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TWO NEW 7-DEHYDROAPORPHINE ALKALOIDS AND ANTIPLATELET ACTION APORPHINES FROM THE LEAVES OF *ANNONA PURPUREA*

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Key Word Index—*Annona purpurea*; Annonaceae; leaves; antiplatelet aggregation; 7-dehydroaporphine; 7-hydroxy-dehydrothalicsimidine; 7-formyl-dehydrothalicsimidine.

Abstract—Bioactivity-directed fractionation led to the isolation of two new 7-dehydroaporphine alkaloids, 7-hydroxy-dehydrothalicsimidine (**1**) and 7-formyl-dehydrothalicsimidine (**2**), along with the five known alkaloids, thalicsimidine (**3**), norpurpureine (**4**), *N*-methyllaurotetanine (**5**), lirinidine (**6**) and *N*-methylasimilobine (**7**), from the leaves of *Annona purpurea*. Structural elucidation of these compounds was established by mass and spectroscopic analyses. Among them, **1**, **3**, **4**, **6** and **7** exhibited significant inhibition of collagen, arachidonic acid and platelet activating factor-induced platelet aggregation; **1** also showed inhibition against thrombin-induced platelet aggregation. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Annona purpurea is a bushy tree native to Middle and South America. Its fruit is edible and its wood is used for the manufacture of paper. Various parts of the tree are employed in traditional medicine. The fruit juice is a remedy for fever, chills and jaundice and a decoction of the inner bark is prescribed in cases of dysentery and edema. The powdered seeds were reported to be insecticide and the CH_2Cl_2 extract of the leaves exhibited strong toxicity towards larvae of the crustacean *Artemia salina* (brine shrimp) and pronounced activity against larvae of the mosquito, *Aedes aegypti*, a vector of yellow fever [1]. In previous phytochemical studies, several aporphine alkaloids and annonaceous acetogenins were reported from this species [1, 2]. As part of our continuing search for novel plant bioactive agents, the methanolic extract of the leaves of *A. purpurea* was found to show significant inhibitory effects on the platelet aggregation induced by several aggregating agents. Bioassay-directed fractionation traced the active fractions to alkaloid components. We report herein on the isolation and characterization of two new alkaloids, 7-hydroxy-dehydrothalicsimidine (**1**) and 7-formyl-dehydrothalicsimidine (**2**), along with five known alkaloids, thalicsimidine (**3**), norpurpureine (**4**), *N*-methyllaurotetanine (**5**), lirinidine (**6**) and *N*-methylasimilobine (**7**) from the leaves of *A. purpurea*. Compounds **1**, **3**, **4**, **6** and **7** exhibited the significant inhibition of collagen, arachidonic acid and platelet activating factor-induced platelet aggregation; **1** also showed an inhibitory effect against thrombin-induced platelet aggregation.

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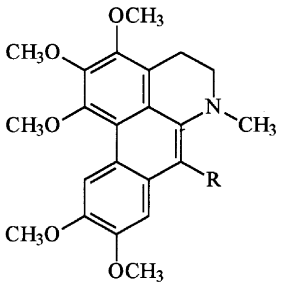
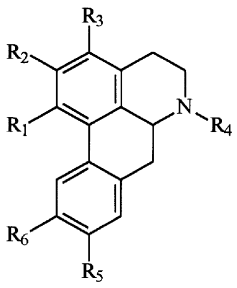
RESULTS AND DISCUSSION

Compound **1** was obtained as a green amorphous powder which was positive to the Dragendorff's test. The HREI mass spectrum showed the $[M]^+$ at m/z 399.1664 corresponding to the molecular formula $C_{22}H_{25}O_6N$ (calcd 399.1682). Its UV absorption maxima at λ 219, 256, 278(*sh*) and 340 nm were characteristic of a 7-dehydroaporphine [3, 4]. The IR absorption at 3500 cm^{-1} and a bathochromic shift of UV absorption while adding alkaline solution indicated the presence of an olefinic hydroxyl group. The ^1H NMR spectrum of **1** exhibited the presence of five aromatic methoxyl groups at δ3.95, 3.96, 4.02, 4.04 and 4.08, two singlets of aromatic protons at δ7.07 and 8.99, two coupled triplets of methylene protons at δ3.25 and 3.29 (each 2H, *t*, J = 6.8 Hz), one methyl group bonded on nitrogen atom at δ3.06 and one D_2O-exchangeable signal at δ6.66. The latter indicated the pre-



R=OH



3. $R_1=R_2=R_3=R_5=R_6=OCH_3$, $R_4=CH_3$

sence of one phenolic function which was also supported by UV and IR spectra. The spectral data suggested that **1** is a 7-dehydroaporphine alkaloid in comparison with literature data [3, 4].

