Alkaloids and Sulphur-containing Amides from *Glycosmis citrifolia* and *Glycosmis elongata*  
(Alkaloid dan Amida Mengandungi Sulfur daripada *Glycosmis citrifolia* dan *Glycosmis elongata*)

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**ABSTRACT**

Air-dried leaves of both *Glycosmis citrifolia* and *Glycosmis elongata* collected from Bogor Botanical Garden, Indonesia were individually extracted with chloroform to give dark viscous extracts after solvent removal. Column chromatographic separation of the extract of *G. citrifolia* yielded 5(6)-glutene-3α-ol, two sets conformers, (E)-dambullin and (Z)-dambullin, and (E)-methyldambullin and (Z)-methyldambullin. Similar treatment of the extract of *G. elongata* gave skimmianine and arborinine. The structures of the compounds were elucidated based on spectroscopic data and comparison with published reports.

**Keywords:** Arborinine; dambullin and methyldambullin; *Glycosmis citrifolia*; *Glycosmis elongata*; skimmianine

**INTRODUCTION**

*Glycosmis* is a genus of small trees of forty species from Rutaceae family commonly found in South East Asia and South China. Fourteen of these species occur in Peninsular Malaysia and grow on limestone rocks and hills. Some of the species were used in traditional medicine preparations for the treatment of various diseases such as to cure fever, cold, indigestion, chest pain diarrhoea and hernia pain (Burkill 1935; Dai & Liu 1999; Gimlette 1939). Many studies on the chemical constituents of *Glycosmis* species have been reported with the identification of compounds including alkaloids, coumarins, chalcones, flavonoids, sulphur-containing amides (Cuong et al. 1999; Greger et al. 1994; Hofer et al. 2000; Jash et al. 1992; Rostagi et al. 1980; Vajrodaya et al. 1998). Some species were also reported to be good antifungal agents for example amides from *Glycosmis mauritiana* (Greger et al. 1996; 1994) and *Glycosmis pentaphylla* (Bandara et al. 1990). In a previous communication we have also reported the identification of three sulphur-containing amides and a flavonoid from related species *Glycosmis chlorosperma* (Rahmani et al. 2004). In continuation of our work on this genus, we report the isolation and structural determination of 5(6)-glutene-3α-ol (1) together with another four known sulphur-containing amides ((E)-dambullin (2), (Z)-dambullin (3), (E)-methyldambullin (4) and (Z)-methyldambullin (5) from *Glycosmis citrifolia*. Instead of isolating similar amides from *Glycosmis elongata*, the plant afforded two alkaloids and elucidated as skimmianine (6) and arborinine (7).

**EXPERIMENTAL**

**GENERAL**

Melting points were determined on a Kofer hot stage microscope model 500X and were uncorrected. The UV spectra were recorded on a Shimadzu UV-160A spectrophotometer. The IR spectra were obtained from a Perkin Elmer FTIR (model 1725X) spectrophotometer. The MS spectra were recorded on Direct Induction Probe (DIP) using a Shimadzu GCMS-QP5050 spectrometer with ionization induced by electron impact at 70 eV. 1H-NMR and 13C-NMR and DEPT spectra were recorded using a JEOL FTNMR 400, operating at 400 and 100 MHZ, respectively.
The spectra were obtained in CDCl₃ with chemical shifts expressed in δ and coupling constant (J) in hertz with tetramethylsilane (TMS) as internal standard.

**PLANT MATERIAL**

The leaves of *G. citrifolia* and *G. elongata* were collected in 2000 from Bogor Botanical Garden, Bogor, Indonesia and voucher specimens were deposited there.

