New Quinazoline Alkaloids from *Glycosmis cochinchinensis*

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Two new quinazoline alkaloids, glycozolone-A (1) and -B (2), and anthranilylamide, glycoamide-A (3) and -B (4) were isolated from leaves of *Glycosmis cochinchinensis* [LOUR.] PIERRE (Rutaceae) collected in Thailand. Structures were characterized on the basis of spectroscopic methods.

Key words quinazoline alkaloid; anthranilylamide; glycozolone; glycoamide; *Glycosmis cochinchinensis*; Rutaceae

The plant genus *Glycosmis* (*G.*) is known to be a rich source of various types of alkaloids. *Glycosmis cochinchinensis* [LOUR.] PIERRE, belonging to the family Rutaceae, is distributed in throughout the Indian-Malayan region. The plants examined in this research were collected in the Saraburi region in Thailand.

The acetone extract of the leaves was subjected to silica gel column chromatography followed by preparative TLC to give two new quinazoline alkaloids called glycozolone-A (**1**) and -B (**2**), in addition to plausible biogenetic precursors, glycoamide-A (**3**) and -B (**4**), respectively.

Glycozolone-A (**1**) was obtained as a racemic colorless oil, $[\alpha]_D$ ±0° ($CHCl_3$), and showed no circular dichroism (CD) absorption in the range from 200 to 400 nm. The molecular formula $C_{16}H_{16}N_2O$ was determined by high resolution (HR) FAB-MS. The IR spectrum showed characteristic absorptions at 3413 and 1664 cm^{-1} due to amide N-H and C=O functions, respectively. The ^{1}H- and ^{13}C-NMR spectra showed the presence of *N*-methyl (δ_H 2.95 and δ_C 36.24) and an amide carbonyl group (δ_C 163.73). ABX-type signals at δ_H 4.69 (m), 3.00 (dd, *J*=4.4, 13.2 Hz), and 2.92 (dd, *J*=4.4, 13.2 Hz) were assigned as methine protons linked to the N-H group and benzylic methylene protons, respectively, by H–H correlation spectroscopy (COSY) analysis. Further, nine aromatic proton signals (Table 1) which were found to be in two sequences of four- and five-spin proton systems by analysis of the H–H COSY spectrum showed the presence of an *ortho*-substituted aromatic ring and a phenyl group. The non-substituted benzyl moiety was found by observation of a base mass fragment peak at *m/z* 161 corresponding to a loss of a benzyl radical from molecular ion in electron impact (EI)-MS. The location of the *N*-methyl and the benzyl moiety were suggested by the results of the nuclear Overhauser effect (NOE) experiments. Irradiation of the *N*-methyl signal at δ_H 2.95 caused 12 and 6% increments of the signal intensity at δ_H 6.64 (H-8) and 4.69 (H-2), respectively. Further, irradiation of H-2 (δ_H 4.69) gave 3% enhancement of the signal at δ_H 7.15 (H-11, 15). In the C–H long-range correlations in ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum, an appearance of a three-bond correlation between a deshielded proton signal at δ_H 7.96 (H-5) and the amide carbonyl carbon signal (C-4) at δ_C 163.73, which also showed a three-bond correlation with a methine proton (H-2, δ_H 4.69), suggested the 4-quinazolone ring system in the molecule. Further, two-bond correlations between benzylic protons (H-9, δ_H 3.00, 2.92) and the carbon signal (C-2) at δ_C 73.43 bearing two nitrogen atoms, together with an appearance of ABX proton signals as mentioned above, confirmed the location of the benzylic side chain at C-2. On the basis of these results, glycozoline-A was assigned to have structure **1**, which was known as a $LiAlH_4$ reduction product of arborine.[1,2] However, it is the first example of the isolation of **1** from a natural source.

Glycozoline-B (**2**) was also isolated as a colorless oil having a molecular formula $C_{17}H_{18}N_2O_2$, with a difference of OCH_2 compared with **1**. The CD spectrum showed no absorption in the range from 200 to 400 nm. In ^{1}H-NMR spectrum, observation of a methoxy signal at δ_H 3.79 and a pair of *ortho* coupled A_2B_2 type signals at δ_H 7.06 and 6.85 (each 2H, d, *J*=8.1 Hz), coupled with the similarity of the signal pattern to that of **1**, except for signals due to the benzyl moiety of **1**, suggested the structure **2** for glycozoline-B. In the EI-MS, the base fragment peak at *m/z* 161, resulting from a loss of the methoxybenzyl moiety from the molecular ion, was observed, as in the case of **1**. The NOE and HMBC experiments of glycozoline-B also supported the structure **2** (see Experimental).