To confirm the structure of **1**, the NOESY spectrum was examined. H-11 and the typical deshielding aromatic proton resonance at δ8.99 (*s*), showed correlations to two methoxyl groups at δ3.95(1-OMe) and 4.04 (10-OMe) (Fig. 1). The sequence of a methoxyl at δ3.96 (3-OMe), methylenes at δ3.25 and 3.29 (H-4 and H-5), the *N*-methyl group at δ3.06, the broad signal of hydroxyl at δ6.66 (7-OH), the aromatic proton at δ7.07 (H-8) and a methoxyl at δ4.02 (9-OMe) were also confirmed by NOESY. According to the above data, the structure of **1** was elucidated as a 7-hydroxy-dehydrothalicsimidine (1,2,3,9,10-pentamethoxy-6-methyl-7-hydroxydehydro-aprophine).

Compound **2** was isolated as yellow needles and also gave a positive Dragendorff's test. The HREI mass spectrum showed the $[M]^+$ at *m/z* 411.1684 corresponding to the molecular formula $C_{23}H_{25}O_6N$ (calcd 411.1682). The UV absorption maxima at 217, 268, 285 and 332 nm suggested the presence of an extended aromatic system as a 7-dehydroaporphine. The IR spectrum of this compound showed an absorption at 1620 cm^{-1} indicating the presence of a typical aromatic aldehyde of a 7-formyl dehydroaporphine [5]. The ^1H NMR revealed the presence of five methoxyl groups at δ3.87, 3.94, 4.03, 4.07 and 4.10, two aromatic singlets at δ8.68 and 8.89, methylenes at δ3.17 and 3.52 (each 2H, *t*, J = 6.8 Hz), *N*-methyl group at δ3.36 and a formyl proton at δ10.26. Due to the close proximity of the 7-formyl group, the chemical shifts of H-8 (δ8.86, *s*) and the *N*-methyl (δ3.36, s) are more down-field than those in the 7-hydroxyl dehydroaporphine **1**.

In the NOESY spectral experiment, the deshielding aromatic proton at δ8.89 (H-11) showed correlations to two methoxyl groups at δ3.87(1-OMe) and 4.03 (10-OMe). The correlations of the methoxyl at δ3.94 (3-OMe), two methylene protons at δ3.17 and 3.52 (H-4 and H-5, each 2H, *t*, J = 6.8), the *N*-methyl at δ3.36, the 7-formyl proton at δ10.26, the aromatic proton at δ8.68 (H-8) and a methoxyl at δ4.07 (9-OMe) were also confirmed by the NOESY spectrum. According to the above data, the structure of **2** was characterized as 7-formyl dehydrothalicsimidine (1,2,3,9,10-pentamethoxy-6-methyl-7-formyl-dehydroaporphine). A 7-formyl-dehydroaporphine readily decomposes when it is exposed in air and the compounds of this type are rare. Only one synthetic and two natural compounds have been studied so far [5–7].

The antiplatelet aggregation activity of the isolated compounds was assayed. Compounds **1**, **3**, **4**, **6** and **7** exhibited inhibition of collagen, arachidonic acid and platelet activating factor induced platelet aggregation and compound **1** also showed inhibition against thrombin induced platelet aggregation. Compound **1**, a new 7-hydroxy-dehydroaporphine, exhibited excellent activity against PAF-induced platelet aggregation at low concentration. The results obtained are shown in Table 1.