**EXTRACTION AND ISOLATION**

The air-dried ground leaves of *G. citrifolia* (1.0 kg) were extracted twice with chloroform at room temperature and each lasted for three days. The extract was concentrated under reduced pressure to give 18.56 g of crude dark green viscous material. Part of extract (15.00 g) was separated by vacuum silica gel column chromatography and eluted with hexane with gradual increase in chloroform and methanol to give ten fractions of 250 ml each. Further separation of fraction 5 by column chromatography and eluted with mixture of hexane and chloroform to yield 5(6)-glutene-3α-ol (1). Fraction 7 was further separated by column chromatography and eluted with mixture of hexane and chloroform with gradual increase in ethyl acetate and methanol to give 25 fractions of 100 ml each. Fractions 5-6 were combined and further separated to give white solid and recrystallized with ethyl acetate and hexane as needle-shaped crystals of (E)-dambullin (2). Fraction 15 from the above column was subjected to an aluminium oxide mini column chromatography and eluted with chloroform and methanol to give white solid obtained was recrystallized with acetone to afford (Z)-dambullin (3) as needle-shaped crystals. Fractions 16-17 from the vacuum column above were combined and repeatedly separated by silica gel mini column chromatography eluted with mixture of hexane, chloroform and ethyl acetate to give white solid. The compound was recrystallized with ethyl acetate and hexane to afford mixture of (E)-methyl(dambullin (4) and (Z)-methyl(dambullin (5) as needles.

Similar extraction of the air-dried ground leaves of *G. elongata* (377.0 g) gave 12.62 g of dark crude extract. Part of the extract (8.28 g) was separated by column chromatography and eluted with mixture of hexane, chloroform and methanol to give 18 fractions of 200 ml each. Fraction 11-12 were combined and rechromatographed on silica gel eluted with chloroform with increasing amounts of ethyl acetate and methanol to give 16 fractions. Further separation of fractions 5 and 6 gave yellow solid which on recrystallization with chloroform and ethyl acetate yielded yellow needle-shaped crystals of arborinine (6). Fractions 15-18 were combined and further separated with chromatography to give orange solid which was subsequently recrystallized with chloroform and ethyl acetate to afford orange needle-shaped crystals of skimmianine (7).

The compound was obtained as white needle-shaped crystals, m.p. 209-210 °C (Buckingham et al. 1994, m.p. 210-211.5 °C). IR ν₃⁰⁺ cm⁻¹ (KBr disc): 3400, 2918, 2850, 1638, 1464, 1062, 940, 730 and 722. EIMS m/z (% intensity): 426 (4.53), 408 (1.95), 393 (2.02), 274 (80.18), 259 (100), 245 (2.02), 231 (218) (1.28), 189 (13.16), 173 (0.86), 163 (15.60), 150 (28.42), 134 (62.80), 119 (45.89), 109 (54.27), 95 (27.22), 81 (19.64) and 55 (12.89).

**1H-NMR** (400 MHz, CDCl₃): δ 5.62 (d, 8.78 Hz, 1H, H-6), 3.46 (br s, 1H, H-3), 1.16 (s, 3H, H-28), 1.13 (s, 3H, H-23), 1.09 (s, 3H, H-26), 1.06 (s, 3H, H-24), 1.03 (s, 3H, H-27), 0.99 (s, 3H, H-30), 0.96 (s, 3H, H-29), 0.83 (s, 3H, H-25).

**13C-NMR** (100 MHz, CDCl₃) ppm: 141.76 (C-5), 122.11 (C-6), 76.33 (C-3), 49.63 (C-10), 47.41 (C-8), 43.00 (C-18), 40.83 (C-4), 39.27 (C-14), 38.95 (C-22), 37.80 (C-13), 35.97 (C-19), 35.06 (C-16), 34.82 (C-9), 34.59 (C-15), 34.52 (C-30), 33.08 (C-11), 32.39 (C-28), 32.03 (21), 30.33 (C-12), 30.09 (C-29), 29.72 (C-17), 28.94 (C-23), 28.24 (C-20), 27.79 (C-2), 25.46 (C-24), 23.62 (C-7), 19.62 (C-27), 18.44 (C-26), 18.20 (C-21) and 16.19 (C-25).

