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Antiplatelet Aggregation Constituents from *Annona purpurea*

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Bioactivity-directed fractionation led to the isolation of 19 compounds, including three oxoaporphines, oxopurpureine (**5**), oxonuciferine (**6**), and oxoglaucine (**7**); three aporphines, (+)-predicentrine (**8**), (−)-glaucine (**9**), and thalbaicalidine (**10**); one aporphine sensu stricto, *N*-formyl-purpureine (**11**); one proaporphine, glaziovine; one phenanthrene, thalicpureine (**12**); two 6a,7-dehydroaporphines, dehydrolirinidine (**13**) and 7-hydroxy-dehydroglaucine (**14**); four flavonoids, quercetin-3-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside, isorhamnetin-3-*O*-rhamnoside, and tanarixetin-3-*O*-rhamnoside; one purine, adenine; one lactam amide, squamolone; and two steroids, β-sitosterol and β-sitosterol-β-D-glucoside from the MeOH extract of the leaves of Formosan *Annona purpurea*. Among them, **11**−**14** were characterized as new compounds and alkaloids, **5**−**8**, **10**, and **12**−**14** exhibited significant antiplatelet aggregation activity.

In previous phytochemical studies, several aporphine alkaloids[1] and annonaceous acetogenins[2] had been isolated from *Annona purpurea* L. (Annonaceae). As a result of our continuing search for bioactive agents from natural sources, the methanolic extract of the leaves of Formosan *A. purpurea* was found to exhibit significant antiplatelet aggregation activity against *in vitro* collagen factor, and from which were isolated the aporphines, 7-hydroxy-dehydrothalicsimidine (**1**), 7-formyl-dehydrothalicsimidine (**2**), thalicsimidine (**3**), norpurpureine (**4**), *N*-methyl-laurotetanine, lirinidine, and dehydrothalicsimidine.[3] Further investigation had led to the isolation and characterization of 19 compounds: three oxoaporphines, oxopurpureine (**5**),[1] oxonuciferine (**6**),[4] and oxoglaucine (**7**);[1] three aporphines, (+)- predicentrine (**8**),[5] (−)-glaucine (**9**),[6] and thalbaicalidine (**10**); [7] one aporphine sensu stricto, *N*-formyl-purpureine (**11**); one proaporphine, glaziovine;[1] one phenanthrene, thalicpureine (**12**); two 6a,7-dehydroaporphines, dehydrolirinidine (**13**) and 7-hydroxy-dehydroglaucine (**14**); four flavonoids, quercetin-3-*O*-rhamnoside,[8,9] kaempferol-3-*O*-rhamnoside,[8,9] isorhamnetin-3-*O*-rhamnoside,[8,9] and tanarixetin-3-*O*-rhamnoside;[8,9] one purine, adenine;[10] one lactam amide, squamolone;[11] and two steroids β-sitosterol[12] and β-sitosterol-β-D-glucoside.[12] The isolation and structure elucidation of four new compounds, **11**−**14**, are presented herein. For those known compounds, the structures were established by spectral methods and compared with those published in the cited references.

Results and Discussion

Guided by an *in vitro* assay testing for inhibition of antiplatelet aggregation activity, a methanolic extract of the air-dried leaves of *A. purpurea* was fractionated mainly by combining open column, MPLC, HPLC, preparative TLC, and Sephadex LH-20 chromatographic techniques. The fractionation procedures resulted in the isolation of four new compounds, **11**−**14**, along with 15 known ones.

