Cytotoxic Benzophenanthridine and Benzylisoquinoline Alkaloids from *Argemone mexicana*

Yuh-Chwen Chang[a,b], Fang-Rong Chang[a], Ashraf T. Khalil[a], Pei-Wen Hsieh[a], and Yang-Chang Wu[a,*]

[a] Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan. Fax: +886-7-3114773. E-mail : yachwu@kmu.edu.tw
[b] Department of Chemical Engineering, Kao Yuan Institute of Technology, Kaohsiung county 821, Taiwan

* Author for correspondence and reprint requests

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Fractionation of the chloroform extract from the aerial part of *Argemone mexicana* led to the isolation of two benzophenanthridine-type alkaloids, *N*-demethyloxysanguinarine and pancorine; three benzylisoquinoline-type alkaloids, (+)-1,2,3,4-tetrahydro-1-(2-hydroxymethyl-3,4-dimethoxyphenylmethyl)-6,7-methylenedioxyisoquinoline, (+)-higenamine and (+)-reticuline. Among them, *N*-demethyloxysanguinarine is a new compound, and (+)-1,2,3,4-tetrahydro-1-(2-hydroxymethyl-3,4-dimethoxyphenylmethyl)-6,7-methylenedioxy-isoquinoline was isolated form a natural source for the first time, to which was assigned a trivial name, (+)-argenaxine. In addition, six known non-alkaloidal compounds were also isolated and identified. All compounds were characterized on the basis of their spectral data and chemical evidences. Some isolated alkaloids from this species were evaluated for their cytotoxicity to human nasopharyngeal carcinoma (HONE-1) and human gastric cancer (NUGC) cell lines. Chelerythrine was found to exhibit significant activity against NUGC cell line, while angoline inhibited both types. (+)-Argenaxine showed moderate activity against the NUGC cell line.

Key words: Argemone mexicana, *N*-Demethyloxysanguinarine, Cytotoxicity

Introduction

Argemone mexicana (Papaveraceae) is a spiny herbaceous annual plant that grows mainly in subtropical countries. It is widely used in folk medicine to alleviate several ailments especially for its analgesic effects (Capasso *et al.*, 1997). Chemical investigations of this plant have revealed the presence of alkaloids (Hussain *et al.*, 1983; Nakkady *et al.*, 1988), amino acids (Dinda *et al.*, 1986), phenolics (Harborne *et al.,* 1983) and fatty acids (Gunstone *et al.,* 1977). Recently, fifteen alkaloids have been isolated from the same species by our group; some with anti-HIV activity (Chang *et al.*, 2003). Our continuous search for bioactive constituents from Formosan Papaveraceae plants as well as the diverse biological activities of benzophenanthridine and benzylisoquinoline type alkaloids (Capasso *et al.,* 1997 ; Chang *et al.*, 2003; Krane *et al.*, 1984; Chen *et al.*, 1997; Kim *et al.*, 1999; Shin *et al.*, 1999; Tanaka *et al.*, 2001) has motivated us to continue the study of this species. In this communication, we report the isolation of two benzophenanthridine-type alkaloids, *N*-demethyloxysanguinarine (**1**) and pancorine (**2**); three benzylisoquinoline-type alkaloids, (+)-argenaxine (**3**), (+)-higenamine (**4**), and (+)-reticuline (**5**). Alkaloid **1** is a new compound, and **3** was isolated for the first time from a natural source, which we named (+)-argenaxine. In addition, six known compounds were isolated and identified, *viz.* α-tocopherol (**6**), phytol (**7**), stigma-4-en-3,6-diene (**8**), adenine (**9**), adenosine (**10**), and isorhamnetin-3-O-β-D-glucopyranoside (**11**). All compounds were identified on the basis of their chemical characters and spectral data especially NMR. Some of isolated alkaloids were subjected to cytotoxic assay against human nasopharyngeal carcinoma (HONE-1) and human gastric cancer (NUGC) cell lines.

