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CHEMICAL CONSTITUENTS FROM THE RHIZOMES OF *ADENIA CARDIOPHYLLA* (MAST.) ENGL.

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Summary

Chemical Constituents from the Rhizomes of *Adenia cardiophylla* (Mast.) Engl.

Adenia cardiophylla is a species in the genus *Adenia* in the Passifloraceae family. The plant's rhizomes have a slightly sweet taste, and they have been used in ethnic medicine in An Giang province to treat lung inflammatory diseases. Five compounds were isolated and identified as 2,3-epoxydeca-4,6,8-triyn-1-ol (**1**), vanillic acid (**2**), demethoxypinoresinol (**3**), succinic acid (**4**), a mixture of deidaclin (**5a**) and tetraphyllin A (**5b**) from the rhizomes of *Adenia cardiophylla*. Their structures were determined by using spectroscopic methods (MS, 1D, and 2D NMR). In addition, the absolute configurations of deidaclin and tetraphylin were identified by comparing experimentally and calculated electronic circular dichroism (ECD) analysis methods.

Keywords: *Adenia cardiophylla*, Passifloraceae, *Cyanogenic glycoside, ECD calculations.*

1. Introduction

Adenia cardiophylla (Mast.) Engl. is a vascular plant in the Passifloraceae family. It is a canopy liana with tendrils up to 20 cm, primarily woody vines 25 m long, and rarely shrubs or trees. It is distributed in the Southeast region of Vietnam, such as Binh Duong, Dong Nai, Ba Ria-Vung Tau, An Giang, and Kien Giang provinces [1],[2],[3]. Several studies on the genus *Adenia* explored the main chemical components of the plants in this genus: toxic proteins [4], cyanogenic compounds [5],[6],[7], lectins [8],[9], polyacetylenes [10], and flavonoids [5]. In addition, species in the genus *Adenia* were reported to possess remarkable biological activities such as anti-oxidative [11], anti-hyperglycaemic [12], anti-spasmodic [13], anti-coagulation [14], anti-depressant [15], anti-anxiety [15], and cytotoxicity effects [9-10]. Until now, most publications on the chemical composition of species in the genus *Adenia* have been carried out on *A. digitata*, *A. gummifera, A. volkensii*, *A. cissampeloides*, *A. globosa*, *A. lobata* and *A. mannii* [4],[5],[6],[7],[8],[9],[10],[11],[12],[13],[14,[15]. To our knowledge, no research has been reported on the phytochemical and pharmacological effects of *Adenia cardiophylla*. Our present study on phytochemical constituents from the ethyl acetate fraction of the rhizomes of *A. cardiophylla* led to the isolation of five compounds. Their structures were identified by spectroscopic data and compared to those reported in the literature. Herein, experimental and calculated ECD spectra analysis confirmed the absolute configuration of compounds **5a**.

2. Experimental

2.1. Materials and Reagents

The rhizomes of *Adenia cardiophylla* (Mast.) Engl. were collected from Tinh Bien town, An Giang province, Vietnam, in November 2018. The voucher specimen (AC112018.AG) was deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City (UMP), Vietnam. The plant was identified by Dr. Vo Van Leo (Department of Pharmacognosy, UMP) and compared to the morphological characteristics as described in botanical references [11]. The solvents used for isolation and extraction were all in analytical reagent (AR) grade: ethanol (96%), *n*-hexane, ethyl acetate, chloroform, methanol, and *n*-butanol (Scharlau, Spain).

2.2. General experimental procedures

Silica gel (40-63 µm, Merck) and Sephadex LH-

20 (GE Healthcare, Sweden) were used for column chromatography (CC). TLC was performed using *silica gel* 60 F_{254} plates (Merck). Spots were detected by UV and 5% vanillin-concentrated sulfuric acid in absolute ethanol or 1% $FeCl_3$ solution in 95% ethanol. ECD spectra were obtained on a Chirascan Plus (Applied Photophysics Ltd., UK). ESI-MS spectra were obtained from a Thermo MSQ plus mass spectrometer (Thermo Scientific, USA), and HR-ESIMS data were acquired on an Agilent 6350 Q-TOF mass spectrometer (Agilent, USA). NMR spectra were measured on a Bruker Avance Neo 400 MHz spectrometer, using TMS as an internal standard. NMR solvents were purchased from Sigma-Aldrich (USA).