Glycoamide-A (**3**), a colorless oil, was found to have the molecular formula $C_{16}H_{18}N_2O$ by HR-EI-MS. IR bands at λ_{max} 3456, 3367, and 1641 cm^{-1} together with NMR signals at δ_H 2.84 (3H, s) and δ_C 29.63, 169.79 in the ^{1}H- and ^{13}C-NMR spectra suggested the presence of an *N*-methyl and an amide carbonyl group in the molecule. Observation of two sequences of four-spin and five-spin proton signals (see Table 1) in the aromatic proton region in the H–H COSY spectrum indicated the presence of *ortho*-substituted and mono-substituted aromatic rings in the molecule. Remaining proton signals at δ_H 3.65 (2H, q, *J*=7.0 Hz) and 2.91 (2H, t, *J*=7.0 Hz) were assignable to protons on the directly linked two methylene groups. The connectivities of partial structures were confirmed by the results of three-bond C–H long-range correlations in the HMBC spectrum. The cross peak of the amide carbonyl carbon (C-7) at δ_C169.79 to H-6 (δ_H

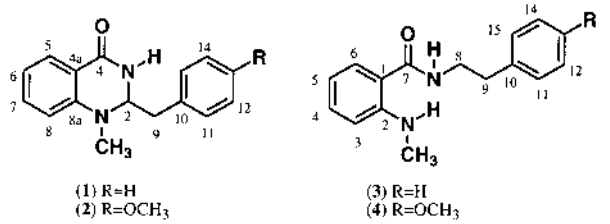

Chart 1. Structures of Alkaloids Isolated from the Leaves of *Glycosmis cochinchinensis*

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Glycozolone-A (**1**) and -B (**2**) and Glycoamide-A (**3**) and -B (**4**)

Position	Glycozolone-A (**1**)		Glycozolone-B (**2**)		Glycoamide-A (**3**)		Glycoamide-B (**4**)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1						115.21		114.43
2	4.69 (m)	73.43	4.63 (m)	73.51		150.48		150.50
3					6.65 (br d, 7.7)	111.04	6.65 (br d, 7.7)	111.05
4		163.73		163.75	7.29 (be t, 7.7)	132.73	7.30 (br t, 7.7)	132.73
4a		115.94		115.92				
5	7.96 (dd, 1.5, 7.7)	128.68	7.96 (br d, 7.7)	128.70	6.53 (br t, 7.7)	114.43	6.54 (br t, 7.7)	114.43
6	6.86 (br t, 7.7)	117.95	6.85 (br t, 7.7)	117.91	7.16 (dd, 1.5, 7.7)	127.00	7.17 (br d, 7.7)	127.00
7	7.43 (br t, 7.7)	134.33	7.42 (br t, 7.7)	134.33		169.79		169.79
8	6.64 (br d, 7.7)	112.18	6.64 (br d, 7.7)	112.15	3.65 (2H, q, 7.0)	40.78	3.62 (2H, q, 7.0)	40.96
8a		146.69		146.75				
9	3.00 (dd, 4.4, 13.2)	38.95	2.95 (dd, 4.4, 13.2)	38.04	2.91 (2H, t, 7.0)	35.74	2.85 (2H,t, 7.0)	34.83
	2.92 (dd, 8.8, 13.2)		2.85 (dd, 8.8, 13.2)					
10		135.56		127.42		138.95		130.91
11	7.15 (d, 7.7)	129.63	7.06 (d, 8.1)	130.62	7.23 (d, 7.7)	128.78	7.15 (d, 8.1)	129.74
12	7.32 (t, 7.7)	128.90	6.85 (d, 8.1)	114.37	7.32 (t, 7.7)	128.68	6.87 (d, 8.1)	114.13
13	7.25 (t, 7.7)	127.02		158.72	7.24 (t, 7.7)	126.54		158.38
14	7.32 (t, 7.7)	128.90	6.85 (d, 8.1)	114.37	7.32 (t, 7.7)	128.68	6.87 (d, 8.1)	114.13
15	7.15 (d, 7.7)	129.63	7.06 (d, 8.1)	130.62	7.23 (d, 7.7)	128.78	7.15 (d, 8.1)	129.74
13-OCH_3			3.79 (3H, s)	55.30			3.80 (3H, s)	55.27
N-Me	2.95 (3H, s)	36.24	2.96 (3H, s)	36.21	2.84 (3H, s)	29.63	2.85 (3H, s)	29.69
N-H	6.07 (br)		5.92 (br)		7.42 (br)		7.41 (br)	
					6.05 (br)		6.02 (br)	

Values are in (δ) ppm. Figures in parentheses are coupling constants (J) in Hz. All signals correspond to ^{1}H in the ^{1}H-NMR spectrum, unless otherwise stated. NMR spectra were recorded in $CDCl_3$.