Results and Discussion

Compound **1** gave an orange color with Dragendorff's reagent indicating its alkaloidal nature and its EIMS revealed a molecular ion peak $[M]^+$ at m/z 333 corresponding to the molecular formula

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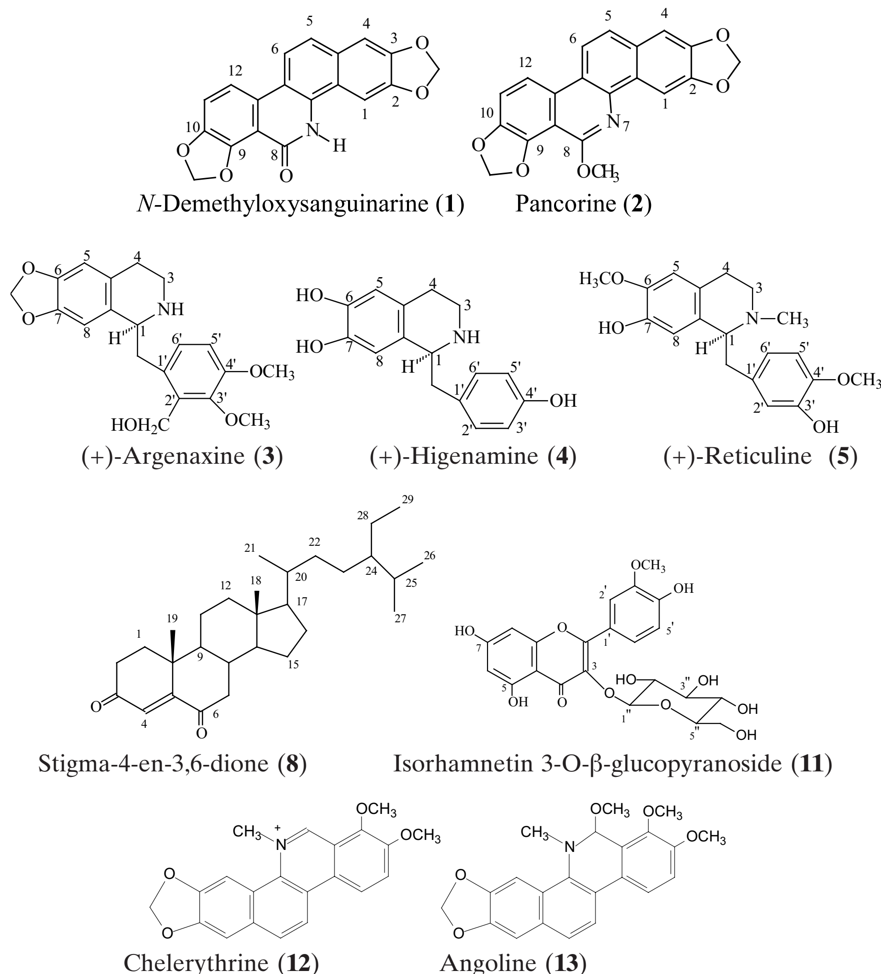

Fig. 1. Structures of the isolated compounds.

$C_{19}H_{11}NO_5$. The double bond equivalence was calculated as 15. The IR spectrum of **1** showed an absorption band at 1665 cm^{-1} indicating the presence of amide carbonyl (Pandey *et al.,* 1979). The ^1H NMR showed six aromatic protons and two methylenedioxy groups at δ 6.11 and 6.30 (each 2H, s), suggesting the presence of benzophenanthridine nucleus (Krane *et al.,*1984). The NOESY spectrum (Fig. 2) showed two peaks between δ 7.14 (1H, s, H-4) and δ 7.55 (1H, d, J = 8.8 Hz, H-5) and between δ 7.65 (1H, d, J = 8.8 Hz, H-12) and δ 7.82 (1H, d, J = 8.8 Hz, H-6), indicating that the two methylenedioxy groups were attached to C-2/C-3 and C-9/C-10. Comparing the NMR data of **1** with those of oxysanguinarine (Williams *et al.*, 1993), it was found that they are similar except for the disappearance of the signal attributed to *N*-methyl group. Based on the results mentioned above, it was concluded that **1** is N-demethyloxysanguinarine.

