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Indole and monoterpene alkaloids from the leaves of *Kopsia dasyrachis*

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Abstract

A new monoterpene alkaloid, kinabalurine G, in addition to 11 indole alkaloids and the catechine–skytanthine compound, kopsirachine, was obtained from the leaf extract of *Kopsia dasyrachis*. The structure of the novel alkaloid, danuphylline, was confirmed by an X-ray analysis.

Keywords: *Kopsia* species; Apocynaceae; Indole and monoterpene alkaloids

1. Introduction

We recently reported the alkaloidal composition of the stem bark extract of *Kopsia dasyrachis* Ridl., one of about seventeen Malaysian *Kopsia* species, which is found in Sabah, Malaysian Borneo (Kam, Subramaniam & Chen, 1999). A previous study of the leaf alkaloids of *K. dasyrachis* from Borneo yielded three new indoles, *viz*., kopsidasine, kopsidasine *N*-oxide, kopsidasinine (Homberger & Hesse, 1982) and in addition, kopsirachine, which is constituted from the union of catechine and two units of skytanthine (Homberger & Hesse, 1984). We have also reported the structure elucidation (Kam, Lim, Choo & Subramaniam, 1998), as well as a biomimetic, electrochemically-mediated semisynthesis (Kam, Lim & Choo, 1999) of the novel pentacyclic indole, danuphylline, which was obtained in minute amount from the leaf extract of this plant. We now report the full alkaloidal composition of the leaf, including the isolation of a new monoterpene alkaloid, as well as confirmation of the structure of danuphylline by X-ray analysis.

2. Results and discussion

The ethanol extract of the leaves furnished a basic fraction which upon extensive chromatography yielded a total of 13 alkaloids, *viz*., methyl chanofruticosinate **1** (Chen, Li, Kirfel, Will & Breitmaier, 1981), methyl 11,12-methylenedioxychanofruticosinate **2** (Chen et al., 1981; Kam, Tan, Hoong & Chuah, 1993), methyl *N*-decarbomethoxychanofruticosinate **3** (Chen et al., 1981; Kam et al., 1993), methyl 11,12-methylenedioxy-*N*-decarbomethoxychanofruticosinate **4** (Chen et al., 1981; Kam et al., 1993), kopsamine **5** (Crow & Michael, 1962; Feng, Kan, Potier, Kan & Lounasmaa, 1983; Gilbert, 1965; Kam & Sim, 1998; Zheng, Zhou & Huang, 1989), 11,12-dimethoxykopsamine **6** (Kam & Sim, 1998; Zheng et al., 1989), kopsamine *N*(4)-oxide **7** (Kam & Sim, 1998; Zheng et al., 1989), pleiocarpine **8** (Homberger & Hesse, 1982), 12-methoxypleiocarpine **9** (Kam & Sim, 1998), kopsifine **10** (Kam et al., 1999; Kam & Subramaniam, 1998), kopsirachine **11** (Homberger & Hesse, 1984), danuphylline **12** (Kam

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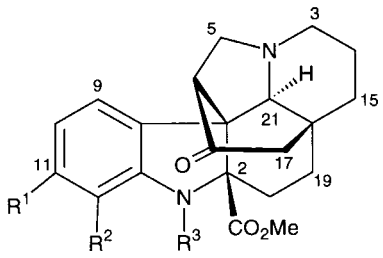
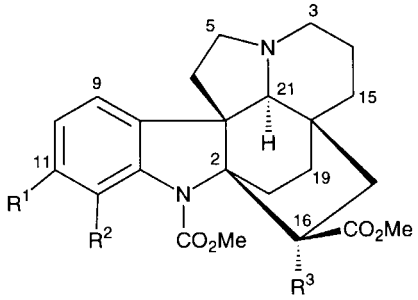


