Chemical Constituents of *Glycosmis pentaphylla*. Isolation of A Novel Naphthoquinone and A New Acridone Alkaloid

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Chemical constituents of MeOH–CH_2Cl_2 extract of the stem of *Glycosmis pentaphylla* RETZ (Rutaceae) collected in Papua New Guinea were studied. A novel naphthoquinone and a new acridone alkaloid called glycoquinone (1) and glycocitrine-III (2), respectively, were isolated along with twelve known compounds, and their structures were elucidated by spectrometric analyses. This is the first isolation of a naphthoquinone derivative from the *genus Glycosmis*.

Key words *Glycosmis pentaphylla*; naphthoquinone; acridone alkaloid; glycoquinone; glycocitrine-III; Rutaceae

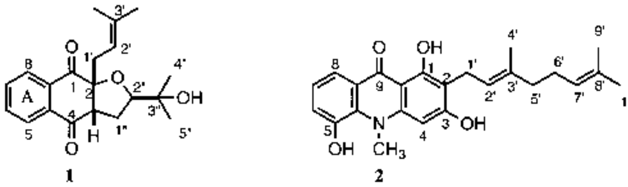
In our previous papers[1,2)] on biologically active natural products from medicinal plants in Papua New Guinea, the isolation and structural elucidation of a dibenzofuran, a depsidone, and some xanthones from *Calophyllum panciflorum* A. C. SMITH, *Garcinia assigu* LANTB., *Garcinia dulcis* (ROXB.) KURZ., and *Garcinia latissima* MIQ. belonging to the Guttiferae were described. In our further studies on this project, extracts of some kinds of medicinal plants traditionally used in Papua New Guinea were tested for their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) activation.[3)] The extract of *Glycosmis pentaphylla* was found to exhibit the most significant anti-tumor promoter activity. This paper describes the studies of the constituents of *G. pentaphylla* and isolation and structural elucidation of new components called glycoquinone (**1**) and glycocitrine-III (**2**).

Results and Discussion

The dried stem of *G. pentaphylla* was extracted with a mixture of methanol and dichloromethane at room temperature. The extract was dissolved in acetone, and the soluble portion was submitted to a combination of silica gel column chromatography and preparative TLC to give a novel naphthoquinone and a new acridone alkaloid along with twelve known compounds.

Structure of Glycoquinone (1) **1** was obtained as a pale yellow oil. CD spectrum showed no absorption in the range from 200 to 400 nm. The molecular formula was determined as $C_{20}H_{24}O_4$ by high-resolution (HR)-MS. The IR spectrum exhibited bands at ν_{max} 3377 (br) and 1691 cm^{-1} due to hydroxyl and carbonyl groups, respectively. Observation of symmetrically oriented four-spin AA′BB′ type signals at δ_H 8.09 and 7.78 including a deshielded 2H proton signal in the aromatic proton region of the ^1H-NMR spectrum and two carbonyl carbon signals at δ_C 195.51 and 198.63 in the ^{13}C-NMR spectrum coupled with UV absorptions at λ_{max}: 227, 255, 300 nm suggested the presence of a dihydro-1,4-naphthoquinone skeleton having no substituent in the A-ring. The presence of a prenyl moiety in the molecule was indicated by ^1H-NMR signals at δ_H 2.58 (1H, dd, J=14.3, 7.7 Hz, H-1′), 2.53 (1H, dd, J=14.3, 8.1 Hz, H-1′), 5.03 (1H, m, H-2′), 1.56 (3H, s, H-5′), and 1.51 (3H, s, H-4′) and appearance of a base mass fragment peak at m/z 260 [$M^+ - \cdot CH_2CH{=}C(CH_3)_2 + \cdot H$] in the electron impact-mass spectra (EI-MS). Further, two 3H singlets at δ_H 1.29 and 1.14 and an oxygen-linked quarternary carbon signal at δ_C 71.80 together with a mass fragment ion at m/z 242 produced by loss of water from the base fragment ion revealed the presence of a 1-hydroxy-1-methylethyl side chain in the molecule. Remaining signals observed were one set of clearly separated four-spin proton signals at δ_H 3.93 (1H, dd, J=4.4, 9.2 Hz, H-2″), 3.41 (1H, dd, J=9.5, 10.8 Hz, H-3), 2.46 (1H, ddd, J=4.4, 9.5, 13.4 Hz, H-1″), and 2.00 (1H, ddd, J=9.2, 10.8, 13.4 Hz, H-1″). Consideration of their chemical shifts, multiplicities, and J-values of each signal together with the results of H–H COSY spectrum indicated the presence of a partial structure [–O–CH–CH_2–CH–C=O] in the molecule. A deshielded proton at δ_H 2.46 was believed to locate at the *peri*-position of the carbonyl group.