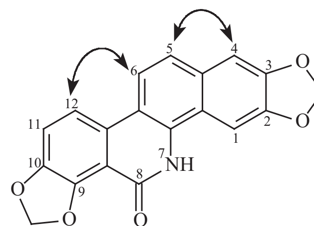

Fig. 2. NOESY cross-peaks observed for compound **2**.

The EIMS of **3** revealed a $[M-H_2O]^+$ ion at *m/z* 339 corresponding to the molecular formula

$C_{20}H_{23}NO_5$, and the UV absorption at 225 and 285 nm, suggesting the presence of an aromatic alkaloid with an attached alcoholic group. In the aromatic region, the ^1H NMR showed an AB pattern at δ 6.95 (1H, d, J = 8.5 Hz, H-5′) and δ 7.03 (1H, d, J = 8.5 Hz, H-6′), and two aromatic proton singlets at δ 6.76 (H-5) and 6.82 (H-8). The spectral data of **3** suggested the presence of a benzylisoquinoline nucleus with a methylenedioxy group as indicated by a signal at δ 5.93 (2H, s) and two methoxyl groups as evidenced from the two singlets at δ 3.82 and 3.86 (each 3H, s). The methylenedioxy group was most likely located on the aromatic ring at positions C-6 and C-7, thus allowing H-5 and H-8 to be *para*-located. The presence of hydroxymethyl group was deduced from the two spin-coupled doublets at δ 4.86, 4.79 (each d, J = 11.5 Hz, CH_2OH). All spectral data of **3** were consistent with the reported values (Hanaoka *et al.,* 1983). The above data confirmed that **3** has the structure of 1,2,3,4-tetrahydro-1-(2-hydroxymethyl-3,4-dimethoxyphenyl-methyl)-6,7-methylenedioxyisoquinoline. Although **3** was previously synthesized (Hanaoka *et al.,* 1983), this is the first detection of this alkaloid in a natural source. We named it (+)-argenaxine. It could be an intermediate in the biogenesis pathway of protoberberine-type alkaloids.

Furthermore, alkaloids **2** (Krane *et al.,* 1984), **4** (Xu and Lin, 1999), **5** (Shamma *et al.,* 1983; Castro *et al.,* 1985), **6** (Mozawa *et al.,* 2000), **7** (Bjornland *et al.,*1989), **8** (Itokawa *et al.,* 1973), **9** (Kawahara *et al.,*1992), **10** (Chen *et al.*, 1997), and **11** (Harborne and Mabry, 1982) were identified by direct comparison of their physical and spectral data with the literature values.

Moreover, some isolated alkaloids of our current and previous (Chang *et al*., 2003) investigation were evaluated for their cytotoxicity to human nasopharyngeal carcinoma (HONE-1) and human gastric cancer (NUGC) cell lines (Table I). Alkaloid **12** was found to be active against NUGC cell line, while **13** inhibited both types. Alkaloid **3** showed moderate activity against NUGC cell line.

Table I. Cytotoxicity of the alkaloids isolated from *Argemone mexicana.*

Sample (150 μM)	% of control	
	HONE-1	NUGC
N-Demethyloxysanguinarine (**1**)	91	104
Pancorine (**2**)	96	100
(+)-Argenaxine	92	**64**
(+)-Higenamine (**4**)	95	97
(+)-Reticuline (**5**)	96	90
Chelerythrine (**12**)	109	**1**
Angoline (**13**)	**1**	**0**
O-Methylzanthoxyline	–	95
Norchelerythrine	–	86
Sanguinarine	–	100
6-Acetonyldihydrosanguinarine	–	105
6-Acetonyldihydrochelerythrine	–	98
Aronttianamide	–	93
Berberine	–	128
Dihydrocheilantifoline	–	128

– Not tested.

Experimental

General experimental procedures for instrumentation, plant information, source of alkaloids, and preliminary fractionation, see Chang *et al.* (2003).