1 $R^1 = R^2 = H$, $R^3 = CO_2Me$

2 $R^1, R^2 = OCH_2O$, $R^3 = CO_2Me$

3 $R^1 = R^2 = R^3 = H$

4 $R^1, R^2 = OCH_2O$, $R^3 = H$



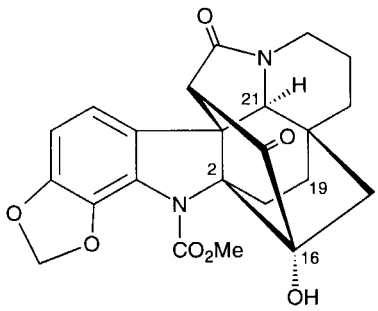
5 $R^1, R^2 = OCH_2O$, $R^3 = OH$

6 $R^1 = R^2 = OMe$, $R^3 = OH$

7 $R^1, R^2 = OCH_2O$, $R^3 = OH$, $N(4)\rightarrow O$

8 $R^1 = R^2 = R^3 = H$

9 $R^1 = R^3 = H$, $R^2 = OMe$



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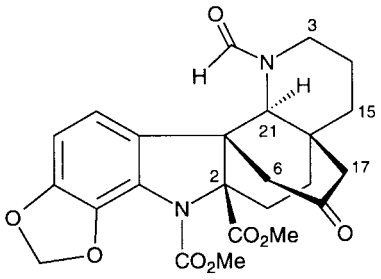

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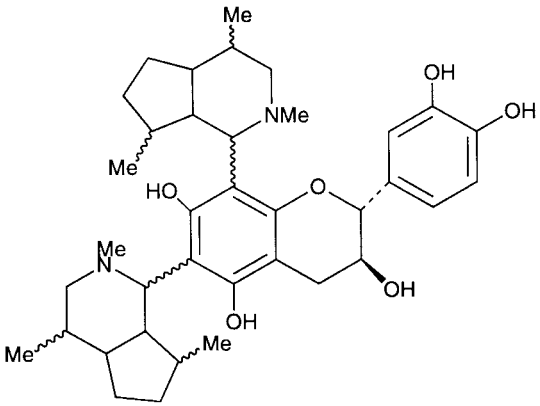

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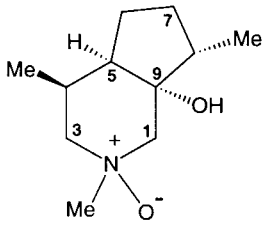
et al., 1998, 1999), and kinabalurine G **13**, which is the *N*-oxide of a hydroxyskytanthine. Compound **2** is the major alkaloid found in the leaves, and as with compounds **1**, **3** and **4**, have been encountered previously in other *Kopsia* species (Chen et al., 1981; Kam et al., 1993). Danuphylline **12** represents a new indole alkaloid possessing a novel pentacyclic carbon skeleton (Kam et al., 1998). We have carried out an electrochemically-mediated semisynthesis starting from **2** based on the proposal that its probable origin is *via* unravelling, through a retro-aldol sequence, of an intermediate carbinol amine **14**, derived in turn from an iminium ion precursor (Kam et al., 1999). We have now carried out an X-ray diffraction analysis which has confirmed the structure reported earlier based on spectral data.

The crystals of **12** are orthorhombic belonging to



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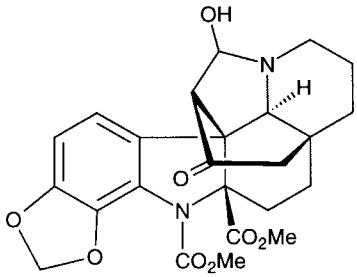

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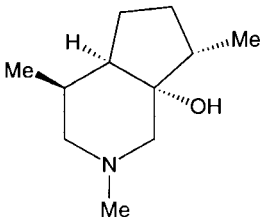

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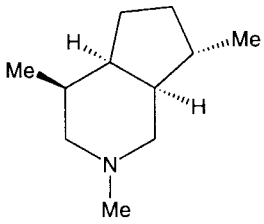

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the space group $P2_12_12_1$, with a = 7.2558 (5) Å, b = 16.0230 (10) Å, c = 18.798 (2) Å; $\alpha=\beta=\gamma=90°$; V = 2185.4 (3) Å^3; D_X = 1.430 Mg m^{-3} and Z = 4. The structure was solved by the direct method SAPI-91 (Fan, Yao, Zheng, Gu & Qian, 1991), and refined by the full matrix least squares method. The final R-factor was 0.0769. As shown in the perspective diagram of Fig. 1, compound **12** does indeed possess the novel ring system proposed earlier based on spectral analysis (Kam et al., 1998). Furthermore the results also confirm the *trans* disposition of the *N*(4) lone pair and H-21, as well as the location of the formamide-H within the anisotropic influence of the aromatic ring, resulting in the anomalously high field resonance of the formamide proton in ^{1}H NMR.