7.16), which correlates to C-2 (δ_C 150.48) having a three-bond correlation with the *N*-methyl protons was observed. Further, the amide carbonyl carbon (C-7) correlated to the methylene proton (H-8) at δ_H 3.65, which correlated to C-10 (δ_C 138.95), indicating the structure of a phenethyl group attached to the amide nitrogen. Based on these results coupled with appearance of the base mass fragment ion at *m/z* 134 which was caused by the cleavage of the amide bond in the EI-MS, the structure of glycoamide-A was assigned formula **3**, corresponding to the *des*-*N*-methyl derivative of doisuthine from *G. ovoidea*.[3)]

Glycoamide-B (**4**), a colorless oil, $C_{17}H_{20}N_2O_2$. The NMR spectrum was found to be similar to that of **3**, except for signals due to the aromatic protons on the phenethyl moiety. The presence of *p*-methoxyphenethyl moiety attached to the amide nitrogen, instead of non-substituted phenethyl group in **3** was suggested by the following spectral data: observations of 1) a methoxy signal at δ_H 3.80 (3H, s) and δ_C 55.27, 2) A_2B_2-type signals at δ_H 7.15 (2H, d, J=8.1 Hz) and 6.87 (2H, d, J=8.1 Hz), 3) a base fragment peak at *m/z* 134 ($M^+ - \cdot NH\text{-}CH_2CH_2\text{-}C_6H_4\text{-}OCH_3$), 4) an 11% enhancement of H-12, 14 at δ_H 6.87 on irradiation of the methoxyl signal at δ_H 3.80, 5) a three-bond C–H long-range correlation between the carbonyl carbon at δ_C 169.79 (C-7) and methylene protons (H-8) at δ_H 3.62. From the aforementioned results, the structure of glucoamide-B was concluded to be **4**.

Biosynthetic studies of arborine, 2,3-dehydro derivative of glycozoline-A (**1**), in *G. arborea* showed that the alkaloid is produced *via* *N*-methyl-*N*-(phenylacetyl)anthranilic acid from *N*-methylanthranilic acid, phenylacetic acid derived from phenylalanine, and ammonia.[4,5)] Another reasonable biogenetic pathway through the *N*-methylanthranilyl amide from *N*-methylanthranilic acid and phenylethylamine was excluded. However, co-occurrence of glycoamide **3** and **4** and corresponding cyclized quinazolones **1** and **2**, respectively, in *G. cochinchinensis* is of interest to us from a biogenetic viewpoint.[6)]

Known components, doisuthine[3)] and arborinine[7—9)] were also isolated and characterized by spectrometric methods.

Experimental

^{1}H- and ^{13}C-NMR, H–H COSY, NOE, and HMBC spectra were recorded on an A-400 or A-600 (JEOL) spectrometer, in $CDCl_3$. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. EI- and HR-EI-MS were taken with a Hitachi M-80 spectrometer having a direct inlet system. FAB- and HR-FAB-MS were measured with a JMS-HX 110 spectrometer. UV spectra were recorded on a V-550 UV/VIS spectrophotometer (Jasco) in MeOH, IR spectra on an IR-230 (Jasco) in $CHCl_3$, optical rotations on a DIP-370 (Jasco) in $CHCl_3$ at 25 °C, and CD spectra on a J-600 (JASCO) in MeOH. Preparative TLC was done on Kiesel gel $60F_{254}$ (Merck).

Plant Material Leaves of *G. cochinchinensis* [Lour.] Pierre (Rutaceae) were collected at Saraburi province, Thailand in 1996. Authentication was achieved by comparison with the herbarium specimen in the Royal Forest Department, Ministry of Agriculture and Cooperative, Thailand. The herbarium specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and Isolation The dried leaves (410 g) of the plant were extracted with acetone at room temperature. The acetone extract was subjected to silica gel column chromatography eluted with hexane, hexane–acetone (8 : 1, 4 : 1, 2 : 1, 1 : 1, 1 : 2), acetone, and MeOH, successively, to give 8 fractions. The fraction eluted with hexane–acetone (2 : 1) and (1 : 2) was repeatedly subjected to preparative TLC with appropriate combinations of hexane, EtOAc, benzene, acetone, $CHCl_3$, CH_2Cl_2, and MeOH as developing solvents to give glycoamide-A (**3**, 28 mg) and -B (**4**, 1.2 mg) from the former fraction, and glycozolone-A (**1**, 3.5 mg) and -B (**2**, 2.6 mg) from the latter one, along with known alkaloids, doisuthine (28.4 mg) and arborine (3.3 mg), respectively.

