NITROGEN-CONTAINING COMPOUNDS FROM *PSEUDERANTHEMUM CARRUTHERSII* VAR. *ATROPURPUREUM*

HỢP CHẤT CHỨA NITROGEN TỪ CÂY XUÂN HOA ĐỎ *PSEUDERANTHEMUM CARRUTHERSII* VAR. *ATROPURPUREUM*

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ABSTRACT

*Genus Pseuderanthemum (Acanthaceae) has 10 species in Vietnam. Pseuderanthemum genus is attracting the attention of many research groups in pharmacology and chemistry. One of these species is Pseuderanthemum carruthersii (Seem.) Guill. var. atropurpureum (Bull.) Fosb. used in traditional remedies to heal the wounds due to its anti–inflammatory activity. Using column chromatographic method with absorbents including silica gel and sephadex LH20, four nitrogen-containing compounds were isolated from the dried leaves of Pseuderanthemum carruthersii (Seem.) Guill. var. atropurpureum (Bull.) Fosb. (Acanthaceae). They included indole 3–carboxaldehyde (**1**), uracil (**2**), adenine (**3**), and betaine (**4**). The chemical structures of these compound were elucidated by using spectroscopic analysis (nuclear magnetic resonance spectroscopy, mass spectrometry and infrared spectroscopy) and reflecting with reported data in the literature. To the best of our knowledge, the former three mentioned substances, indole 3–carboxaldehyde (**1**), uracil (**2**) and adenine (**3**), were isolated for the first time from this genus.*

Keywords: *nitrogen-containing compound, Pseuderanthemum,* indole 3–carboxaldehyde, uracil, adenine, betaine.

TÓM TẮT

*Chi Pseuderanthemum (Acanthaceae) có 10 loài hiện diện ở Việt Nam. Chi Pseuderanthemum đang thu hút sự quan tâm của nhiều nhóm nghiên cứu về các lĩnh vực y dược và hóa học. Loài Pseuderanthemum carruthersii (Seem.) Guill. var. atropurpureum (Bull.) Fosb. là một trong những loài thuộc chi Pseuderanthemum được sử dụng trong dân gian để làm lành vết thương do hoạt tính kháng viêm của nó. Bằng cách sử dụng phương pháp sắc ký với các chất hấp phụ silica gel và sephadex LH20, bốn hợp chất chứa nitrogen đã được cô lập từ lá khô cây Xuân hoa đỏ, Pseuderanthemum carruthersii (Seem.) Guill. var. atropurpureum (Bull.) Fosb. (Acanthaceae). Các hợp chất đó là indole 3–carboxaldehyde (**1**), uracil (**2**), adenine (**3**), và betaine (**4**). Cấu trúc hóa học của chúng được xác định dựa vào các phương pháp phổ nghiệm (phổ cộng hưởng từ hạt nhân, khối phổ và phổ hồng ngoại) và so sánh với tài liệu tham khảo. Theo sự hiểu biết của chúng tôi, ba hợp chất indole 3–carboxaldehyde (**1**), uracil (**2**) và adenine (**3**), được cô lập lần đầu tiên từ loài này.*

Từ khóa: *hợp chất có chứa nitrogen, Pseuderanthemum,* indole 3–carboxaldehyde, uracil, adenine, betaine.

1. INTRODUCTION

Genus *Pseuderanthemum* (Acanthaceae) has 10 species in Vietnam. One of these species is *Pseuderanthemum carruthersii* (Seem.) Guill. var. *atropurpureum* (Bull.) Fosb. used in traditional remedies to heal the wounds.[1] Previous phytochemical

investigations on this species revealed the presence of phenylethanoid, iridoid, triterpnenoid, lignan and flavonoid compounds.[2-3]

In this paper, the isolation of four nitrogen-containing compounds from the leaves of this plant and the structure elucidation of these compounds were reported.

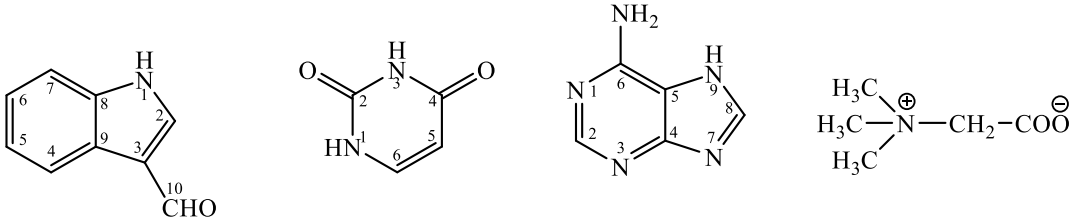

Figure 1. *Chemical structures of the isolated nitrogen-containing compounds*

2. MATERIALS AND METHOD

2.1 Materials

Pseuderanthemum carruthersii leaves were collected in Phuoc Hai Commune, Dat Do District, Ba Ria - Vung Tau Province, Vietnam. The plant was identified by Dr. Hoang Viet, Faculty of Biology, university of Natural Sciences of Ho Chi Minh City, Vietnam.