Extraction and isolation

The air-dried whole plants (1.5 kg) of *A. mexicana* were extracted repeatedly with MeOH (20 l × 5) at room temperature, and concentrated *in vacuo* to yield a dark residue (310 g). The MeOH extract showed cytotoxic activity. The residue was dissolved in H_2O–MeOH (5:1 v/v) mixture (1.5 l) then successively extracted with $CHCl_3$ (1.5 l × 3) and then with *n*-BuOH (1.5 l × 3) till exhaustion. Each extract was separately concentrated *in vacuo* to yield $CHCl_3$ (48.3 g) and *n*-BuOH (14.7 g) residues. The $CHCl_3$ extract was subjected to column chromatography on Si gel with a gradient solvent system of *n*-hexane–$CHCl_3$–MeOH to give **1** (3 mg, $CHCl_3$/MeOH [50:1 v/v], R_f 0.44), **2** (4.5 mg, $CHCl_3$/MeOH [50:1], R_f 0.49), **3** (12 mg, $CHCl_3$/MeOH [8:1], R_f 0.43), **6** (25 mg, *n*-hexane/$CHCl_3$ [5:1], R_f 0.55), **7** (35 mg, *n*-hexane/$CHCl_3$ [5:1], R_f 0.50), and **8** (15 mg, $CHCl_3$/MeOH [99:1], R_f 0.65). The *n*-BuOH extract was subjected to column chromatography on silica gel with a gradient system of $CHCl_3$–EtOAc–MeOH to produce eleven fractions. Fraction No. 9 was re-chromatographed on Si gel column using $CHCl_3$/MeOH for gradient elution followed by purification on Sephadex LH-20 using MeOH/$CHCl_3$ as a solvent to afford **4** (15 mg, $CHCl_3$/MeOH [10:1 v/v], R_f 0.42), **5** (9 mg, $CHCl_3$/MeOH [10:1], R_f 0.52). Fractions No. 6–8 were repeatedly chromatographed on Si gel column

using $CHCl_3$/MeOH for gradient elution followed by purification on preparative TLC using $CHCl_3$/MeOH [10:1] to offer **9** (25 mg, $CHCl_3$/MeOH [10:1 v/v], R_f 0.56), **10** (35 mg, $CHCl_3$/MeOH [10:1], R_f 0.49) and **11** (15 mg, $CHCl_3$/MeOH [10:1], R_f 0.46).

N-Demethyloxysanguinarine or 2,3-methylenedioxy-9,10-methylenedioxy-benzo[c]phenanthridin-8-one (**1**)

White amorphous powder, UV (EtOH), λ_{max} (log ε), 222 (4.02), 283 (3.36), and 327 (4.13) nm. IR (KBr) ν_{max}, 1665 (carbonyl group), 943, 1032 (methylenedioxyl group) cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 6.11 (2H, s, $C_{2\&3}-OCH_2O-$), 6.30 (2H, s, $C_{9\&10}-OCH_2O-$), 7.14 (1H, s, H-4), 7.29 (1H, d, J = 8.8 Hz, H-11), 7.55 (1H, d, J = 8.8 Hz, H-5) , 7.65 (1H, d, J = 8.8 Hz, H-12), 7.82 (1H, d, J = 8.8 Hz, H-6), 7.86 (1H, s, H-1); EI-MS, *m/z* (rel. int. %) : 333 (57) $[M]^+$, 322 (13), 248 (10), 161 (21), 69 (70), 55 (100), $C_{19}H_{11}NO_5$.

Pancorine (**2**)

White amorphous powder, UV (EtOH) λ_{max} (log ε), 220 (3.85), 278 (3.36), and 325 (4.08) nm; IR (KBr) ν_{max}, 940, 1035 (methylenedioxyl group) cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 3.91 (3H, s, C_8-OCH_3), 6.10 (2H, s, $C_{2\&3}-OCH_2O-$), 6.27 (2H, s, $C_{9\&10}-OCH_2O-$), 7.16 (1H, s, H-4), 7.25 (1H, d, J = 8.8 Hz, H-11), 7.52 (1H, d, J = 8.8 Hz, H-5), 7.57 (1H, s, H-1), 7.76 (1H, d, J = 8.8 Hz, H-12) , 7.98 (1H, d, J = 8.8 Hz, H-6); EI-MS, *m/z*: 347 $[M]^+$, $C_{20}H_{13}NO_5$.