Compound **11** was obtained as a brown amorphous powder, $[\alpha]^{24}_{D}$ −135° (*c* 0.2, $CHCl_3$), positive to Dragendorff's test. Its molecular weight was determined by the HREIMS $[M]^+$ ion at *m*/*z* 399.1659, which corresponded to the molecular formula of $C_{22}H_{25}O_5N$ (calcd 399.1682). The UV absorptions maxima at λ 225, 268, 290 sh, and 316 nm suggested **11** as a 1,2,3,9,10-pentaoxygen-bearing substi-

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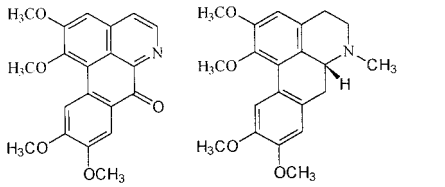
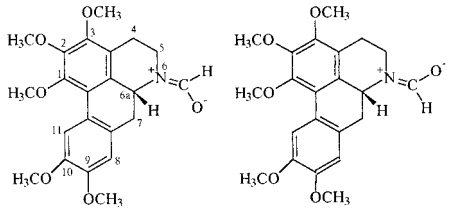
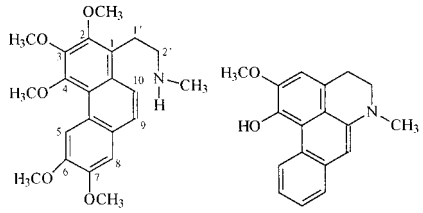


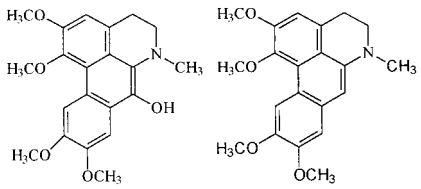
7. **9.**



11a. *Z*-form **11b**. *E*-form



12. **13.**



14. **15.**

tuted aporphine.[13] The IR absorption at ν 1660 cm^{-1} indicated the presence of a carbonyl group. The ^{1}H NMR spectrum of **11** at 400 MHz was complex. This arose from the resonances of two rotational isomers that occur due to restricted rotation about the *N*-formyl group. Amidic aporphines, whether *N*-formyl or *N*-acetyl, always exist as a mixture of enolates, as clearly demonstrated by their NMR studies.[13] In each instance, for the major isomer, **11a**, however, the oxygen of the amidic function lies *syn* to C-6a, and *anti* to C-5, a phenomenon most probably associated with steric factors.[13] Two separate sets of signals in the ratio of 3:1 indicated slow exchange on the NMR time scale between the two rotational isomers in solution. The ^{1}H NMR of the major component showed a typical absorption of *N*-formyl proton at δ 8.02, two aromatic protons at δ 8.03 and 6.80, and five methoxyls at δ 3.74, 3.90, 3.92, 3.93, and 3.98 (each 3H, s). Because the compound possessed the aporphine skeleton, the signals at δ 8.03 and 3.74 were assigned to the chemical shift of H-11 and OMe-1, which were deshielding and shielding because of the ring-current effect of rings A and D, respectively. The remaining positions of one aromatic proton and four methoxyl groups needed to be assigned on the structure of **11a**. The COSY and NOESY spectra of **11a** were taken to confirm the structure. A signal at δ 4.91, assigned to H-6a of the major isomer, was coupled to signals at δ 3.02 (J = 4.4 Hz) and 2.76 (J = 14.0 Hz); these coupling constants were compatible with axial−equatorial and axial−axial relationships, respectively. Therefore, H-6a was axially disposed, and the H-7 axial proton was identified at δ 2.76. The proton at δ 6.80 is characterized to H-8, which was found to couple to the H-7 equatorial proton at δ 3.02 and a methoxyl at δ 3.98 for OMe-9 in NOESY spectrum. The proton at δ 2.62 for H-4$_{eq}$ was

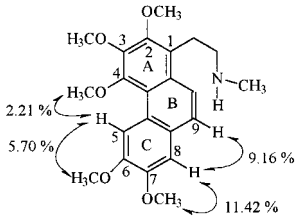


Figure 1. Two 1D NOE-DIF correlations of **12** obtained while irradiating at H-5 and H-8.

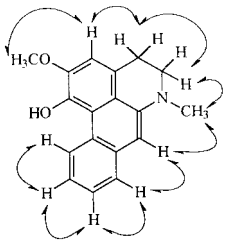


Figure 2. The 2D NOESY response of **13**.