Finally, connectivities of these structural units were established by results of analyses of the ^1H-detected heteronuclear multiple bond connectivity (HMBC) spectrum shown by arrows in Fig. 1. The significant C–H long-range correlations for structure determination are described below. One of the carbonyl carbons at δ_C 198.63 (C-1) showed three-bond correlations with the methylene protons (δ_H 2.58, 2.53, H-1′) on the prenyl moiety and the methine (δ_H 3.41, H-3), which further correlated with the methylene carbon (δ_C 34.53, C-1′) on the prenyl moiety. The quarternary carbon at δ_C 90.05 (C-2) showed a three-bond correlation with the methylene proton at δ_H 2.46 (H-1″) on the four-spin proton system which correlated with the quarternary carbon of the 1-hydroxy-1-methylethyl moiety at δ_C 71.80 (C-3″), and also showed two-



1 **2**

Chart 1

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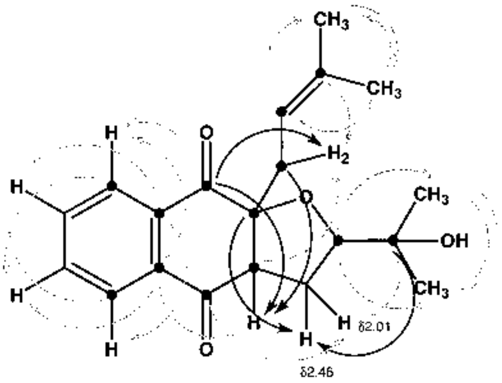

Fig. 1. C–H Three Bond Long-Range Correlations in the HMBC Spectrum of **1** in $CDCl_3$

bond correlations with H-1′ (δ_H 2.58, 2.53) on the prenyl group and H-3 (δ_H 3.41) (see Experimental). These correlations suggested the presence of a tetrahydrofuran ring fused to the 2,3-dihydro-1,4-naphthoquinone skeleton and locations of the prenyl and 1-hydroxy-1-methylethyl moieties at C-2 and C-2″, respectively. Furthermore, the relative stereochemistries of three asymmetric centers in the molecule were proposed by observations of nuclear Overhauser effect (NOE) enhancements between the methine proton signal at δ_H 3.41 (H-3) and an olefinic proton signal at δ_H 5.03 (H-2′) on the prenyl moiety, and the methyl protons at δ_H 1.14 (H-5″) on the side chain and one of methylene protons at δ_H 2.46 (H-1″) which also have NOE with the methine proton at δ_H 3.41 (H-3) (see Experimental). On the basis of the foregoing spectral data, structure **1** was assigned to be glycoquinone.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1** in $CDCl_3$

	1	
	δ_H	δ_C
1	—	198.63 (s)
2	—	90.05 (s)
3	3.41 (dd, 9.5, 10.8)	55.02 (d)
4	—	195.51 (s)
4a	—	134.18 (s)
5	8.09 (m)	127.17 (d)
6	7.78 (m)	134.69 (d)
7	7.78 (m)	134.64 (d)
8	8.09 (m)	127.17 (d)
8a		133.60 (s)
1′	2.58 (dd, 14.3, 7.7) 2.53 (dd, 14.3, 8.1)	34.53 (t)
2′	5.03 (m)	116.54 (d)
3′		138.07 (s)
4′	1.51 (3H)	17.95 (q)
5′	1.56 (3H)	25.65 (q)
1″	2.46 (ddd, 4.4, 9.5, 13.4) 2.01 (ddd, 9.2, 10.8, 13.4)	31.85 (t)
2″	3.93 (dd, 9.2, 4.4)	83.99 (d)
3″	—	71.80 (s)
4″	1.29 (3H)	26.74 (q)
5″	1.14 (3H)	24.64 (q)

Values in (δ_H and δ_C) ppm. The coupling constants (J) in parentheses are in Hz. All signals correspond to 1H in the ^{1}H-NMR spectrum, and were observed as singlets, unless otherwise stated.