(+)-Argenaxine or 1,2,3,4-tetrahydro-1-(2-hydroxymethyl-3,4-dimethoxy-phenylmethyl)-6,7-methylenedioxyisoquinoline (**3**)

Colorless prisms; m.p. 157–158 °C; $[\alpha]_D^{24}$ +95° (c 0.34, $CHCl_3$). UV (MeOH) λ_{max} (log ε), 225 (4.14), 285 (3.38) nm. IR (KBr) ν_{max}, 3381 (OH), 1620, 970 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.28 (2H, m, H-4), 3.31 (2H, m, CH_2-C_1), 3.48 (1H, m, H-3a), 3.70 (1H, m, H-3b), 3.82, 3.86 (each 3H, s, OCH_3 X 2), 3.94 (1H, m, H-1), 4.86, 4.79 (each d, J = 11.5 Hz, CH_2OH), 5.93 (2H, s, $C_{6\&7}-OCH_2O-$), 6.76 (1H, s, H-5), 6.82 (1H, s, H-8), 6.95 (1H, d, J = 8.5 Hz, H-5′), 7.03 (1H, d, J = 8.5 Hz, H-6′); EI-MS, *m/z* (rel. int. %): 357 $[M]^+$ (2), 339 $[M-H_2O]^+$ (42), $C_{20}H_{23}NO_5$.

(+)-Higenamine (**4**)

Colorless amorphous powder, m.p. 255–256 °C; $[\alpha]_D^{24}$ +85°(c 0.74, $CHCl_3$), UV λ_{max} (MeOH) nm (log ε), 225 (4.52), 285 (4.53). IR (KBr) ν_{max} cm^{-1}, 3400 (OH), 1613, 945 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 3.02 (2H, m, CH_2-C_1), 3.26 (2H, m, H-4a&H-4b), 3.35 (1H, m, H-3a), 3.44 (1H, m, H-3b), 4.58 (1H, br.s, H-1), 6.62 (1H, s, H-5), 6.63 (1H, s, H-8), 6.81 (2H, d, J = 8.0 Hz, H-3′&H-5′), 7.15 (2H, d, J = 8.0 Hz, H-2′&H-6′); EI-MS, *m/z* (rel. int. %): 271 $[M]^+$ (4), 164 (100), 149 (18), 107 (34), 77 (29), $C_{16}H_{17}NO_3$.

(+)-Reticuline (**5**)

White needles, m.p 200–202 °C; $[\alpha]_D^{24}$ +112° (c 0.22, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε), 258 (4.50), 285 (4.39), 334 (2.17). IR (KBr) ν_{max}, 3400,1608, 940 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.50 (3H, s, $N-CH_3$), 2.65 (1H, dd, J = 13.8, 4.6 Hz, CH_2-C_1), 2.76 (1H, dd, J = 13.8, 6.8 Hz, CH_2-C_1), 2.81 (2H, m, H-4a & H-4b), 3.10 (1H, m, H-3a), 3.17 (1H, m, H-3b), 3.74 (1H, t, J = 6.8 Hz, H-1), 3.82 & 3.84 (each 3H, s, OCH_3 X 2), 6.27 (1H, s, H-5), 6.50 (1H, s, H-8), 6.54 (1H, d, J = 8.4 Hz, H-5′), 6.71 (1H, dd, J = 8.4, 2.4 Hz, H-6′), 6.77 (1H, d, J = 2.4 Hz, H-2′). EI-MS, *m/z* (rel. int. %): 329 (52) $[M]^+$ (5), 192 (100), 177 (5), 105 (18), 57 (61), 43 (78), $C_{19}H_{23}NO_4$.

α-Tocopherol (**6**)

Yellowish oil, UV λ_{max} (EtOH) nm (log ε), 295 (4.05). IR (Nujol) ν_{max}, 3450, 2960, 2875, 1030 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ 0.85 (12H, m, CH_3-4′a,8′a,12′a,13′), 1.05–1.62 (19 H, m, H-2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′), 1.80 (2H, m, H-3), 2.11 (6H, s, CH_3-5 & CH_3-7), 2.16 (8H, m, H-4, CH_3-8, CH_3-2a), 2.61 (2H, t, J = 6.7 Hz, H-1′, 4.20 (1H, br.s., OH-6). EI-MS, *m/z* (rel. int. %): 430 $[M]^+$ (53), 207 (26), 165 (74), 95 (35), 83 (32), 81 (51), 57 (62), 55 (73), 43 (100), $C_{29}H_{50}O_2$.