2.2 Apparatus

NMR spectra were measured on a Bruker Avance III spectrometer, at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C-NMR, using residual solvent signal as internal reference: D_2O δ_H 4.79; or on a Bruker Avance at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, using residual solvent signal as internal reference: CD_3OD δ_H 3.31, δ_C 49.0.

Melting point was determined on a Buchi B–540 melting point apparatus.

The IR spectra were recorded on a Vector22– Bruker FT–IR spectrometer.

The CI–MS spectra were recorded on a Finnigan Mat SSQ–7000 CI–MS spectrometer. The APCI–MS spectra were measured on a LC–MSD–Trap–SL APCI–MS spectrometer.

2.3 Method

The dried and powdered leaves of *Pseuderanthemum carruthersii* (1kg) were exhaustively extracted at room temperature (maceration, 2L x 7 times) with petroleum ether (60-90^{o}C), dichloromethane, ethyl acetate and methanol to obtain concentrated extracts: petroleum ether extract (17g), dichloromethane extract (22g), ethyl acetate extract (7g) and methanol extract (100g).

The dichloromethane extract (22g) was subjected to silica gel column, eluted with petroleum ether and acetone to give 9 fractions. Fraction 6 was re-chromatographed by silica gel columns with dichloromethane:methanol to obtain compound **1**.

The ethyl acetate extract (7g) was applied to silica gel column with dichloromethane: ethyl acetate, to give 21 fractions. Fraction 16 was passed through sephadex LH20 chromatography column with chloroform: methanol 1:1, and then silica gel column with chloroform:methanol to obtain compound **2**. Fraction 20 was purified by sephadex LH20 column chromatography eluted with chloroform:methanol (1:1) and silica gel column chromatography eluted with chloroform:methanol to give compound **3**.

The methanol extract (100g) was subjected to silica gel column, eluted with a mixture of acetone and methanol, then a mixture of methanol and water to give 3 fractions. Fraction 2 was re-chromatographed by silica gel columns to obtain compound **4**.

Indole-3-carboxaldehyde **(1)**: Pale brown powder. m.p. 194^{o}C. CI–MS m/z 146 $[M+H]^{+}$, ^{1}H-NMR (CD_3OD, 400 MHz), δ_H 9.89 (s, 1H, H–10), 8.16 (ddd, J=7.6, 1.2, 0.8, 1H, H–4), 8.09 (s, 1H, H–2), 7.48 (ddd, J=7.6, 1.2, 0.8,

1H, H–7), 7.27 (m, 2H, H–5 and H–6). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 187.4 (C–10), 139.6 (C–3), 138.9 (C–2), 125.7 (C–8), 125.0 (C–5), 123.6 (C–6), 122.4 (C–4), 120.1 (C–9), 113.1 (C–7).

Uracil **(2)**: m.p. 249^{o}C (decompose), CI–MS m/z 113 $[M+H]^+$. IR ν_{max} (KBr) cm^{-1}: 3113, 1717, 1678, 1454, 1418, 1390, 1235, 1097, 822, 760, 584, 564, 544, 435 ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 7.38 (d, J=8.0, 1H, H–6), 5.60 (d, J=8.0, 1H, H–5). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 167.3 (C–4), 153.5 (C–2), 143.5 (C–6), 101.7 (C–5).

Adenine **(3)**: m.p. 342^{o}C. CI-MS m/z 136 $[M+H]^+$, IR ν_{max} (KBr) cm^{-1}: 3303, 3123, 2970, 2797, 2695, 2601, 1670, 1605, 1455, 1416, 1369, 1025, 940. ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 8.19 (s, 1H, H–2), 8.12 (s, 1H, H–8). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 156.8 (C–6), 153.6 (C–2), 151.5 (s, C–4), 141.3 (C–8), 117.4 (C–5).

Betaine **(4)**: APCI–MS m/z 118,1. ^{1}H-NMR (D_2O, 500 MHz) δ_H 3.85 (s), 3.30 (s). ^{13}C-NMR (D_2O, 125 MHz) 168.7, 67.3, 53.8.

3. RESULTS AND DISCUSSION

3.1 Structural elucidation of compound 1

The ^{1}H–NMR of compound **1** displayed a single signal at δ_H 9.89 (s) for a formyl group. Furthermore, the ^{1}H–NMR showed three olefin proton signals at tại δ_H 8.16 (1H; ddd; J = 7.6; 1.2; 0.8 Hz; H–4); 7.48 (1H; ddd; J = 7.6; 1.2; 0.8 Hz; H–7) and 7.27 (2H; m; H–5 and H–6) for an *ortho* two-substituted aromatic ring. The single signal at 8.09 (1H; s; H–2) demonstrated a proton of another aromatic ring.