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for **2** in $CDCl_3$

	2	
	δ_H	δ_C
1-OH	14.89	
1	—	161.99 (s)
2	—	106.33 (s)
3		162.48 (s)
4	6.30	91.50 (d)
4a	—	145.89 (s)
5		144.86 (d)
6	7.08 (d, 8.1)	120.28 (d)
7	7.07 (t, 8.1)	121.65 (d)
8	8.03 (d, 8.1)	119.11 (d)
8a		124.14 (s)
9		180.59 (s)
9a		105.35 (s)
N-Me	3.98 (3H)	40.80 (q)
10a		133.82 (s)
1′	3.52 (d, 7.3)	21.45 (t)
2′	5.34 (m)	121.68 (d)
3′		139.69 (s)
4′	1.85 (3H)	16.28 (q)
5′	2.11 (2H, m)	39.77 (t)
6′	2.11 (2H, m)	26.45 (t)
7′	5.07 (m)	123.79 (d)
8′		132.06 (s)
9′	1.60 (3H)	25.64 (q)
10′	1.68 (3H)	17.69 (q)
Others	6.27 (br, OH)	

Values in (δ_H and δ_C) ppm. The coupling constants (J) in parentheses are in Hz. All signals correspond to 1H in the ^{1}H-NMR spectrum, and were observed as singlets, unless otherwise stated.

Structure of Glycocitrine-III (2) **2** was obtained as a yellow oil, $C_{24}H_{27}NO_4$. The UV absorptions (λ_{max}: 214, 228, 255, 267, 284, 308sh, 336sh, 401 nm) and IR bands (ν_{max} 3396 (br), 1635, 1600 cm^{-1}) suggested the 1-hydroxy-9-acridone skeleton.[4,5] The ^{1}H-NMR spectrum showed aromatic proton signals of a three-spin system at δ_H 8.03 (1H, d, J=8.1 Hz), 7.07 (1H, t, J=8.1 Hz), 7.08 (1H, d, J=8.1 Hz) and a 1H singlet at δ_H 6.30 in addition to a chelated hydroxyl signal at δ_H 14.89. Among these three-spin proton signals, the lower field signal at δ_H 8.03 was assignable to H-8 deshielded by 9-carbonyl group, and consequently, these signals could be assigned as H-8, H-7, and H-6, respectively. The signal at δ_H 3.98 in the ^{1}H-NMR and δ_C 40.80 in the ^{13}C-NMR spectra indicated the presence of an *N*-methyl group. In differential NOE experiments, irradiation of *N*-methyl signal at δ_H 3.98 enhanced a lone aromatic singlet at δ_H 6.30 suggesting the location of this proton at C-4. The remaining signals at δ_H 5.34 (1H, m, H-2′), 5.07 (1H, m, H-7′), 3.52 (1H, d, J=7.3 Hz, H-1′), 2.11 (4H, m, H-5′, 6′), 1.85 (3H, s, H-4′), 1.68 (3H, s, H-10′), 1.60 (3H, s, H-9′) in the ^{1}H-NMR spectrum, coupled with two characteristic mass fragment ions at *m/z* 324 and 270 arising from loss of $[C_5H_9]$ and $[C_9H_{15}]$ from the molecular ion in EI-MS, respectively, and appearance of NOE enhancement between H-1′ (δ_H 3.52) and the methyl signal (δ_H 1.85) suggested that this new acridone (**2**) contained a geranyl moiety $[-CH_2CH=C(CH_3)-CH_2CH_2CH=C(CH_3)_2]$ in the molecule. The location of the geranyl moiety at C-2 was proposed by significant C–H three- or two-bond correlations between a hydrogen bonded proton at δ_H 14.89 (1-OH) and a carbon (C-2) at δ_C 106.33 having a correlation with the methylene protons (H-1′, δ_H 3.52) on the geranyl moiety, respectively, and by an additional correlation of this methylene proton with the carbon

(C-1) bearing a hydrogen bonded hydroxyl group in the HMBC spectrum (see Experimental). Based on these results, we assigned structure **2** to glycocitrine-III.

Known acridone alkaloids, noracronycine,[6] *des-N*-methylnoracronycine,[6] *des-N*-methylacronycine,[6] citracridone-I,[7] arborinine,[8—10] 5-hydroxyarborinine,[11,12] and acrifoline,[13] furoquinoline alkaloids, skimmianine[14] and kokusaginine,[14] and naphthoquinone analogues, avicenol-B,[15] avicequinone-C,[15] and avicenone[15] were also isolated and characterized. Determination of the anti-tumor promoting activities of the isolated compounds is now in progress.

Experimental

^{1}H- and ^{13}C-NMR, H–H COSY, NOE, HMQC, and HMBC (J=8 Hz) spectra were recorded on an A-400 or A-600 (JEOL) spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. All mass spectra were taken under electron impact (EI) conditions, unless otherwise stated, using an M-80 (Hitachi) spectrometer having a direct inlet system. UV spectra were recorded on a V-550 UV/VIS spectrophotometer (JASCO) in MeOH, IR spectra on a FT/IR-230 (JASCO) in $CHCl_3$, and 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 Kieselgel 60 F_{254} (Merck).