2.3. Extraction and isolation

The rhizomes of *A. cardiophylla* (7.0 kg) were ground and percolated with 70% ethanol (105 L). The solvents were evaporated under reduced pressure to obtain the concentrated ethanol extract (2 L). The ethanol extract was successively partitioned with *n*-hexane (2L x 3), ethyl acetate (2L x 3), and saturated *n*-BuOH (2L x 3), then the solvents were removed to obtain 48 g *n*-hexane fraction (H), 45 g ethyl acetate fraction (E), 37 g *n*-BuOH fraction (B), and 254 g water residue (W), respectively. The ethyl acetate fraction (E, 35.0 g) was subjected to *silica gel* CC (500 g) and eluted with $CHCl_3$-MeOH (10:0 to 5:5, v/v) to obtain fourteen sub-fractions (E1 - E14). Fraction E4 (3.0 g) was separated by *silica gel* CC (100 g) and eluted with *n*-hexane-EtOAc (9:1 to 5:5, v/v) to obtain fourteen sub-fractions (E4.1 - E4.14). Fraction E4.3 (120 mg) was further purified by Sephadex LH-20 CC (MeOH) to obtain compound **1** (10.4 mg). Fraction E4.5 (116.4 mg) was crystallized in MeOH and filtered to give compound **2** (32.3 mg). Fraction E4.9 (160 mg) was subjected to Sephadex LH-20 CC ($CHCl_3$: MeOH, 9:1, v/v) to achieve compound **3** (14.7 mg). Fraction E4.11 (240 mg) was loaded on Sephadex LH-20 CC (MeOH) to yield three sub-fractions (E4.11.1 - E4.11.3). Fraction E9 (3.03 g) was evaporated in *vacuo* and precipitated to obtain compound **4** (177.6 mg). Fraction E11 (2 g) was subjected to *silica gel* CC (80 g) and eluted with saturated ethyl acetate in water to obtain eight sub-fractions (E11.1 - E11.8). Compounds **5a** and **5b** (217.7 mg) were obtained in a mixture from sub-fraction E11.7 (513.9 mg).

2,3-Epoxydeca-4,6,8-triyn-1-ol (1): colorless amorphous powder. ESI-MS: *m/z* = 159.0302 $[M-H]^-$, $C_{10}H_8O_2$. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 4.85 (1H, d, *J* = 5.2 Hz, H-3), 3.81 (1H, q, *J* = 5.2 Hz, H-2), 3.65 (1H, dt, *J* = 11.2, 5.2 Hz, H-1a), 3.62 (1H, dt, *J* = 11.2, 5.2 Hz, H-1b), 1.99 (3H, s, CH_3-10). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 77.7 (C-9), 74.8 (C-2), 71.5 (C-5), 71.2 (C-4), 64.5 (C-7), 63.3 (C-8), 62.4 (C-1), 57.2 (C-6), 49.9 (C-3), 2.4 (C-10)

Vanillic acid (2): colorless amorphous powder, ESI-MS: *m/z* = 167.00 $[M-H]^-$, $C_8H_8O_4$. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.58 (1H, d, *J* = 2.0 Hz, H-2), 7.57 (1H, dd, *J* = 8.8, 2.0 Hz, H-6), 6.88 (1H, d, *J* = 8.8 Hz, H-5), 3.91 (3H, s, 3-OCH_3). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 168.6 (C-7), 151.3 (C-4), 147.2 (C-3), 123.9 (C-6), 121.6 (C-1), 114.4 (C-5), 112.3 (C-2), 54.9 (3-OCH_3).