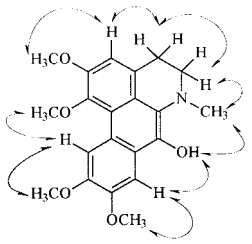


Figure 3. The 2D NOESY response of **14**.

correlated with the proton at 3.01 for H-4$_{ax}$, the methoxyl at δ 3.92 for OMe-3, and δ 3.45 and 3.80 for H-5. The protons in aliphatic region could be recognized clearly from examination of the data and comparison with the literature.[13−15] The ^{1}H NMR of **11** is summarized in Table 1. The major structure **11a** was elucidated as *Z*-form *N*-formylaporphine. In a similar way, the structure of the minor component **11b** was characterized as *E*-form *N*-formylaporphine. The complex signals prevented assignments of most coupling constants of protons of **11b** in the aliphatic region of ^{1}H NMR. Thus, compound **11**, elucidated as a mixture of two isomers, constructed a new compound, which we named *N*-formyl-purpureine.

Compound **12** was obtained as yellow needles, positive to the Dragendorff's test. Its molecular weight was determined by the HREIMS [M]$^+$ ion at *m*/*z* 385.1887, which corresponded to the molecular formula $C_{22}H_{27}O_5N$ (calcd 385.1889). The UV absorptions maxima at λ 220, 264, 308, 326, 345, and 358 nm suggested **12** was a phenanthrene alkaloid.[16] The ^{1}H NMR spectrum showed four proton signals falling into the aromatic region. Two coupling protons at δ 7.59 (1H, d, J = 9.1 Hz) and 7.86 (1H, d, J = 9.1 Hz) were characteristic of H-9 and H-10, where the aromatic proton at δ 9.15 (1H, s) corresponded to the H-5 in the phenanthrene ring system. H-5 was shifted downfield to δ 9.15 because of the ring-current effect of ring A in the phenanthrene skeleton. Two 1D NOE difference experiments were employed in order to confirm the assignment of ^{1}H NMR, and the results are given in Figure 1. Positive NOE enhancements of 11.42% and 9.16% were calculated for the methoxyl at δ 4.08 for OMe-7 and δ 7.59 for H-9, while the signal at δ 7.17 was irradiated in the 1D-NOE difference spectrum. That is, the chemical shift at δ 7.17 was assigned to H-8. With irradiation H-5 also showed 2.21% and 5.70% positive NOE enhancements to two methoxyls at δ 3.94 and 4.07, respectively. Five

Table 1. ^{1}H NMR Data of **11a** and **11b**[a]

proton	*N*-formyl-purpureine	
	Z-form (major)	*E*-form (minor)
OMe-1	3.74 (3H, s)	3.72 (3H, s)
OMe-2, 3, 10	3.90, 3.92, 3.93 (each 3H, s)	3.90, 3.92, 3.93 (each 3H, s)
H-4$_{ax}$	3.01 (1H, ddd, J = 16.0, 12.4, 4.5 Hz)	2.54 (1H, m)
H-4$_{eq}$	2.62 (1H, ddd, J = 16.0, 2.8, 1.6 Hz)	2.59 (1H, m)
H-5$_{ax}$	3.45 (1H, ddd, J = 12.4, 12.4, 2.8 Hz)	3.10 (1H, m)
H-5$_{eq}$	3.80 (1H, ddd, J = 12.4, 4.5, 1.6 Hz)	4.40 (1H, m)
CHO-N	8.02 (1H, d, J = 0.8 Hz)	8.38 (1H, d, J = 0.8 Hz)
H-6a$_{ax}$	4.91 (1H, dd, J = 14.0, 4.4 Hz)	4.48 (1H, dd, J = 14.0, 4.4 Hz)
H-7$_{eq}$	3.02 (1H, dd, J = 14.0, 4.4 Hz)	3.15 (1H, m)
H-7$_{ax}$	2.76 (1H, dd, J = 14.0, 14.0 Hz)	2.74 (1H, m)
H-8	6.80 (1H, s)	6.76 (1H, s)
OMe-9	3.98 (3H, s)	3.97 (3H, s)
H-11	8.03 (1H, s)	7.96 (1H, s)