**1H-NMR** (400 MHz, CDCl₃) and **13C-NMR** (100 MHz, CDCl₃) (see Table 1)

The white solid obtained was recrystallized with ethyl acetate to give white needle-shaped crystals with Rf value of 0.41 (40 % CHCl₃, 60 % EtOAc) and m.p 146-147 °C (Greger et al. 1994, m.p. 145-147 °C). UV λmax nm (log ε), EtOH: 276.8 (sh), 224.2 (2.39) and 211.6 (sh). IR ν₃⁰⁺ cm⁻¹ (KBr disc): 3580, 3300, 3072, 2926, 1658, 1633, 1555, 1510, 1455, 1285, 1245, 1130, 977, 944, 782. EIMS m/z (% intensity): 337 (7.60), 282 (3.23), 269 (17.12), 254 (3.09), 204 (7.07), 188 (9.90), 162 (2.45), 150 (3.31), 133 (15.85), 120 (100.00), 107 (66.67), 91 (24.12), 71 (32.67), 69 (76.12), 53 (43.05), 41 (76.98). 1H-NMR, (400 MHz, CDCl₃) and 13C-NMR (100 MHz, CDCl₃) (see Table 1)

**1H-NMR** (400 MHz, CDCl₃) and **13C-NMR** (100 MHz, CDCl₃) (see Table 1)

The white solid obtained was recrystallized with acetone to give white needles with m.p. 124-126 °C with Rf value 0.38 (40 % CHCl₃, 60 % EtOAc). UV λmax nm (log ε), EtOH: 277.4 (sh), 224.0 (2.05), 210.8 (sh). IR ν₃⁰⁺ cm⁻¹ (KBr disc): 3582, 3304, 3068, 2922, 1654, 1552, 1512, 1450, 1308, 1298, 1128, 1140, 974, 944. EIMS m/z (% intensity): 337 (2.05), 282 (1.52), 269 (2.72), 172 (1.56), 162 (2.23), 150 (1.09), 133 (2.62), 120 (100), 107 (22.88), 91 (6.41), 71 (1.97), 69 (2.62). 1H-NMR (400 MHz, CDCl₃) and 13C-NMR (100 MHz, CDCl₃) (see Table 1)

**1H-NMR** (400 MHz, CDCl₃) and **13C-NMR** (100 MHz, CDCl₃) (see Table 1)

This mixture of conformers was obtained as white needle-shaped crystals with m.p. 75-77 °C (Greger et al. 1994,
after extensive column chromatography amides were isolated in which two of them were obtained 17S-glutene-3α-ol (1) and four sulphur-containing separation technique, one triterpene identified as (OMe), 61.9 (OMe), 141.2 (C-8a), 104.6 (C-1'), 143.4 (C-2'), 59.4 (OMe), 56.7 (C-4a), 118.2 (C-5), 112.6 (C-6), 153.0 (C-7), 142.2 (C-8), 141.2 (C-8a), 104.6 (C-1'), 143.4 (C-2'), 59.4 (OMe), 56.7 (OMe), 61.9 (OMe).

SKIMMIANINE (7)
Skimmianine was obtained as orange needle-shaped crystals with m.p. 179-181°C (Bowen et al. 1978, m.p. 174-176°C) and Rf value of 0.61 (80% chloroform: 20% ethyl acetate). UV λmax nm (log ε), EtOH: 272.5 (2.38), 397.0 (0.31), 265 (sh), 321.0 (sh); IR νmax cm⁻¹ (KBr disc): 3436, 2920, 2850, 1562, 1529, 1462, 1322, 1190, 1108, 1060. EIMS m/z (% intensity): 292 (100), 285 (76.54), 270 (54.15), 256 (49.73), 244 (44.73), 230 (32.12), 216 (23.15), 213 (11.86), 201 (45.33), 176 (75.54), 129 (54.15), 115 (67.78). 1H-NMR (400 MHz, CDCl3) δ: 8.02 (d, 1H, 9.27 Hz, H-5), 7.59 (d, 1H, 2.93 Hz, H-6), 7.24 (d, 1H, 9.27 Hz, H-6), 7.05 (d, 1H, 2.93 Hz, H-1'), 4.44 (s, 3H, 3-OMe), 4.12 (s, 3H, 8-OMe), 4.03 (s, 3H, 7-OMe). 13C-NMR (100 MHz, CDCl3) ppm: 180.5 (C-9), 159.1 (C-3), 155.9 (C-1), 141.7 (C-14), 140.3 (C-11), 133.8 (C-6), 129.9 (C-2), 126.3 (C-8), 121.3 (C-7), 120.4 (C-13), 114.5 (C-5), 105.5 (C-12), 86.6 (C-4), 60.7 (2-OMe), 55.9 (3-OMe), 34.0 (N-Me).