(7*S**,8*R**,7′*S**,8′*R**)-**Demethoxypinoresinol (3)**: colorless amorphous powder. ESI-MS: *m/z* = 327.14 $[M-H]^-$, $C_{19}H_{20}O_5$. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.22 (2H, d, *J* = 8.4 Hz, H-2 and H-6), 6.97 (1H, d, *J* = 2.0 Hz, H-2′), 6.83 (1H, dd, d, *J* = 8.4, 2.0 Hz, H-6′), 6.78 (2H, d, *J* = 8.4 Hz, H-3 and H-5), 6.78 (1H, d, *J* = 8.4 Hz, H-5′), 4.72 (1H, d, *J* = 4.4 Hz, H-7), 4.71 (1H, d, *J* = 4.4 Hz, H-7′), 4.25 (1H, dd, *J* = 9.2, 6.8 Hz, H-9a), 4.22 (1H, dd, *J* = 9.2, 6.8 Hz,, H-9a′), 3.88 (3H, s, 3′-OCH_3), 3.84 (1H, dd, *J* = 9.2, 3.6 Hz, H-9b), 3.83 (1H, dd, *J* = 9.2, 3.6 Hz, H-9b′), 3.16 (2H, m, H-8 and H-8′). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 156.9 (C-4), 147.7 (C-3′), 145.9 (C-4′), 132.4 (C-1′), 131.6 (C-1), 127.3 (C-2 and C-6), 118.7 (C-6′), 114.8 (C-3 and C-5), 114.6 (C-5′), 109.5 (C-2′), 86.2 (C-7′), 86.0 (C-7), 71.2 (C-9), 71.1 (C-9′), 54.9 (3′-OCH_3), 54.0 (C-8), 53.8 (C-8′).

Succinic acid (4): colorless amorphous powder. ESI-MS: *m/z* = 117.47 $[M-H]^-$, $C_4H_6O_4$; ^{1}H NMR (400 MHz, CD_3OD) δ_H: 2.58 (4H, s, H_2-2 and H_2-3). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 174.9 (C-1 and C-4), 28.4 (C-2 and C-3).

Compound 5: colorless amorphous powder. ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (+8.23); ESI-MS: *m/z* = 294.09531 $[M+Na]^+$, (calcd for $C_{12}H_{17}NO_6Na$, 294.09536).

Table 1. The ^{13}C (100 MHz, CD_3OD) and ^{1}H NMR (400 MHz, CD_3OD) data of **5a** and **5b**.

C	5a		Deidaclin [7]		5b		Tetraphyllin A [7]	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	83.5	-	84.9	-	82.8	-	84.1	-
2	129.7	5.97 dt (5.6, 2.0)	131.0	5.96 dt (5.6, 2.3)	128.5	6.35 dt (5.6, 2.0)	129.9	6.34 dt (5.6, 2.3)
3	139.3	6.27 dt (5.6, 2.0)	140.7	6.27 dt (5.6, 2.3)	140.6	6.00 dt (5.6, 2.0)	141.9	6.00 dt (5.6, 2.3)
4a	30.2	2.50 m	31.6	2.40-2.65 m	30.6	2.50 m	32.0	2.5-2.6 m
4b		2.58 m				2.58 m		

C	5a		Deidaclin [7]		5b		Tetraphyllin A [7]	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
5a	37.1	2.46 m	38.5		37.7	2.46 m	39.2	
5b		2.55 m				2.55 m		
CN	119.1		120.6		119.6		121.0	
1′	100.1	4.60 d (8.0)	101.4	4.61 d (7.6)	99.5	4.52 d (8.0)	100.9	4.52 d (7.6)
2′	73.3	3.18 m	74.7	3.2 t (7.6)	73.3	3.14 m	74.8	3.2 t (7.6)
3′	76.5	3.29 - 3.36 m	77.9	3.3-3.45 m	76.7	3.29 - 3.36 m	78.0	3.3-3.45 m
4′	69.8		71.3		69.9		71.4	
5′	76.8		78.1		76.8		78.1	
6a′	61.1	3.84 dd (12.5, 2.5)	62.5	3.86 dd (12.0, 2.3)	61.2	3.86 dd (12.0, 2.5)	62.6	3.86 dd (12.0, 2.3)
6b′		3.66 m		3.66 dd (12.0, 5.2)		3.68 m		3.68 dd (12.0, 5.2)