[a] In $CDCl_3$, δ values in ppm relative to TMS (400 MHz, J in Hz). *Z:E* ratio 3:1.

methoxyls at δ 3.94, 3.99, 4.02, 4.07, and 4.08 (each 3H, s, 5 × OMe) were characterized to attach at the positions of C-4, C-3, C-2, C-6, and C-7, respectively. The chemical shifts of two coupling methylenes were shown at δ 3.46 (2H, m, H-1′) and 2.96 (2H, m, H-2′). These evidences indicated the structure of **12** as a new *N*-methyl-2,3,4,6,7-pentamethoxyl phenanthrene, thalicpureine.

Compound **13** was obtained as green needles, positive to the Dragendorff's test. Its molecular weight was determined by the HREIMS $[M]^+$ ion at *m/z* 279.1270, which corresponded to the molecular formula of $C_{18}H_{17}O_2N$ (calcd 279.1259). Its UV absorptions maxima at λ 216, 253, and 312 nm revealed **13** as a 6a,7-dehydroaporphine.[16] The IR spectrum at ν 3600 cm^{-1} and a bathochromic shift of UV absorption with addition of an alkaline solution indicated the presence of a phenolic function. An AA′BB′ aromatic system at δ 7.33 (1H, td, J = 7.0, 1.8 Hz, H-10), 7.44 (1H, td, J = 7.8, 1.4 Hz, H-9), and 7.63 (1H, dd, J = 7.8, 1.8 Hz, H-8) and a typical downfield-shifted proton at δ 9.51 (1H, dd, J = 7.0, 1.4 Hz, H-11) in ring D was recognized by the ^{1}H NMR spectrum. The 2D COSY and NOESY experiments evidenced the assignment of **13**. A signal at δ 6.96 was assigned to H-7, which was correlated to δ 7.17 for H-8 and δ 2.72 for *N*-methyl. A signal at δ 6.60 (H-3) was correlated to δ 4.02 (OMe-2) and 3.23 (H-4), and a methylene at δ 3.35(H-5) showed the cross peaks to the *N*-methyl and H-4 were also observed in NOESY spectrum of **13**. Therefore, two aromatic singlets at δ 6.60 and 6.96, one methoxyl at δ 4.02, two coupling methylenes at δ 3.23 and 3.35 were assigned to H-3, H-7, OMe-2, H-4, and H-5, respectively. The results of the NOESY spectrum are shown in Figure 2. From the above data, the structure of **13** is in agreement as a new 1-hydroxy-2-methoxy-6-methyl-6a,7-dehydroaporphine, named dehydrolirinidine.

Compound **14** was obtained as green needles, positive to the Dragendorff's test. Its molecular weight was determined by the HREIMS $[M]^+$ ion at *m/z* 369.1581, which corresponded to the molecular formula $C_{21}H_{23}O_5N$ (calcd 369.1576). The UV absorptions maxima at λ 222, 260, 278 (sh), and 335 nm are characteristic of **14** as a 6a,7-dehydroaporphine.[3,16] The IR absorption at 3460 cm^{-1} and a bathochromic shift of UV absorption while adding alkaline solution indicated the presence of a hydroxyl group. Four methoxyl signals at δ 3.89, 4.00, 4.01, and 4.02 (each 3H, s); three aromatic signals at δ 6.97 (1H, s, H-3), 7.06 (1H, s, H-8), and 9.09 (1H, s, H-11); and a D_2O exchangeable signal at δ 6.60 revealed the presence of **14** as a 1,2,9,10-tetramethoxy-7-hydroxy-6a,7-dehydroaporphine. A hydroxyl at δ 6.60 (OH-7) exhibited the cross peaks to the *N*-methyl group and H-8 in the NOESY spectrum. This is strong evidence for the presence of a 7-hydroxy-6a,7-dehydroaporphine skeleton. A chemical shift at δ 3.35 was assigned to H-5, which also showed the NOE correlations to 3.26 (H-4) and the deshielding *N*-methyl group at 3.06 (3H, s). This assignment was in agreement with ^{13}C NMR and DEPT values at δ 40.44 (q), and two signals at δ 30.9(t) and 50.41(t), respectively. The COSY and NOESY experiments were obtained to confirm the location of functional groups. The result of the NOESY spectrum is summarized in Figure 3. The structure of **14** was elucidated as a new 1,2,9,10-tetramethoxy-6-methyl-7-hydroxy-6a,7-dehydroaporphine, named 7-hydroxy-dehydroglaucine.