EXPERIMENTAL

General

M.p.s are uncorrected. ^1H NMR were recorded at 400 and 200 MHz and ^{13}C NMR at 100 and

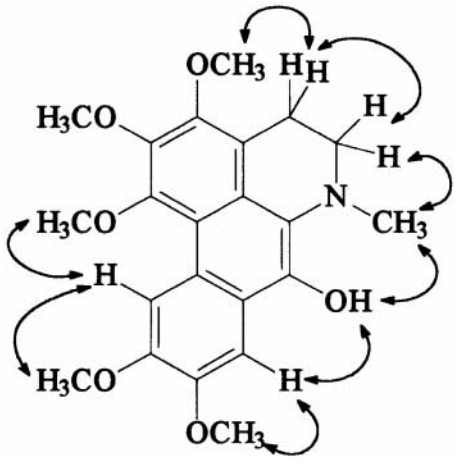


Fig. 1. The NOESY response of **1**.

Table 1. Effects of alkaloids **1**, **3**, **4**, **6** and **7** from *A. purpurea* on the aggregation of rabbit platelets induced by thrombin (Thr), arachidonic acid (AA), collagen (Col) and platelet-activating factor (PAF)[a]

Compound	Concentration (μg ml^{-1})	% Aggregation			
		Thr (0.1 U ml^{-1})	AA (100 μM)	Col (10 μg ml^{-1})	PAF (2 nM)
Control		91.2 ± 1.0(4)	85.0 ± 1.2(6)	86.3 ± 1.2(4)	88.7 ± 0.3(5)
1	100	34.5 ± 18.1(4)[c]	0.0 ± 0.0(4)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]
	50		0.0 ± 0.0(4)[d]		0.0 ± 0.0(3)[d]
	20		67.6 ± 2.8(4)[d]		0.0 ± 0.0(3)[d]
	10		77.4 ± 1.6(4)[b]		0.0 ± 0.0(3)[d]
	5				45.4 ± 8.8(3)[d]
	2				64.9 ± 1.4(3)[b]
	1				77.9 ± 0.6(3)
3	100	87.8 ± 1.8(3)	16.6 ± 13.6(4)[d]	37.6 ± 18.8(3)[b]	59.5 ± 13.3(3)[b]
4	100	68.4 ± 0.3(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]
	50		57.6 ± 5.6(3)[d]		79.4 ± 3.2(3)[b]
	20		83.2 ± 0.5(3)		88.0 ± 1.1(3)
6	100	88.5 ± 0.8(3)	17.8 ± 9.4(4)[d]	0.0 ± 0.0(3)[d]	25.3 ± 13.0(5)[d]
7	100	84.9 ± 1.1(3)[b]	0.0 ± 0.0(4)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]
	50		2.9 ± 2.5(4)[d]		80.7 ± 1.3(3)
	20		55.0 ± 15.2(4)[b]		86.5 ± 3.1(3)
	10		83.6 ± 1.9(4)		

[a]Platelets were preincubated with each compound (100 μg ml^{-1}) or 0.5% DMSO (control) at 37°C for 3 min, then the inducer thrombin (0.1 U ml^{-1}), arachidonic acid (100 μM), collagen (10 μg ml^{-1}) or PAF (2 ng ml^{-1}) was added. Values are presented as means S.E. (n = 3–6).
[b]$P < 0.05$.
[c]$P < 0.01$.
[d]$P < 0.001$, as compared with the respective control.

50 MHz, in $CDCl_3$ using TMS as an internal standard. EIMS were obtained at 70 eV. Silica gel 60 (Macherey-Nagel and Merck), active charcoal (Wako) and Sephadex LH-20 (Pharmacia) were used for the cc. Precoated silica gel plates (Macherey-Nagel, SIL G-25 UV_{254}, 0.25 mm, aluminum) were used for analytical TLC and precoated silica gel plates (Macherey-Nagel, SIL G/UV_{254}, 0.25 mm, Glass) were used for prep. TLC.

Plant material

Annona purpurea L. was collected from Chia-Yi, Taiwan, in June 1993. A voucher specimen (Anno. 8) is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and isolation of alkaloids