2.4. Theoretical ECD calculations

The absolute configuration of compounds **5a/5b** was determined by using density functional theory (DFT) calculations and carried out with the Turbomole 6.5 program. The conformational studies used the MMFF94 force field to obtain energy minimization by the ChemBio3D 21.0.0 software. The MMFF94 conformers were optimized using the DFT/B3LYP exchange-correlation function with the basis set 6-31G(d)/def-SV(P) for compounds **5a** and **5b**. The ECD spectra were simulated by overlapping Gaussian functions for each transition, where σ is the width of the band at 1/*e* height, and Δ*Ei* and R*i* are the excitation energies and rotatory strengths for transitions *I*, respectively. In this case, the value of σ = 0.10 eV was used. The ground-state geometries and the calculated ECD data were performed using the TMolex4.0 software package [16].

3. Results and discussion

Compound **1** was isolated as a colorless amorphous powder. The ESI-MS spectrum showed the negative ion peak with *m*/*z* = 159.0302 $[M-H]^-$, calculated for the molecular formula of $C_{10}H_8O_2$ with seven degrees of unsaturation. The ^{13}C NMR spectrum showed the signals of 10 carbons, including six non-protonated *sp*-hybridized carbons in a conjugated triyne system in the range of δ_C 57-77, two oxymethine groups at δ_C 74.8 (C-2), δ_C 49.9 (C-3), one oxymethylene carbon at δ_C 62.4 (C-1), and one methyl group at δ_C 2.4 (C-10). The ^1H NMR spectrum revealed the signals of one methylene proton at δ_H 3.65 (1H, dt, *J* = 11.2, 5.2 Hz, H-1a) and 3.62 (1H, dt, *J* = 11.2, 5.2 Hz, H-1b), two aliphatic methine protons at δ_H 4.85 (1H, d, *J* = 5.2 Hz, H-3) and 3.81 (1H, q, *J* = 5.2 Hz, H-2), and one methyl proton at δ_H 1.99 (3H, s, CH_3-10). The ^1H NMR and ^{13}C NMR spectra suggested that compound **1** was a polyacetylene derivative with three conjugated triple bonds. Based on the molecular formula, compound **1** possessed one epoxide ring at C-2 and C-3 to satisfy the 7 degrees of unsaturation. The COSY spectrum revealed the correlations of long-range coupling between H_3-10 (δ_H 1.99) and H-2 (δ_H 4.85)/H-3 (δ_H 3.81), between H-3 and H_2-1 (δ_H 3.65 and 3.62). The HMBC spectrum exhibited the correlations from H_2-1 to C-2, C-3; from H-2 to C-3, C-4, C-5; from H-3 to C-2, C-4, C-5, C-6, C-7; and from H_3-10 to C-4, C-5, C-6, C-7, C-8, C-9. Based on the above NMR spectra and comparison with the reference spectroscopic data [17], compound **1** was identified as 2,3-epoxydeca-4,6,8-triyn-1-ol. It is reported from *A. cardiophylla* for the first time.