The isolated alkaloids (including those reported in our previous paper[3]), **1**−**5**, **11**, and **12**, are members of a series of special pentamethoxyl-substituted compounds. The presence of this series of compounds and the pentaoxygenated **10** led us to propose an extension of a hypothesis of biosynthesis pathway (Figure 4) of partial pentaoxygenated aporphine alkaloids in *A. purpurea*.[17−19] It was proposed that the methylation of **10** leads to the formation of the aporphine **3**, and that the phenanthrene **12** is probably derived from the Hofmann elimination of the quaternary aporphine salt of **3**.[17] The relationship of **4** and **11** to **3** can be established by simple chemical reactions, in which **4** is oxidized to obtain **5** which, in turn, can be reduced to obtain **4**.[17] The biological evidence had been reported, the microbial transformation of compound **9** with *Fusarium solani* provided dehydroglaucine (**15**) quantitatively.[18,19] For the same reason, it is possible for **3** to form the intermediate **16**, which can give rise to **1** and **2** after hydroxylation or formylation. The isolation of **16** from this plant would provide strong support for this hypothesis. Unfortunately, it was not isolated during the course of the present study. Based on the presence of Compound **13,** a similar 6a,7-dehydroaporphine-type analogue, it is also possible that intermediate **16** exists in this plant. It is worth noting that air oxidation of **9** gives rise to **7** and **15**.[18] The results suggest that compound **16** can be derived from **3**. Based upon the above reasons, **16** may play a key role in the formation of **1** and **2**.

The antiplatelet aggregation activity[3,20,21] of these compounds has been assayed. Compounds **5**−**8**, **10**, and **12**−**14** showed inhibition of collagen and arachidonic acid (AA)-induced platelet aggregation. Compounds **12**−**14** showed the presence of significant inhibition of platelet activating factor (PAF)-induced platelet aggregation, and **12** and **14** exhibited inhibition against thrombin-induced platelet aggregation. It is interesting that two 7-hydroxy-6a,7-dehydroaporphines, **1**[3] and **14**, exhibited high selectivity against PAF-induced platelet aggregation; the bioactivity