Air-dried leaves of (2.4 kg) were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated and partitioned to yield $CHCl_3$ and aqueous extracts. The bases in the $CHCl_3$ solution were extracted with 3% HCl. The HCl solution was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated to leave a brownish viscous residue (10 g). This was subjected to silica gel cc (40 × 6 cm) and eluted with an *n*-hexane–$CHCl_3$–methanol mixture. 100 fractions (120 ml) were further combined into 6 fr. on the basis of TLC composition. Fr. 3 (1.05 g) eluted with *n*-hexane–$CHCl_3$ (1:1) was further purified by repeated silica gel cc to obtain thalicsimidine (**3**) (152 mg) and norpurpureine (**4**) (20 mg). Fr. 5 (1.23 g) eluted with MeOH–$CHCl_3$ (1:20) was further separated and purified by recrystallization, silica gel cc and prep. TLC (MeOH–$CHCl_3$, 1:10) to give 7-hydroxy dehydrothalicsimidine (**1**) (8 mg), 7-formyl dehydrothalisimidine (**2**) (3 mg) and *N*-methyllaurotetanine (**5**) (15 mg), respectively. Fr. 6 (570 mg) eluted with MeOH–$CHCl_3$ (1:12) was further separated and purified by repeated silica gel cc and prep. TLC (MeOH–$CHCl_3$, 1:8) to give lirinidine (**6**) (32 mg) and *N*-methylasimilobine (**7**) (50 mg), respectively.

7-*Hydroxy-dehydrothalicsimidine* (**1**)

Green amorphous powder with m.p. 223–224°C. UV (C_2H_5OH) λ_{max}: 215, 256, 270 (*sh*), 340 nm. IR (neat) ν_{max}: 3600–3200(OH), 1600, 1580 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.99 (1H, *s*, H-11), 7.07 (1H, *s*, H-8), 4.08 (3H, *s*, 2-OMe), 4.04 (3H, *s*, 10-OMe), 4.02 (3H, *s*, 9-OMe), 3.96 (3H, *s*, 3-OMe), 3.95 (3H, *s*, 1-OMe), 3.29 (2H, *t*, J = 6.8 Hz, H-5), 3.25 (2H, *t*, J = 6.8 Hz, H-4), 3.06 (3H, *s*, N–Me). EIMS (70 eV) m/z (rel. int): 398 (16) $[M-1]^+$, 384 (31), 383 (base peak), 366 (37), 368 (55), 310 (20), 192 (25); HREIMS m/z: 399.1664 $[M]^+$ ($C_{22}H_{25}O_6N$, calcd 399.1682).

*7-Formyl-dehydrothalicsimidine (**2**)*

Yellow needles ($CHCl_3$), m.p. 160–162°C. UV (C_2H_5OH) λ_{max}: 217, 268, 335(*sh*), 432 nm. IR (neat) ν_{max}: 1620(C=O), 1600, 1520 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ10.26 (1H, *s*, CHO), 8.89 (1H, *s*, H-11), 8.68 (1H, *s*, H-8), 4.10 (3H, *s*, 2-OMe), 4.07 (3H, *s*, 9-OMe), 4.03 (3H, *s*, 10-OMe), 3.94 (3H, *s*, 3-OMe), 3.87 (3H, *s*, 1-OMe), 3.52 (2H, *t*, J = 6.8 Hz, H-5), 3.36 (3H, *s*, N–Me), 3.16 (2H, *t*, J = 6.8 Hz, H-4). EIMS (70 eV) *m/z* (rel. int): 411 (100) $[M]^+$, 396 (32), 382 (13), 363 (12), 198 (15); HREIMS *m/z*: 411.1680 $[M]^+$ ($C_{23}H_{25}O_6N$, calcd 411.1682).

*Thalicsimidine (**3**)*

Brown amorphous powder (152 mg) with m.p. 131–132°C. $[\alpha]_D^{25}$ +70.60° (*c* 0.12, $CHCl_3$) [2, 8].

*Norpurpureine (**4**)*

Brown amorphous powder, m.p. 115–117°C. $[\alpha]_D^{25}$ −70.4° (*c* 0.2, $CHCl_3$) [2, 9].

N-*methyllaurotetanine (**5**)*

Yellow needles ($CHCl_3$), m.p. 237–238°C. $[\alpha]_D^{25}$ +76.0° (*c* 0.1, $CHCl_3$) [10].

*Lirinidine (**6**)*

Brown amorphous powder, m.p. 214–215°C $[\alpha]_D^{25}$ −32.8° (*c* 0.1, $CHCl_3$) [11].

N-*methylasimilobine (**7**)*

Yellow needles ($CHCl_3$), m.p. 173–174°C. $[\alpha]_D^{25}$ −60.0° (*c* 0.2, C_2H_5OH) [12, 13].

Assay method for antiplatelet aggregation

Assays were carried out according to procedures described in Refs. [14] and [15]. Results are given in Table 1.

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