Compound **2** was isolated as a colorless amorphous powder. Its negative ESI-MS spectral data exhibited the ion peak at *m*/*z* = 167.00 $[M-H]^-$, consistent with the molecular formula of $C_8H_8O_4$, implying 5 degrees of unsaturation. The ^{13}C NMR spectrum of compound **2** showed the presence of 8 carbons, including one carbonyl carbon (δ_C 168.6, C-7), three aromatic methines (δ_C 123.9 (C-6), 114.4 (C-5), and 112.3 (C-2)), three non-protonated carbons (δ_C 151.3 (C-4), 147.2 (C-3), and 121.6 (C-1)), and one methoxy group at δ_C 54.9 (3-OCH_3). The ^1H NMR and ^1H-^1H COSY spectra exhibited the aromatic *ortho*-coupling between protons H-5 (δ_H 6.86, d, *J* = 8.8 Hz) and H-6 (δ_H 7.58, dd, *J* = 8.8, 2.0 Hz), and the aromatic *meta*-coupling between H-6 and H-2 (δ_H 7.57, d, *J* = 2.0 Hz). The ^1H NMR and ^{13}C NMR spectra suggested that compound **2** was a simple phenolic acid derivative. In the HMBC spectrum, the correlations were observed between H-5 (δ_H 6.86) and C-1 (δ_C 121.6)/C-3 (δ_C 147.2)/C-4 (δ_C 151.3), between H-2 (δ_H 7.57) and C-1/C-3/C-4/C-6 (δ_C 123.9)/C-7 (δ_C 168.6), between H-6 (δ_H 7.58) and C-1/C-2 (δ_C 112.3)/C-4/C-7, and the correlation between methoxy group (δ_H 3.91) and C-3. The HMBC correlations of compound **2** were shown in Fig. 2. Based on the NMR spectra data and compared to reference data [18], compound **2** was determined as vanillic acid.

Compound **3** was obtained as a colorless

amorphous powder. The ESI-MS spectral data exhibited a negative-ion peak at *m/z* = 327.14 $[M-H]^-$, corresponding to the molecular formula of $C_{19}H_{20}O_5$ and 10 degrees of unsaturation. The ^{13}C NMR spectrum of compound **3** showed 19 carbon signals, containing 11 methine carbons (seven aromatic methines at δ_C 127.3 (C-2 and C-6), 118.7 (C-6′), 114.8 (C-3 and C-5), 114.6 (C-5′), and 109.5 (C-2′)), and four aliphatic methines at δ_C 86.2 (C-7′), 86.0 (C-7), 54.0 (C-8), and 53.8 (C-8′)), five non-protonated carbons (δ_C 156.9 (C-4), 147.7 (C-3′), 145.9 (C-4′), 132.4 (C-1′), and 131.6 (C-1)), two oxymethylene carbons (δ_C 71.2, C-9 and 71.1, C-9′), and one methoxy group (δ_C 54.9, 3′-OCH_3). The carbon signals at δ_C 86.2 (C-7′), 86.0 (C-7), 54.0 (C-8), 53.8 (C-8′), 71.2 (C-9), and 71.1 (C-9′) were characterized for the furofuran lignan framework. The ^1H NMR and ^1H-^1H COSY spectra of **3** showed a couple of a symmetric proton signals at δ_H 7.22 (2H, d, *J* = 8.4 Hz, H-2 and H-6) and 6.78 (2H, d, *J* = 8.4 Hz, H-3 and H-5) and signals of a 1,3,4-trisubstituted aromatic ring (δ_H 6.97, d, *J* = 2.0 Hz, H-2′, 6.83, dd, d, *J* = 8.4, 2.0 Hz, H-6′, and 6.78, d, *J* = 8.4 Hz, H-5′). In addition, the COSY spectrum showed the correlations of furofuran spin systems H_2-9/(H-7)/H-8/H-8′/(H-7′)/H_2-9′ (Fig. 2). The HMBC correlations were observed from H-2, H-6 to C-2, C-4, C-5, C-7; from H-3, H-5 to C-4, C-5; from H-7 to C-1, C-2, C-8, C-9; from H-8 to C-1, C-7, C-8′; from H_2-9 to C-7, C-8; from H-2′ to C-1′, C-3′, C-4′, C-6′, C-7, from H-6′ to C-2′, C-4′, C-7′, from H-7′ to C-1′, C-6′, C-8′, C-9′, from H-8′ to C-1′, C-7′, C-8, and from methoxy group to C-3′ (Fig. 2). Based on the NMR data and compared to the reference data [19], compound **3** was determined as demethoxypinoresinol, which was first reported in *A. cardiophylla*. The *α*-orientations of H-8 and H-8′ was inferred from the coupling constant (*J* = 9.2, 3.6 Hz) of H-9b and H-9b′ based on the references data [19],[20]. Furthermore, the relative stereochemistry of **3** at C-7, C-7′, C-8, and C-8′ was compared with the known compound, pinoresinol by the coupling constants (*J* = 4.4 Hz) of H-7/H-8 and H-7′/H-8′ in **3,** and H-7/H-8 and H-7′/H-8′ (*J* = 4.5 Hz) as mentioned in the references data [19],[20]. Thus, compound **3** was determined to be (7*S**,8*R**,7′*S**,8′*R**)-demethoxypinoresinol.