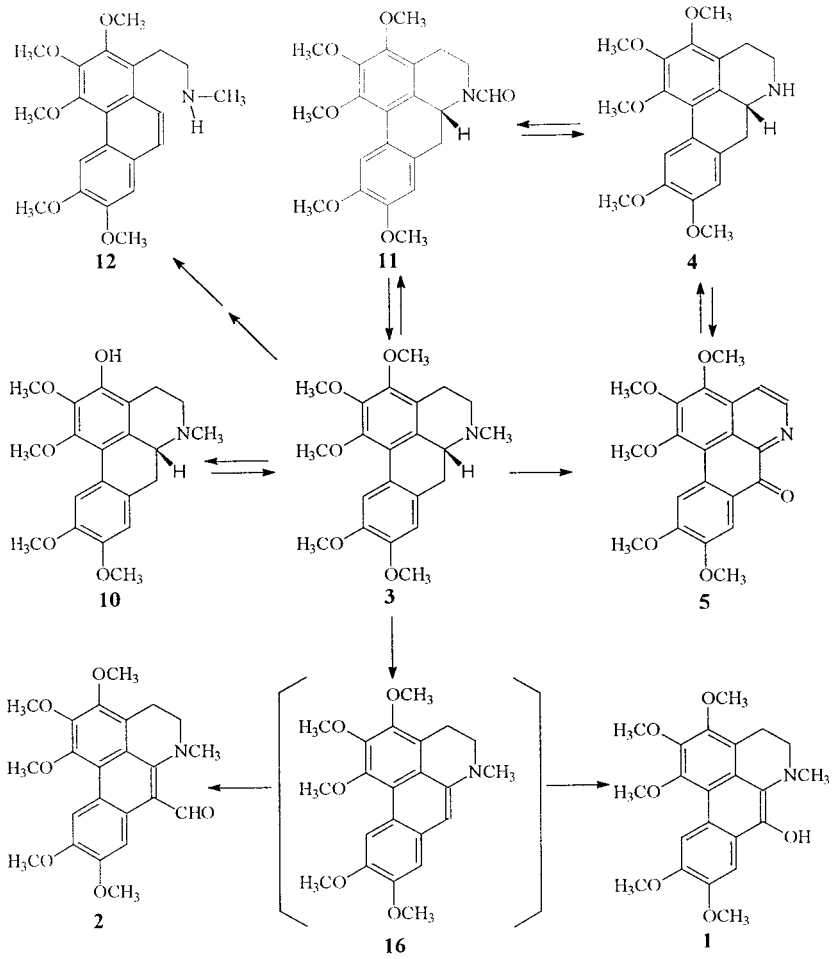


Figure 4. A hypothetical biosynthesis pathway of partial aporphine alkaloids in *A. purpurea*. Compound **16** was not found in this study.

of this type of alkaloid needs to be studied further. The results of antiplatelet aggregation assay are presented in Table 2.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micro-melting point apparatus and were uncorrected. The UV spectra were obtained on a Hitachi 200−20 spectrophotometer, and IR spectra were measured on a Hitachi 260−30 spectrophotometer. ^{1}H NMR spectra were recorded with Varian NMR spectrometer at 400 and 200 MHz, and ^{13}C NMR spectra were recorded with Varian NMR spectrometer at 100 and 50 MHz, in $CDCl_3$ using TMS as internal standard. EIMS were obtained with JEOL JMS−SX/SX 102A mass spectrometer and Quattro GC/MS spectrometer at 70 eV. Si gel 60 (Macherey-Nagel and Merck), active charcoal (Wako), and Sephadex LH-20 (Pharmacia) were used for chromatographic column, precoated Si gel plates (Macherey-Nagel, SIL G-25/UV_{254}, 0.25 mm, aluminum) were used for analytical TLC, and precoated Si gel plates (Macherey-Nagel, SIL G/UV_{254}, 0.25 mm, glass) were used for preparative TLC.

Plant Material. *Annona purpurea* L. leaves were collected from Chia-Yi, Taiwan, June 1993. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The air-dried leaves of *Annona purpurea* L. (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 to yield the $CHCl_3$ layer (Part A) and the HCl layer. The HCl layer was basified with NH_4OH and then extracted with $CHCl_3$ (Part B). Part B was dried and evaporated to leave a brown viscous residue (10 g). The residue was chromatographed on a Si gel (406 cm) column using *n*-hexane, $CHCl_3$, and $CHCl_3$−MeOH mixtures of increasing polarity to yield 100 fractions of 120 mL each, which were further combined into six fractions according to their TLC patterns. Each fraction was re-chomatographed over Si gel and Sephadex LH-20 and purified by further Si gel column chromatography, recrystalization, or preparative TLC to yield **5** (1.89 g, $CHCl_3$−MeOH 9:0.5, R_f 0.60), **6** (35 mg, $CHCl_3$−MeOH 9:0.5, R_f 0.57), **7** (153 mg, $CHCl_3$−MeOH 9:0.5, R_f 0.50), **8** (56 mg, $CHCl_3$−MeOH 9:1, R_f 0.51), **9** (6.7 mg, $CHCl_3$−MeOH 9:1, R_f 0.45), **10** (39.8 mg, $CHCl_3$−MeOH 9:1, R_f 0.46), **11** (5 mg, $CHCl_3$−MeOH 9:1, R_f 0.43), **12** (25 mg, $CHCl_3$−MeOH 9:1, R_f 0.38), **13** (8 mg, $CHCl_3$−MeOH 9:1, R_f 0.40), and **14** (9 mg, $CHCl_3$−MeOH 9:1, R_f 0.36) were obtained from these fractions, respectively.