Plant Materials The plant material used in this study, *Glycosmis pentaphylla* RETZ (Rutaceae) was collected in the Rigi River area, Central Province of Papua New Guinea, during August—September 1997. A voucher specimen was deposited at the herbarium of the University of Papua New Guinea.

Extraction and Isolation The dried stems (940 g) were extracted with MeOH–CH_2Cl_2 (1 : 1) at room temperature. The extract was dissolved in acetone. The soluble portion was submitted to silica gel column chromatography eluted successively by hexane–acetone (19 : 1—1 : 1), acetone, CH_2Cl_2–MeOH (3 : 1), and MeOH. Further treatment by silica gel column and preparative TLC gave glycoquinone (**1**) (2.0 mg), noracronycine (0.5 mg), *des-N*-methylnoracronycine (0.7 mg), avicenol-B (4.5 mg), avicequinone-C (1.0 mg), and avicenone (0.9 mg) from hexane–acetone (7 : 3) fraction, glycocitrine-III (**2**) (2.5 mg), kokusaginine (3.1 mg), skimmianine (5.6 mg), citracridone-I (0.7 mg), and 5-hydroxyarborinine (1.4 mg) from hexane–acetone (1 : 1) fraction, and arborinine (1.5 mg), *des-N*-methylacronycine (1.3 mg), and acrifoline (1.3 mg) from acetone fraction.

Glycoquinone (**1**): Pale yellow oil. $[\alpha]_D$ ±0° (c=0.068). CD (MeOH, 200—400 nm): no absorption. UV λ_{max} nm: 227, 255, 300. IR ν_{max} cm^{-1}: 3377 (br), 1691, 1597. MS m/z (%): 328 (M$^+$, 29), 260 (100), 242 (31), 239 (27), 227 (26), 189 (30), 173 (44), 159 (29), 149 (78). HR-MS Calcd for $C_{20}H_{24}O_4$: 328.1674. Found: 328.1715. Differential NOE: Irradiation of H-3 (δ_H 3.41) gave 5% NOE at H-1″ (δ_H 2.46) and 2% NOE at H-2′ (δ_H 5.04); irradiation of H-5″ (δ_H 1.14) gave 4% NOE at H-1″ (δ_H 2.46) and 4% NOE at H-2″ (δ_H 3.93); irradiation of H-2″ (δ_H 3.93) gave 6% NOE at H-1″ (δ_H 2.00); irradiation of H-1″ (δ_H 2.00) gave 3% NOE at H-2″ (δ_H 3.93) and 4% NOE at H-1″ (δ_H 2.46); irradiation of H-1″ (δ_H 2.46) gave 4% NOE at H-3 (δ_H 3.41) and 13% NOE at H-1″ (δ_H 2.00); irradiation of H-4″ (δ_H 1.29) gave 3% NOE at H-2″ (δ_H 3.93). HMBC: C–H 2-bond correlations: C-2→H-3, H-1′; C-3→H-1″; C-4→H-3; C-1′→H-2′; C-2′→H-1′; C-3′→H-4′, H-5′; C-1″→H-3; C-2″→H-1″.

Glycocitrine-III (**2**): Yellow oil. UV λ_{max} nm: 214, 228, 255, 267, 284, 308sh, 336sh, 401. IR ν_{max} cm^{-1}: 3396 (br), 1635, 1601. MS m/z (%): 393 (M$^+$, 34), 324 (84), 308 (25), 294 (11), 282 (15), 270 (100), 257 (28). HR-MS Calcd for $C_{24}H_{27}NO_4$: 393.1938. Found: 393.1965. Differential NOE: Irradiation of N-CH_3 (δ_H 3.98) gave 2% NOE at H-4 (δ_H 6.30); irradiation of H-4′ (δ_H 1.85) gave 2% NOE at H-1′ (δ_H 3.52). HMBC C–H 3-bond correlations: C-1→H-1′; C-2→1-OH, H-4; C-3→H-1′; C-4a→N-CH_3; C-5→H-7; C-6→H-8; C-8a→H-7; C-9a→1-OH, H-4; C-10a→N-CH_3, H-8; C-2′→H-4′; C-3′→H-1′; C-4′→H-2′; C-5′→H-2′, H-4′; C-7′→H-9′, H-10′; C-9′→H-10′; C-10′→H-9′.

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15) Recently, these naphthoquinones were also isolated by us from some *Avicennia* plants belonging to the Avicenniaceae. Full details of the structure elucidation of these compounds will be reported elsewhere.