Glycozolone-A (**1**): Colorless oil, $[\alpha]_D$ ±0° (c=0.113). CD (MeOH, 200—400 nm): no absorption. UV λ_{max} (log ε): 205 (4.25), 226 (4.30), 262 (3.60), 355 (3.22) nm. IR ν_{max} cm^{-1}: 3413, 1664, 1612. EI-MS *m/z* (%): 161 (base peak, $M^+ - \cdot C_7H_7$), 91 (14, C_7H_7). FAB-MS *m/z* 253 $[M+H]^+$. ^{1}H- and ^{13}C-NMR see Table. NOE: irradiation of NCH_3 (δ 2.95) gave 12% NOE at H-8 (δ 6.64) and 6% NOE at H-2 (δ 4.69); irradiation of H-2 (δ 4.69) gave 4, 4, 2, and 3% NOE at NCH_3 (δ 2.95), H-9 (δ 3.00), NH (δ 6.07), and H-11, 15 (δ 7.15), respectively. HMBC (C–H long-range correlations): C-

2→NCH_3, H-9; C-4→H-2, H-5; C-4a→H-6, H-8; C-5→H-7; C-6→H-8; C-7→H-5; C-8→H-6; C-8a→H-5, H-7, NCH_3, H-2; C-9→H-11, H-15; C-10→H-9, H-12, H-14; C-11→H-9, H-13, H-15; C-12→H-14; C-13→H-11, H-15; C-14→H-12; C-15→H-9, H-11, H-13. HR-FAB-MS Calcd for $C_{16}H_{17}N_2O$: 253.1341. Found: 253.1318.

Glycozolone-B (**2**): Colorless oil, $[\alpha]_D$ ±0° (c=0.0235). CD (MeOH, 200—400 nm): no absorption. UV λ_{max} (log ε): 203 (4.40), 226 (4.37), 266 (3.75), 319 (3.41), 367 (3.30), 411 (3.03) nm. IR ν_{max} cm^{-1}: 3413, 1662, 1610. EI-MS *m/z* (%): 161 (base peak, $M^+ - \cdot C_8H_9O$), 134 (4), 121 (8, C_8H_9O). FAB-MS *m/z* 283 $[M+H]^+$. ^1H- and ^{13}C-NMR see Table. NOE: irradiation of NCH_3 (δ 2.96) gave 12% NOE at H-8 (δ 6.64) and 5% NOE at H-2 (δ 4.63); irradiation of OCH_3 (δ 3.79) gave 15% NOE at H-12, 14 (δ 6.85). HMBC (C–H long-range correlations): C-2→NCH_3, H-9; C-4→H-5; C-4a→H-6, H-8; C-5→H-7; C-6→H-8; C-7→H-5; C-8→H-6; C-8a→H-5, H-7, NCH_3; C-9→H-11, H-15; C-10→H-9, H-12, H-14; C-11→H-9, H-15; C-13→H-11, H-15, OCH_3; C-15→H-9, H-11. HR-FAB-MS Calcd for $C_{17}H_{19}N_2O_2$: 283.1446. Found: 283.1451.

Glycoamide-A (**3**): Colorless oil. UV λ_{max} (log ε): 211 (4.43), 256 (4.00), 342 (3.65) nm. IR ν_{max} cm^{-1}: 3456, 3367, 1641. EI-MS *m/z* (%): 254 (M^+, 18), 150 (8), 134 (base peak, $M^+ - \cdot C_8H_{10}N$), 105 (29), 91 (54). ^1H- and ^{13}C-NMR see Table. HMBC (3-bond C–H long-range correlations): C-1→H-3, H-5; C-2→H-4, H-6, NCH_3; C-3→H-5; C-4→H-6; C-6→H-4; C-7→H-6, H-8; C-9→H-11, H-15; C-10→H-8, H-12, H-14; C-11→H-9; C-12→H-14; C-13→H-11, H-15; C-14→H-12; C-15→H-9. HR-EI-MS Calcd for $C_{16}H_{18}N_2O$: 254.1418. Found: 254.1438.

Glycoamide-B (**4**): Colorless oil. UV λ_{max}: 219, 259, 279, 341 nm. IR ν_{max} cm^{-1}: 3359 (br), 1639. EI-MS *m/z* (%): 284 (M^+, 33), 150 (82), 134 (base peak, $M^+ - \cdot C_9H_{12}NO$), 121 (40), 105 (40), 91 (31). ^1H- and ^{13}C-NMR see Table. NOE: irradiation of NCH_3 (δ 2.85) gave 5% NOE at H-8 (δ 6.65); irradiation of OCH_3 (δ 3.80) gave 11% NOE at H-12, 14 (δ 6.87). HMBC (3-bond C–H long-range correlations): C-1→H-3, H-5; C-2→H-4, H-6, NCH_3; C-3→H-5; C-4→H-6; C-5→H-3; C-7→H-6, H-8; C-9→H-11, H-15; C-10→H-8, H-12, H-14; C-11→H-9, H-15; C-12→H-14; C-13→H-11, H-15, OCH_3; C-14→H-12; C-15→H-9, H-11. HR-EI-MS Calcd for $C_{17}H_{20}N_2O_2$: 284.1525. Found: 284.1541.

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