***N*-Formyl-purpureine (11):** obtained as a brown amorphous powder; mp 212−213 °C; $[\alpha]^{24}_D$ −135.0° (*c* 0.2, $CHCl_3$), UV (C_2H_5OH) λ_{max} 225, 268, 290, 316 nm; IR (neat) λ_{max} 1660 cm^{-1} (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; EIMS (70 eV) *m*/*z* (rel int %) 399 ([M]$^+$, 36), 368 (39), 367 (82), 341 (100); HREIMS *m*/*z* 399.1659 ($C_{22}H_{25}O_6N$, calcd 399.1682).

Thalicpureine (12): purified as yellow needles ($CHCl_3$); mp 196−197 °C; UV (EtOH) λ_{max} 220, 264, 308, 326, 345, 358 (sh) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) 9.15 (1H, s, H-5), 7.86 (1H, d, J = 9.1 Hz, H-10), 7.59 (1H, d, J = 9.1 Hz, H-9), 7.17 (1H, s, H-8), 4.08 (3H, s, OMe-7), 4.07 (3H, s, OMe-6), 4.02 (3H, s, OMe-2), 3.99 (3H, s, OMe-3), 3.94 (3H, s, OMe-4), 3.46 (2H, m, H-1′), 2.96 (2H, m, H-2′), 2.72 (3H, s, Me-N); ^{13}C NMR ($CDCl_3$, 100 MHz) 24.1 (C-2′), 62.1 (C-1′), 48.4 (Me-N), 55.6, 55.7, 60.4, 61.2, 61.3 (5 × OMe); EIMS (70 eV) *m*/*z* (rel int %) 385 ([M]$^+$, 11), 343 (27), 342 (100), 341 (43); HREIMS *m*/*z* 385.1887 ($C_{22}H_{27}O_5N$, calcd 385.1889).

Table 2. Effects of the Alkaloids **5**−**8**, **10**, and **12**−**14** from *A. purpurea* on the Aggregation of Rabbit Platelets Induced by Thrombin (Thr), Arachidonic Acid (AA), Collagen (Col), and Platelet-Activating Factor (PAF)[a]

	% aggregation			
compound	Thr (0.1 U/mL)	AA (100 μM)	Col (10 μg/mL)	PAF (2 nM)
control	91.2 ± 1.0 (4)	85.0 ± 1.2 (6)	86.3 ± 1.2 (4)	88.7 ± 0.3 (5)
5				
100 μg/mL	85.6 ± 3.6 (3)	43.7 ± 9.6 (3)[d]	53.5 ± 17.4 (4)[b]	87.2 ± 0.8 (3)
6				
50 μg/mL	88.9 ± 1.5 (3)	16.3 ± 0.8 (3)[d]	55.2 ± 14.9 (4)[b]	83.7 ± 0.6 (3)
7				
50 μg/mL	87.0 ± 1.6 (3)	55.8 ± 7.0 (3)[d]	74.4 ± 6.4 (4)	84.4 ± 4.1 (3)
8				
100 μg/mL	88.9 ± 1.1 (3)	0.0 ± 0.0 (3)[d]	0.0 ± 0.0 (3)[d]	74.0 ± 1.8 (3)
50 μg/mL		62.0 ± 7.6 (3)[b]		
20 μg/mL		83.2 ± 0.5 (3)		
10				
100 μg/mL	86.3 ± 0.6 (3)	4.2 ± 3.8 (4)[d]	0.0 ± 0.0 (3)[d]	73.6 ± 4.8 (3)
50 μg/mL		72.2 ± 4.9 (4)		
20 μg/mL		82.2 ± 2.5 (4)		
12				
100 μg/mL	33.6 ± 17.5 (4)[b]	0.0 ± 0.0 (4)[d]	0.0 ± 0.0 (3)[d]	0.0 ± 0.0 (3)[d]
50 μg/mL		0.0 ± 0.0 (4)[d]		3.9 ± 3.2 (3)[d]
20 μg/mL		41.9 ± 10.9 (4)[c]		55.5 ± 8.3 (3)[b]
10 μg/mL		74.2 ± 3.0 (4)		78.8 ± 6.0 (4)
5 μg/mL		83.7 ± 2.8 (4)		82.4 ± 4.5 (4)
13				
100 μg/mL	86.3 ± 0.6 (4)	0.0 ± 0.0 (4)[d]	0.0 ± 0.0 (3)[d]	51.7 ± 8.3 (3)[b]
50 μg/mL		23.8 ± 19.4 (4)[d]		
20 μg/mL		77.6 ± 2.2 (4)		
10 μg/mL		82.8 ± 2.6 (4)		
14				
100 μg/mL	78.8 ± 3.4 (4)	21.8 ± 9.7 (4)[d]	12.7 ± 6.3 (4)[d]	0.0 ± 0.0 (3)[d]
50 μg/mL				0.0 ± 0.0 (3)[d]
20 μg/mL				25.2 ± 12.8 (3)[d]
10 μg/mL				64.9 ± 1.4 (3)[b]
5 μg/mL				77.9 ± 2.3 (3)