RESULTS AND DISCUSSION
From the chloroform extract of the air-dried ground leaves of G. citrifolia after extensive column chromatography separation technique, one triterpene identified as 5(6)-glutene-3α-ol (1) and four sulphur-containing amides were isolated in which two of them were obtained as mixture of a pair of conformers. Previous works on the plant reported the identification of flavonoids, amidosulphoxides and quinolone alkaloids (Wu et al. 1995). Similar separation treatment of the second plant extract, G. elongata, furnished two alkaloids. There has been no previous report on the phytochemical investigation of G. elongata.

(E)-DAMBULLIN (2) AND (Z)-DAMBULLIN (3)
Both compounds were isolated as individual white needle-shaped crystals after recrystallization with ethyl acetate and acetone, respectively. The molecular formula of the compounds were assigned as C18H24NO5S based on examination of the MS. 1H-NMR and 13C-NMR spectral data of both compounds also showed similar pattern but there are some differences in their physical and spectroscopic behaviour. It is interesting to note that the melting point of (Z)-conformer (124-126°C) is much lower than the (E)-conformer (146-148°C) in which the later melting point is identical to those as reported by Greger et al. (1994). The 1H-NMR spectra of both compounds showed similar pattern but the main difference is the resonances for the amide proton (Table 1). In (E)-dambullin (3) this proton resonates at δ 5.91 while the other one occurred at much lower field δ 8.71. This might be due to the restricted rotation about the amide C-N bond due to the presence of lone pair electron on nitrogen atom. Three pairs of doublets were observed in the aromatic region of the 1H-NMR of both conformers and two of the pairs belong to the protons at H-10/H-14 and H-11/H-13. The other pairs were assigned to the two trans-methine protons at H-3 and H-4. The chemical shifts of the prenyloxy side chain attached to the C-12 of the aromatic ring exhibited similar values. In addition, the 13C-NMR spectral data of the two compounds were also quite similar and literature search indicated that both compounds were previously reported to occur in Glycosmis angustifolia (Greger et al. 1994).

(E)- METHYLDAMBULLIN (4) AND (Z)- METHYLDAMBULLIN (5)
The melting point of the needle-shaped mixture of the conformers was found to be 75-77°C (Greger et al. 1994, m.p. 70-73°C). Attempts were made to separate the mixture by column chromatography but was unsuccessful. The UV spectra of the compounds were identical with absorption maxima at 274.4, 224.2 and shoulder at 211.0 nm typical characteristic of sulphur-containing amides. The EIMS of the compounds showed molecular ion peak at m/z 351 which is consistent with the molecular formula of C18H24NO5S and the base peak at m/z 120. In the 1H-NMR spectrum, the chemical shifts of the two pairs of equivalent aromatic protons at H-10/H-14 and H-11/H-13 in both conformers occurred at about identical positions.
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*NR* (br t)
However, the chemical shifts of the methine protons H-3 and H-4 resonances at different locations (Table 1). For the s-cis conformer, they occurred as doublets at δ 7.02 and 6.88, while for the s-trans conformer the two signals occurred at δ 7.34 and 7.11, respectively. The three protons singlet at δ 3.05 and 2.87 indicated the existence of –N-Me and –SO₂-Me groups of the s-cis form, respectively. The chemical shifts for these methyl protons in the s-trans form occurred at δ 2.99 and 3.02, respectively. The sequence of Me-SO₂-CH=CH-CO-NR-CH₂-aryl which is characteristic of sulphones derived from methylthiopropenoic acid amide was further supported by the COSY spectrum. The ¹³C-NMR also provided evidence for the existence of eighteen carbon atoms and their chemical shifts values were closely matched except for the N-Me groups (Table 1). In one isomer the methyl group occurred at 34.1 ppm, while in the other one this signal shifted to slightly lower field at 36.4 ppm. The ratio of the distribution of the two conformers was found to be 64:36 as revealed by the ¹³C-NMR spectrum. These data are in complete agreement with those compounds isolated from the related Glycosmis angustifolia as reported by Greger et al. (1994). This is the first report the identification of the two conformers from G. citrifolia.