Compound **4** was obtained as a colorless amorphous powder. The negative ESI-MS spectral data showed the pseudomolecular ion at *m/z* = 117.47 $[M-H]^-$, corresponding to the molecular formula of $C_4H_6O_4$ with 2 degrees of unsaturation. The ^{13}C NMR spectrum data showed the signals of a carbonyl group at δ_C 174.9 and a methylene group at δ_C 28.4. The ^1H NMR revealed the signal of only one methylene proton at δ_H 2.58, with the aid of the molecular formula compound **4** was determined as a symmetric dicarboxylic acid. Based on the spectroscopic data, compound **4** was identified as succinic acid [21].

Compound **5** was isolated as a colorless amorphous powder. The ^{13}C NMR of compound **5** showed 12 carbon signals, including one non-protonated nitrile carbon at δ_C 119.2, one non-protonated sp^3-hybridized carbon at δ_C 83.5 (C-1), two olefinic carbons (δ_C 139.3, C-2 and 129.7, C-3), five oxymethine carbons (δ_C 100.1 (C-1′), 76.8 (C-5′), 76.5 (C-3′), 73.3 (C-2′), and 69.8 (C-4′)), and three methylene carbons (one oxymethylene at δ_C 61.1 (C-6′) and two aliphatic methylenes at δ_C 37.1 (C-5) and 30.2 (C-4)). All the carbon signals were typical of a cyanogenic glycoside. The ^1H NMR showed the signals of olefinic protons at δ_H 6.27 (1H, dt, *J* = 5.6, 2.0 Hz, H-2) and 5.97 (1H, dt, *J* = 5.6, 2.0 Hz, H-3), and two methylene protons (δ_H 2.58 (H-4b), 2.55 (H-5b), 2.50 (H-4a), and 2.46 (H-5a)). The signals of an anomeric proton at δ_H 4.60 (1H, d, *J* = 8.0 Hz, H-1′), oxymethine, and oxymethylene protons in the range of δ_H 3.18-3.84, demonstrated a *β*-hexosyl sugar moiety attached to the cyanogenic aglycon. The ^1H-^1H COSY spectrum showed the presence of two independent spin system correlations incorporating all methine and methylene protons: H-2/H-3/H_2-4/H_2-5/, and H-1′/H-2′/H-3′/H-4′/H-5′/H_2-6′. The HMBC spectrum exhibited correlations from H-2 to carbons C-1, C-3, C-4, C-5; from H-3 to carbons C-1, C-2, C-4, C-5; from H_2-4 to C-1, C-2, C-3, C-5; from H_2-5 to C-1, C-2, C-4, C-6. Furthermore, the HMBC correlations from H-1′ to C-1/C-2′/C-3′, from H-2′ to C-1′/C-3′, and from H_2-6′ to C-4′/C-5′ revealed the linkage of the *β*-D-glucopyranosyl group to C-1. The molecular formula of **5** was determined as $C_{12}H_{17}NO_6$ from the positive HRESI-TOF/MS ion peak at *m/z* = 294.09531 $[M+Na]^+$ (calcd. for $C_{12}H_{17}NO_6Na$, 294.09536), consistent with 5 degrees of unsaturation. The ^{13}C NMR and ^1H NMR spectra data of **5** showed the mixture of two epimers at C-1 with the ratio of 4/1. By comparing the NMR spectral data with reference [6],[7], compound **5** was identified as a mixture of deidaclin (1*R*-form) (**5a**) and tetraphyllin A (1*S*-form) (**5b**) as shown in Fig. 3. Comparison of the calculated and experimental ECD spectra suggested that the major enantiomer of 1*R*-form predominates in the mixture (Fig. 4).