trans-Phytol or (2E)-3,7,11,15-tetramethy-2-hexadecen-1-ol (**7**)

Colorless oil, UV λ_{max} (EtOH) nm (log ε), 210 (3.99), 235 (4.22). IR (Neat) ν_{max}, 3500, 2980, 1650, 1590 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.84 (6H, dd, *J* = 6.8, 1.6 Hz, CH_3-18, 19), 0.88 (6H, d, *J* = 6.0 Hz, CH_3-16, 20), 1.10–1.50 (20H, m), 1.66 (3H, s, CH_3-17), 1.99 (2H, br.t, *J* = 7.2 Hz, H-4), 4.15 (2H, d, *J* = 7.0 Hz, H-1), 5.39 (1H, br.t, *J* = 7.0, 1.2 Hz, H-2). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 16.0 (C-17), 19.6 (C-18,19), 22.6 (C-16,20), 24.4 (C-9), 24.7 (C-13), 25.1 (C-5), 27.9 (C-15), 32.6 (C-7), 32.7 (C-11), 36.6 (C-6), 37.2 (C-12), 37.3 (C-8,10), 39.3 (C-14), 39.8 (C-4), 59.0 (C-1), 123.3 (C-2), 139.4 (C-3); EI-MS, *m/z* (rel. int. %): 296 $[M]^+$ (3), 279 (17), 149 (100).

Stigma-4-en-3,6-dione (**8**)

Colorless needles ($CHCl_3$), m.p. 137–139 °C; $[\alpha]_D^{24}$ −38° (c 0.1, $CHCl_3$), UV λ_{max} (EtOH) nm (log ε), 255 (4.38), 322 (3.25). IR (KBr) ν_{max}, 1685 (conj. C=O), 1610 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.72 (3H, s, H-18), 0.81 (3H, d, *J* = 6.4 Hz, H-26), 0.82 (1H, m, H-24), 0.83 (3H, d, *J* = 6.1 Hz, H-27), 0.85 (3H, t, *J* = 6.9 Hz, H-29), 0.95 (3H, d, *J* = 6.5 Hz, H-21), 1.16 (3H, s, H-19), 1.10~1.59 (11H, m, H-11, 12a, 14, 15a, 16a, 17, 20, 22), 1.85~1.95 (3H, m, H-1a&8&16b), 2.00~2.20 (6H, m, H-23&7a&12b&28), 2.30 (1H, m, H-1b), 2.46 (1H, m, H-2a), 2.58 (1H, m, H-2b), 2.68 (1H, dd, *J* = 15.6, 3.8 Hz, H-7b), 6.17 (1H, s, H-4). ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 11.7 (C-29), 11.9 (C-18), 17.3 (C-19), 18.5 (C-21), 18.6 (C-27), 19.2 (C-26), 20.7 (C-11), 22.9 (C-28), 23.8 (C-15), 25.8 (C-23), 27.8 (C-16), 28.9 (C-25), 33.6 (C-22), 33.7 (C-2), 33.9 (C-10), 35.3 (C-20), 35.8 (C-1), 38.9 (C-8), 39.6 (C-12), 42.3 (C-13), 45.6 (C-24), 46.5 (C-7), 50.7 (C-9), 55.5 (C-14), 56.4 (C-17), 125.2 (C-4), 160.8 (C-5), 199.2 (C-3), 201.9 (C-6); EI-MS, *m/z* (rel. int. %): 426 $[M]^+$ (20), 411$[M-CH_3]^+$ (5), 408 $[M-H_2O]^+$ (10), 398 (18), 384 (9), 285 (18), 243 (37), 137 (100), 109 (41), 91 (43), 81 (75), 69 (98), $C_{29}H_{46}O_2$.