The ^{13}C and DEPT–NMR of compound **1** revealed 9 carbon signals. The quaternary carbon signal at δ_C 187.4 proved the presence of the formyl group. Moreover, the ^{13}C–NMR also displayed five olefin methines and three olefin quaternary carbons in the region of δ_C 110.0–140.0.

The CI–MS exhibited a pseudo molecular ion peak at m/z 146 $[M+H]^+$ appropriated for molecular formula of C_9H_7NO.

Based on the spectroscopic analysis and comparison with the published data [4], compound **1** was assigned for indole 3–carboxaldehyde.

3.2 Structure elucidation of compound 2

The IR of compound **2** showed an absorption band at 3113 cm^{-1}. This band was attributed to N-H stretching vibration of the secondary amide. The bands at 1717 and 1678 cm^{-1} referred to the secondary amide C=O stretching.

The ^{1}H–NMR of compound **2** displayed two coupling double signals at δ_H 7.38 (1H; d; J = 8.0 Hz; H–6) and δ_H 5.60 (1H; d; J = 8.0 Hz; H–5).

The ^{13}C and DEPT–NMR showed four signals, including two quaternary olefin carbons at δ_C 167.3 (C–4) and 153.5 (C–2); and two methine olefin carbons at δ 143.5 (C–6) and 101.7 (C–5).

The CI–MS of compound **2** revealed a pseudo molecular ion peak at m/z 113 $[M+H]^+$ established for the molecular formula of $C_4H_4N_2O_2$.

Based on the comparison of the spectroscopic data of compound **2** and the published data [5], the structure of compound **2** was assigned for uracil.

3.3 Structure elucidation of compound 3

The IR of compound **3** displayed a N-H stretching band at 3303 cm^{-1} of secondary amine. The peaks at 1670 and 1605 cm^{-1} denoted N-H scissoring and N-H bending bands of primary and secondary amines, respectively. The ^{1}H–NMR of compound **3** exhibited the presence of two single signals at δ_H 8.19 (1H; s; H–2) and 8.12 (1H; s; H–8) for protons of aromatic rings.

The ^{13}C and DEPT–NMR displayed three carbons of the aromatic rings, including a quaternary olefin carbon at δ_C 156.8 (C–6) and two olefin methine carbons at δC 153.7 (C–2) and 141.3 (C–8). Although two signals at δ_C 151.5 (C–4) and 117.4 (C–5) did not appear clearly in ^{13}C–NMR spectrum, they were demonstrated by HMBC spectrum.

The HMBC showed correlations from δ_H 8.12 (1H; s; H–8) to δ_C 151.5 (C–4) and 117.4 (C–5) and from δ_H 8.19 (1H; s; H–2) to δ_C 156.8 (C–6).

The CI–MS exhibited the pseudo molecular ion peak at m/z 136 $[M+H]^+$ corresponding for molecular formula $C_5H_5N_5$.

Based on the spectroscopic analysis and comparison of published data [6], the structure of compound **3** was assigned as adenine.

3.4 Structural elucidation of compound 4

The ^{1}H–NMR of compound **4** showed two single signals at δ_H 3.85 (2H; s; H–2) and 3.30 (9H; s; 3x H_3C–N).

The ^{13}C and DEPT–NMR demonstrated the presence of carbon carboxyl at δ_C 168.7. Furthermore, there were some signals at δ_C 67.3 (C–2) and 53.8 (H_3C–N).

The APCI–MS displayed the simple ion peak at m/z 118.1 ($[M+H]^+$) corresponding to a molecular formula $C_5H_{11}NO_2$. The other ion peaks were polymerized molecular peaks, including a dimer peak at ($[2M+H]^+$; 235.0), trimer peak ($[3M+H]^+$; 352.0), tetramer peak ($[4M+H]^+$; 469.1) and pentamer peak ($[5M+H]^+$; 586.0).

The spectroscopic analysis and comparison of published data [7] assigned the structure of compound **4** as betaine.

4. CONCLUSION

From the dried leaves of *Pseuderanthemum carruthersii* (Seem.) Guill. var. *atropurpureum* (Bull.) Fosb. (Acanthaceae), collected in Phuoc Hai Commune, Dat Do District, Ba Ria-Vung Tau Province, Vietnam, four nitrogen-containing compounds were isolated including indole 3–carboxaldehyde (**1**)**,** uracil (**2**), adenine (**3**), and betaine (**4**). Their chemical structures were elucidated by spectroscopic methods as well as comparing with data in the literature. Three first substances were isolated for the first time from this genus.

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