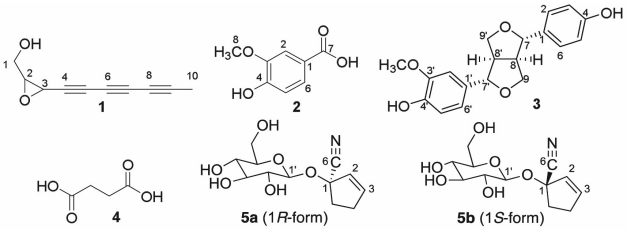

Fig.1. Chemical structures of compounds **1-5a and 5b**

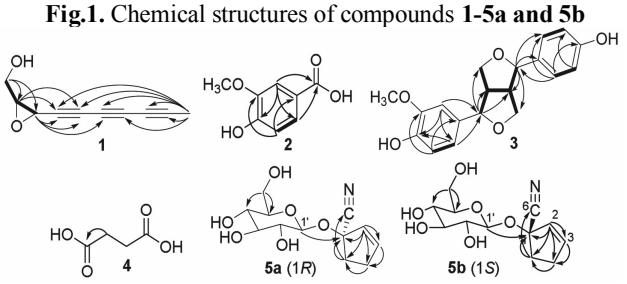

Fig.2. The key HMBC (⟶) and COSY (▬) correlations of compounds **1-5a/5b**.

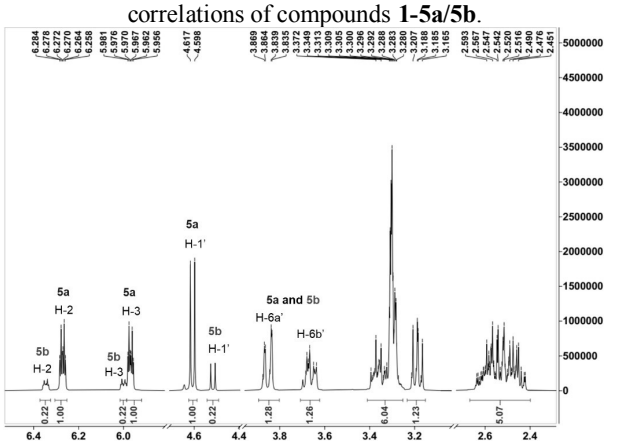

Fig. 3. ^{1}H NMR spectrum of the mixture of **5a/5b** (ratio of 4:1)