[a] Platelets were preincubated with each compound (100 μg/mL) or 0.5% DMSO (control) at 37 °C for 3 min, then the inducer Thr (0.1 U/ml), AA (100 μM), Col (10 μg/mL), or PAF (2 ng/mL) 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.

Dehydrolirinidine (13): characterized as green needles ($CHCl_3$); mp 189−190 °C; UV (EtOH) λ_{max} 216, 253, 312 nm; UV (EtOH + KOH) λ_{max} 222, 275, 335 nm; IR (neat) ν_{max} 3600 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 9.51 (1H, dd, J = 7.0, 1.4 Hz, H-11), 7.63 (1H, dd, J = 7.8, 1.8 Hz, H-8), 7.44 (1H, td, J = 7.8, 1.4 Hz, H-9), 7.33 (1H, td, J = 7.0, 1.8 Hz, H-10), 6.96 (1H, s, H-7), 6.60 (1H, s, H-3), 4.02 (3H, s, OMe-2), 3.35 (2H, m, H-5), 3.23 (2H, m, H-4), 3.08 (3H, s, Me-N); ^{13}C NMR ($CDCl_3$, 100 MHz) 30.8 (C-4), 40.5 (Me-N), 50.5 (C-5), 56.7 (OMe-2), 101.9 (C-7); EIMS (70 eV) m/z (rel int %) 279 ($[M]^+$, 6), 257 (11), 173 (23), 154 (84); HREIMS m/z 279.1270 ($C_{18}H_{17}O_2N$, calcd 279.1259).

7-Hydroxy-dehydroglaucine (14): green needles ($CHCl_3$); mp 242−243 °C; UV (EtOH) λ_{max} 222, 260, 335 nm; UV (EtOH + KOH) λ_{max} 222, 265, 340 nm; IR (neat) ν_{max} 3460 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 9.09 (1H, s, H-11), 7.06 (1H, s, H-8), 6.97 (1H, s, H-3), 6.60 (1H, s, OH-7), 3.89 (3H, s, OMe-1), 4.00, 4.01, 4.02 (each 3H, s, 3 × OMe), 3.35 (2H, m, H-5), 3.26 (2H, m, H-4), 3.06 (3H, s, Me-N); ^{13}C NMR ($CDCl_3$, 100 MHz) 30.9 (C-4), 50.4 (C-5), 40.4 (Me-N), 55.5, 55.7, 56.3, 59.9 (4 × OMe); EIMS (70 eV) m/z (rel int %) 369 ($[M]^+$, 7), 354 (29), 353 (100), 338 (65); HREIMS m/z 369.1581 ($C_{21}H_{23}O_5N$, calcd 369.1576).

Assay Method for Antiplatelet Aggregation. The assays were carried out according to procedures in the literature.[20,21] Results are given in Table 2.

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