Compound **13** was the most polar alkaloid isolated and was also obtained in minute amount as a colourless oil. The mass spectrum showed a molecular ion at m/z 199 which analyzed for $C_{11}H_{21}NO_2$, with other fragment peaks at m/z 183, 166, 110, 84 and 58. The latter three fragments are characteristic of a skytanthine type alkaloid (Cordell, 1977; Kam, Yoganathan & Chen, 1997). The IR spectrum showed the presence of a hydoxyl group (3404 cm^{-1}), while the ^{1}H NMR spectrum indicated the presence of two CH_3CH groups and an *N*-methyl group, which was rather deshielded at δ 3.17. This observation, coupled with the polar nature of this compound, and the observation of a strong M-16 fragment in the mass spectrum, suggested that compound **13** is an *N*-oxide. This was readily confirmed by $FeSO_4$ reduction of **13** which yielded the parent monoterpene alkaloid **15**. The *N*-methyl signal is now shifted upfield to δ 2.30, while the resonances of the two α-carbons, C-1 and C-3, are also shifted upfield from δ 67.3 and 67.2 to δ 62.5 and 57.7 respectively (Table 1). The presence of a low field, quaternary carbon signal at δ_C 79.1, indicated that the hydroxyl function is attached to a quaternary carbon, i.e.

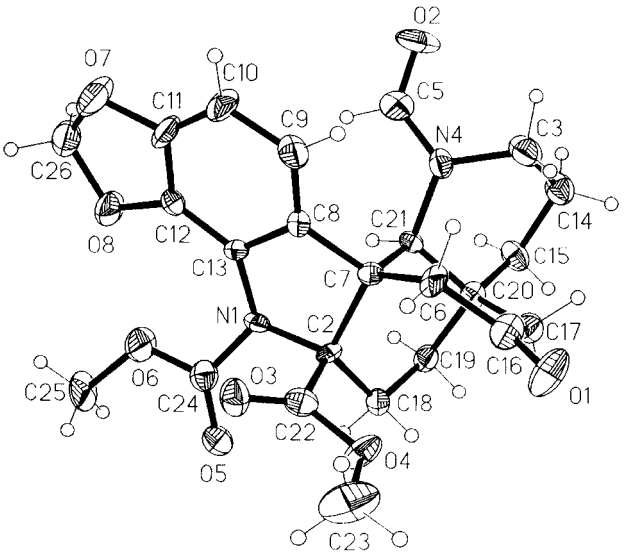

Fig. 1. X-Ray structure of **12**.

Table 1
^{1}H and ^{13}C NMR spectral data[a] for compounds **13**, **15** and **16**

Position	**13**[b]		**15**	**16**[c]
	δ_H	δ_C	δ_C	δ_C
1α	3.04 dt (12.5,2)	67.3	62.5	57.1
1β	2.95 d (12.5)			
3α	3.11 ddd (11,4,2)	67.2	57.7	57.9
3β	2.89 t (11)			
4α	3.02 m	24.0	29.1	46.2
5α	2.17 m	44.3	44.4	40.2
6	1.17 tdd (13,11,8)	20.3	19.9	31.3
6	1.70 dtd (13,9,3)			
7	1.37 dddd (14,10,8,3)	28.8	29.7	22.2
7	1.86 dddd (14,11,9,3)			
8β	1.76 m	39.6	39.1	36.5
9	–	79.1	78.2	30.8
N-Me	3.17 s	61.0	45.9	46.5
8-Me	1.02 d (7)	16.6	17.1	22.7
4-Me	0.96 d (7)	15.9	16.8	17.5

[a] $CDCl_3$, 400 MHz.
[b] assignments based on COSY, HMQC and HMBC.
[c] From Ref. (Homberger & Hesse, 1984).