Adenine (**9**)

Colorless needles (MeOH), m.p. 173–174 °C; orange color with Dragendorff's reagent. UV λ_{max} (MeOH) nm, 260, 295, 323, 346. IR (KBr) ν_{max}, 3380 (N–H st.), 3200 (C=C st) cm^{-1}. ^{1}H-NMR (200 MHz, CD_3OD), δ 8.13 (1H, s, H-2), 8.20 (1H, s, H-8). EI-MS, *m/z*: 135 $[M]^+$, 119 $[M-NH_2]^+$, $C_5H_5N_5$.

Adenosine (**10**)

Colorless needles (MeOH), m.p. 196–198 °C; orange color with Dragendorff's reagent. UV λ_{max} (MeOH) nm (log ε), 220 (4.48), 253 (4.39). IR (KBr) ν_{max}, 3480 (O–H st), 3120 (C=C st), 1600, 1495 cm^{-1}. ^{1}H-NMR (200 MHz, CD_3OD), δ 3.60–5.14 (5H, m, H-2′,3′,4′,5′), 5.96 (1H, d, *J* = 6.4 Hz, H-1′), 8.18 (1H, s, H-2), 8.30 (1H, s, H-8); EI-MS, *m/z* (rel. int. %): 267 $[M]^+$ (53), 237 (32), 178 (18), 164(38), 148 (39), 135 (21), $C_{10}H_{13}N_5O_4$.

Isorhamnetin 3-O-β-glucopyranoside (**11**)

Yellow needles (MeOH), m.p.: 278–280 °C; $[\alpha]_D^{24}$ −85°(c 0.5, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε), 252 (4.67), 298 (4.13), 377 (4.55). IR (KBr) ν_{max}, 3300 (OH), 1650 (conj.C=O), 1600 cm^{-1}. ^{1}H-NMR (400 MHz, CD_3OD), δ 3.24 (1H, m, H-3″), 3.45 (1H, m, H-4″), 3.47 (1H, m, H-5″), 3.57 (1H, m, H-2″), 3.74 (1H, dd, *J* = 12.0; 2.4 Hz, Ha-6″), 3.65 (1H, dd, *J* = 12.0; 5.5 Hz, Hb-6″), 3.94 (3H, s, $C_{3'}$–OCH_3), 5.40 (1H, d, *J* = 7.6 Hz, H-1″), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.28 (1H, d, *J* = 2.0 Hz, H-8), 6.90 (1H, d, *J* = 8.6 Hz, H-5′), 7.58 (1H, d, *J* = 2.0 Hz, H-2′), 7.92 (1H, dd, *J* = 8.6; 2.0 Hz, H-6′). ^{13}C-NMR (50 MHz, CD_3OD), δ 58.8 ($C_{3'}$–OCH_3), 62.6 (C-6″), 71.5 (C-4″), 75.9 (C-2″), 78.1 (C-5″), 78.5 (C-3″), 94.7 (C-8), 99.9 (C-6), 103.6 (C-1″), 104.2 (C-10), 114.4 (C-2′), 116.0 (C-5′), 123.1 (C-1′), 123.9 (C-6′), 135.3 (C-3), 148.4 (C-4′), 150.8 (C-3′), 158.4 (C-2), 158.9 (C-3), 163.0 (C-9), 165.9 (C-7), 179.4 (C-4). EI-MS, *m/z* (rel. int. %): 316 (100) [M–glucosyl]$^+$, 301 (8), 287 (12), $C_{22}H_{22}O_{12}$.

Biological assays

The compounds isolated from A. mexicana were tested against two human cancer cell lines, Hone-1 (nasopharyngeal carcinoma) and NUGC (human gastric cancer cells) [The cell lines were deposited in the National Health Research Institute, Taipei, Taiwan, with deposit no. 60259 and 60621, respectively]. Cell lines were maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal calf serum (Gibco). Cultures were established in 96-well plates for 24 h. After a 72-h

exposure to the test compounds (50 μg/ml each), the percentage of cell viability was determined using the MTS [3-(4,5-methylthiazol-2-yl)-5-(3-carboxy- methoxyphenyl)-2-(4-sulfenyl)- 2H-tetrazolium, inner salt] colorimetric assay. Actinomycin D (100% ~ 98% inhibition at the concentration of 50 μg/ml) was used as a positive control, according to a previously described procedure (Malich *et al.,* 1997)

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