presence of a singlet olefin resonated at δ_H 5.87 (1H, s, H-22), a signal doublet of doublets displayed at δ_H 2.77 (1H, dd, J = 9.0, 5.5 Hz, H-17). The signal doublet of doublets presented at δ_H 4.98 (1H, dd, J = 18.0 Hz; 1.0 Hz, H-21a) and 4.79 (1H, dd, J = 18.0 Hz; 1.0 Hz, H-21b). Resonance signals from 1.20 to 2.10 ppm are those of other methylene protons.

The ^{13}C–NMR spectrum of compound **2** shows the resonance signal of 23 carbons, of which two methyl groups displayed at δ_C 18.9 (C-19) and 15.8 (C-18). There are nine methylene groups from 21.0 to 74.0 ppm, and seven methylene groups have a chemical shift from 36.0 to 118.0. Carbon methine attached with the electron attraction group resonates at δ_C 68.2 (C-3) and 73.4 (C-1), a carbon olefin methine resonates at δ_C 117.9 (C-22); five quaternary carbons, including one quaternary carbon resonating at δ_C 85.4 (C-14). In addition, there exits an α,β-unsaturated γ-lactone ring at δ_C 174.2 (C-23), 117.9 (C-22), and 174.4 (C-20). The HMBC spectroscopy shows the correlations between protons δ_H 4.97 (H-21), olefinic carbons at δ_C 117.9 (C-22) and 174.4 (C-20), and carbon of the α,β-unsaturated γ-lactone ring at δ_C 174.2 (C-23). In addition, protons δ_H 2.77 (H-17) correlate with carbons at δ_C 174.4 (C-20), 117.9 (C-22), 85.4 (C-14), and 73.4 (C-21), demonstrating the presence of unsaturated lactone rings attached at the C-17 position. Besides, the methyl group proton at δ_H 0.89 (H-18) and proton at δ_H 2.77 (H-17) correlated with oxygenated carbon at δ_C 85.4 (C-14), demonstrating the presence of a hydroxyl group at the C-14 position. The methyl group proton at δ_H 1.11 (H-19) correlated with the carbons at δ_C 73.4 (C-1), 40.0 (C-10), and 37.4 (C-9), demonstrating the presence of a hydroxyl group at the C-1 position. Some other HMBC correlations are shown in *Figure 2*. The stereochemistry of compound **2** was determined by comparison with previous spectroscopic data (Kohls et al., 2012, Robien et al., 1987, Tan et al., 2002). The comparison shows an orientation β of α,β-unsaturated γ-lactone ring. The ^{1}H-NMR and ^{13}C-NMR data of compound **2** are similar to data previously

published, the spectral data for compound 1*β*,3*β*,14*β*-trihydroxycard-20(22)-enolide, or acovenosigenin A (Rodrigo et al., 2007). Hence, compound 2 is acovenosigenin A.

The ^{1}H-NMR spectrum of compound **3** is similar to compound **2**, including two singlet methyl at δ_H 0.90 (3H, s, H-18) and 0.96 (3H, s, H-19), one carbinol protons resonate at δ_H 4.19 (1H, m, H-3). In addition, a signal doublet of doublets is displayed at δ_H 5.89 (1H, dd, J=1.0, 1.0 Hz, H-22). Moreover, a signal doublet of doublets exhibited at δ_H 2.77 (1H, dd, *J* = 9.0, 5.5 Hz, H-17). The signal doublet of doublets presented at 5.00 (1H, dd, *J* = 18.0 Hz; 1.5 Hz, H-21a) and 4.98 (1H, dd, *J* = 18.0 Hz; 1.5 Hz, H-21b). Like compound **2**, the ^{13}C–NMR spectrum of compound **3** shows the resonance signal of 23 carbons, including two methyl groups displayed at δ_C 16.8 (C-19) and 15.7 (C-18), ten methylene groups from 21.0 to 74.0 ppm, seven methylene groups having a chemical shift from 36.0 to 118.0, five carbon methines attached to the electron attraction group resonating at δ_C 68.0 (C-3), a carbon olefin methine resonating at δ_C 117.9 (C-22), and six quaternary carbons, including one quaternary carbon resonating at δ_C 85.4 (C-14). Besides, there is also the presence of an *α,β*-unsaturated *γ*-lactone ring at δ_C 174.5 (C-23), 117.7 (C-22), and 174.7 (C-20). As well as compound **2**, the HMBC spectroscopy shows the correlations of protons δ_H 4.98 (H-21) with olefinic carbons at δ_C 117.7 (C-22), 174.7 (C-20), and with the carbon of the *α,β*-unsaturated *γ*-lactone ring at δ_C 174.5 (C-23). Furthermore, protons δ_H 2.80 (H-17) correlate with carbons at δ_C 174.7 (C-20), 117.9 (C-22), 85.4 (C-14), and 73.5 (C-21), showing the presence of unsaturated lactone rings attached at the C-17 position. Additionally, the methyl group proton at δ_H 0.90 (H-18) and proton at δ_H 2.80 (H-17) correlated with oxygenated carbon at δ_C 85.4 (C-14), displaying the presence of a hydroxyl group at the C-14 position. Some other HMBC correlations are shown in *Figure 2*. The stereochemistry of compound **3** was determined similar to compound **2,** concluding that *α,β*-unsaturated γ-lactone ring of compound **3** is *β* orientation. From the analysis of NMR data compound **3**, compared with previously published spectral, it is concluded that compound **3** is periplogenin (Junior & Wichtl, 1979).

4. Conclusions

From the chloroform extraction of leaves and stems vines of *Streptocaulon juventas* Merr, using the chromatography method, three pure compounds were isolated, including two steroids and one triterpene. Their structures were elucidated by the spectroscopic method. Although two steroids were found in the root of *S. juventas*, this is the first time that their structures were discovered in the leaves and stems vines of *S. juventas*. Triterpene **1** is the compound that was first discovered in leaves and stems vines of *Streptocaulon juventas* Merr.

❖ ***Conflict of Interest:*** *Author have no conflict of interest to declare.*

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STEROID VÀ TRITERPENE TỪ DỊCH CHIẾT LÁ VÀ THÂN DÂY LEO CỦA *STREPTOCAULON JUVENTAS MERR*

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TÓM TẮT

Từ dịch chiết chloroform của lá và thân của dây leo của cây Hà thủ ô trắng, bằng các phương pháp sắc kí lớp mỏng và sắc kí cột, chúng tôi đã phân lập được ba hợp chất hữu cơ, bao gồm hai steroid và một triterpenoid với tên gọi là 3β-acetyl-20,24-epoxy-dammaran-25-ol, acovenosigenin A, periplogenin. Cấu trúc của các hợp chất này được xác định bằng các phương pháp phổ nghiệm, chủ yếu là phổ cộng hưởng từ hạt nhân. Đây là lần đầu tiên cấu trúc của các hợp chất này được tìm thấy trong lá và thân của dây leo của cây Streptocaulon juventas.

Từ khóa: lá và thân dây leo; steroid; *Streptocaulon juventas;* triterpene