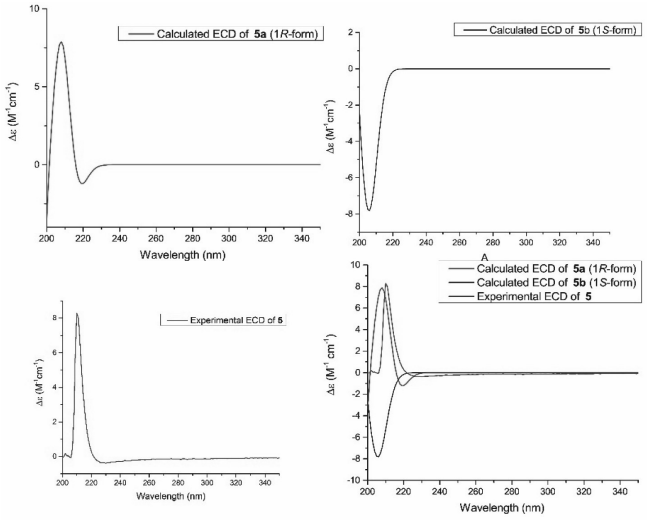

Fig. 4. Experimental and calculated ECD spectra of compounds **5, 5a, and 5b**

4. Conclusion

In summary, five compounds were isolated from the rhizomes of *A. cardiophylla* (Mast.) Engl. Their structures were identified as 2,3-epoxydeca-4,6,8-triyn-1-ol (**1**), vanillic acid (**2**), demethoxypinoresinol (**3**), succinic acid (**4**), deidaclin (**5a**) and tetraphyllin A (**5b**). The absolute configuration of compound **5a** was confirmed by experimental and calculated ECD spectra. Compounds **1-4** were first reported from the genus *Adenia*. Deidaclin (**5a**) and tetraphyllin A (**5b**) were first isolated from *A. cardiophylla* (Mast.) Engl.

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DISCRIMINATION OF SPECIALTY TEA VARIETIES COLLECTED IN NORTHERN VIETNAM BASED ON FREE AMINO ACIDS PROFILE

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Summary

Discrimination of Specialty Tea varieties Collected in Northern Vietnam Based on Free Amino Acids Profile

Green tea (*Camellia sinensis* L.) is one of the most popular drinks in Vietnam and has become an agricultural product with high export value. There are a lot of specialty tea varieties with distinguished flavor and taste; however, little information was found in the literature about the chemical profiles of these varieties. This study focused on the analysis of free amino acid content of *C. sinensis* L. varieties collected in Northern mountainous Agriculture and Forestry Science Institute (NOMAFSI) by using an Amino acid analyzer system and the discrimination of these samples by PCA statistical analysis. Our data contributed to explain the distinction in quality among unprocessed and processed tea leaves from different *C. sinensis* varieties. The results of this study provide a simple tool for further research on the quality control and traceability of tea products.

Keywords: *Camellia sinensis, PCA, Free amino acid, NOMAFSI, Discrimination, Green tea variety.*

1. Introduction

Tea is one of the most popular drinks in Vietnam and has become an agricultural product with high export value. Different tea products are prepared from the young leaves of *Camellia sinensis* L, normally classified as green tea (unfermented tea), Olong tea (semi-fermented tea), and black tea (fully fermented tea). Wide varieties of this species have been breeding, providing abundant resources for producing and manufacturing tea.

In Vietnam, the public institution taking charge of research and development on cultivation techniques, growing and processing green tea is the Northern mountainous Agriculture and Forestry Science Institute (NOMAFSI). This center was thriving on researching the tea propagation by the line selection method, thus generating many precious tea varieties such as VN15, PH11, PH12, Kim Tuyen…The Institute has 250 ha of land for scientific research and has kept a genetic collection with 170 tea varieties [1]. However, research on the chemical composition of these varieties and markers for tea quality is still limited in this country. To date, there is almost no information of the metabolite diversity of *C. sinensis* speciality teas of Vietnam, both raw materials and products.

Free amino acids are crucial in the chemical composition of tea as they are precursors for bioactive compounds and essential proteins [2],[3],[4]. The correlation between amino acids content and the sensory characteristics of tea was revealed by Horie et al. [5]. Amino acids individually contributed to the taste and flavor of tea leaves and also play a role in forming the aroma during tea processing [6],[7]. The amount of each amino acid and the combination of the complete profile directly affect the taste of tea extract and thus, the quality of tea. These indicators and other components like metals were used as input data for the successful geographical classification of tea and other products by chemometric approaches. In these investigations, the Principal component analysis (PCA) technique, one of the most normally used technique to determine how one sample was differentiated from another, were applied for the purpose of classification [2]. As a result, measuring the level of free amino acids in tea samples is considered a critical aspect of quality control. The amino acids content of tea raw materials varied according to genetic resources (varieties or cultivars) and environmental factors such as: geographic region, shade, fertilizer, and climate condition etc. [8],[9]. On the other hand, the amino acids profile of tea products depend on