The UV spectrum showed absorptions at 272.5 and 397.0 nm with shoulders at 265.0 and 321.0 nm characteristic for acridone nucleus (Pakrashi & Bhattacharyya 1965). The strong and broad band at 3436 cm⁻¹ and a sharp peak at 1642 cm⁻¹ in the IR spectrum indicated the presence of hydroxyl and carbonyl groups, respectively. The MS showed the presence of molecular ion peak at m/z 285 consistent with molecular formula C₁₆H₁₅NO₄ and this was further supported by the integration in the ¹H-NMR spectrum which indicated the presence of 15 protons. Except for the occurrence of three singlets at δ 3.91 (OMe), 3.98 (OMe) and 3.76 N-Me) each integrated for three protons, all other resonances occurred in the aromatic region. One of the aromatic protons resonates as a singlet which was assigned to the isolated proton at H-4. The other four aromatic protons belongs to ring C and their positions could be easily be assigned by their correlations in the COSY spectrum. The DEPT spectrum exhibited the occurrence of three methyl, five methine and eight quartenary carbon atoms and this confirmed the suggested molecular formula. The compound was previously reported to occur in G. bilocularis (Bowen et al. 1978).
**SKIMMIANINE (7)**

The compound was obtained as orange needles with m.p. 179-181°C and the MS gave molecular ion peak at m/z 259 which correspond to molecular formula C_{13}H_{21}NO with base peak at m/z 244 due to the loss of a methyl group. The integration in the 1'H-NMR spectrum indicated the existence of 13 protons in which four occurred as two sets of doublets in the aromatic region and three sharp singlets in the high field region. The first set of doublets at δ 8.02 and 7.21 with coupling constant 9.27 Hz were assigned to the two neighbouring protons H-5 and H-6. The other set of doublets at δ 7.05 and 7.60 (2.93 Hz) were due to the two unsaturated protons of furan ring attached to ring A. The three singlets at δ 4.43, 4.15 and 4.02 were assigned to the three methoxyl groups attached to C-4, C-8 and C-7, respectively. Based on this data and comparison with literature report, the compound was assigned as skimmianine isolated previously from *G. arborea* (Chakravarty et al. 1999).

The compounds were tested for cytotoxic activity assayed against T-lymphoblastic leukaemia cell line (CEM-SS). The four amides, (E)-dambullin (2), (Z)-dambullin (3) and mixture of methylambullin ((E)- and (Z)-conformer), exhibited strong activity with IC_{50} values of 1.6, 2.2, 3.0 µg/ml, respectively. For the antioxidant activity using the DPPH free radical, unfortunately none of the compounds displayed significant activity except the mixture of the two methylambullin conformers which showed moderate activity with IC_{50} value of 208.3 µg/ml. Compared with the activity of Vitamin C (IC_{50} = 14.3 µg/ml) and Quercetin (IC_{50} = 78.5 µg/ml), the scavenging activity of the compounds mixture were much weaker.

**CONCLUSION**

The chloroform extracts of both *G. citrifolia* and *G. elongata* have yielded a triterpene (5(6)-glutene-3α-ol (1)), four sulphur-containing amides ((E)-dambullin (2), (Z)-dambullin (3), (E)-methylambullin (4) and (Z)-methylambullin (5)) and two alkaloids (skimmianine (6) and arborinine (7)). The amides were found to be strongly cytotoxic when tested against T-lymphoblastic leukaemia cell line (CEM-SS) with IC_{50} values ranging from 1.6 - 3.0 µg/ml.

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