C-5 or C-9, based on a skytanthine-type carbon skeleton. Detailed analysis of the ^{1}H and ^{13}C NMR spectral data (COSY, HMQC, HMBC, NOE) and comparison with δ-skytanthine **16** (Homberger & Hesse, 1984), enabled placement of the OH function on C-9 and allowed full assignment of the NMR spectral data. For instance, the COSY spectrum revealed the partial structure $CH_3CHCH_2CH_2CH$ corresponding to the Me(8)-C(8)-C(7)-C(6)-C(5) fragment, while the HMBC spectrum showed two-bond correlations from C-9 to H-5, H-1, and H-8, and three-bond correlations to H-4, H-6, H-7, and 8-Me which are consistent with the proposed structure. The COSY spectrum showed long range W-coupling between H-1 and H-3 (2 Hz, see Table 1), which is only possible between H-1α and H-3α (Kam et al., 1997). A similar W-coupling was also observed between H-1α and H-5 (2 Hz), which is possible only if H-5 is also α, and the ring junction stereochemistry is *cis*. The stereochemistry of the 4-methyl group is deduced to be β from the observed $J_{3\beta\text{-}4}$ value of 11 Hz, requiring H-4 and H-3β to be in a *trans*-diaxial arrangement (Kam et al., 1997). Finally, irradiation of H-1β causes NOE enhancement of H-8 and vice versa, which establishes the stereochemistry of the 8-methyl group as α. Based on these results, the structure of kinabalurine G is as shown in **13**. The parent monoterpene, 9-hydroxy-δ-skytanthine **15**, is unknown, and was not detected in the present study, although a 9-hydroxyskytanthine of unknown stereochemistry as well as a β-skytanthine *N*-oxide, have been previously reported from *Tecoma stans* (Dickinson & Jones, 1969) and *Skytanthus acutus* (Streeter, Adolphen & Appel, 1969) respectively. The occurrence of monoterpene alkaloids has also been previously observed in two other *Kopsia* species, *viz*., *K. pauciflora* (Kam et al., 1997) and *K. macrophylla* (Kan et al., 1995).

3. Experimental

3.1. Plant material

Plant material was collected from Sabah, Malaysia and was identified by Dr. K. M. Wong. Voucher specimens are deposited at the Herbarium of the Sabah Forest Department, Sandakan, Sabah, Malaysia.

3.2. Extraction and Isolation

Extraction of alkaloids was carried out in the usual manner as described in detail elsewhere (Kam & Tan, 1990). Essentially, the ground leaf material was exhaustively extracted with 95% EtOH at ambient temperature. The EtOH extract was then concentrated under reduced pressure, partitioned into dilute HCl, basified with concentrated ammonia solution, and the liberated alkaloids were then taken into chloroform to give a basic fraction. The alkaloids were isolated by repeated fractionation using CC and centrifugal TLC on SiO_2. Solvent systems used for chromatography were $CHCl_3$ with increasing proportions of MeOH (CC) and Et_2O, Et_2O-hexane, $CHCl_3$, $CHCl_3$-MeOH (Centrifugal TLC). The yields (g kg^{-1}) of the alkaloids (**1**–**13**) from the leaf extract were: **1** (0.032), **2** (0.281), **3** (0.004), **4** (0.004), **5** (0.046), **6** (0.028), **7** (0.056), **8** (0.003), **9** (0.006), **10** (0.002), **11** (0.313), **12** (0.004) and **13** (0.004).

3.3. Kinabalurine G (*13*)

$[\alpha]_D + 9^\circ$ ($CHCl_3$, *c* 0.129). EIMS, *m*/*z* (rel. int.): 199 [M^+,$C_{11}H_{21}NO_2$] (16), 183 (35), 166 (36), 156 (19), 150 (11), 139 (25), 126 (21), 110 (24), 96 (24), 84 (17) and 58 (100). HREIMS, [M^+], found 199.1574, calcd for $C_{11}H_{21}NO_2$, 199.1572. ^{1}H and ^{13}C NMR: see Table 1.

3.4. Reduction of kinabalurine G (*13*)

Compound **13** (4 mg) was stirred in aqueous ferrous sulfate (2.5%, 2 mL) at 80°C for 0.5 h. The mixture was then extracted with $CHCl_3$ and chromatography over SiO_2 gave the parent monoterpene, 9-hydroxy-δ-skytanthine (**15**) (2 mg, 56%), API-LCMS, MH^+, *m*/*z* 184 ($C_{11}H_{21}NO + H$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, d, J = 7 Hz, 4-Me), 1.00 (3H, d, J = 7 Hz, 8-Me), 1.84 (1H, t, J = 11.5 Hz, H-3), 2.04 (1H, d, J = 12 Hz, H-1), and 2.30 (3H, s, *N*-Me). ^{13}C NMR: see Table 1.

3.5. X-ray diffraction analysis of danuphylline **12**

A total of 2333 reflections were collected by the ω scan method up to θ_{max} of 25.47° on a CAD4 diffractometer at 27°C using $MoK_{\alpha}(\lambda = 0.71073$ Å) radiation. The crystal dimensions are 0.4 × 0.1 × 0.1 mm. A total of 985 reflections with $I > 2\sigma(I)$ were observed and were corrected for the Lorentz-polarization effect, but not for absorption. The structure was solved by using the direct method SAPI-91 (Fan et al., 1991). All non-hydrogen atoms were refined anisotropically by full matrix least squares refinement on an IBM 486 PC to $R = 0.0769$, $wR = 0.1268$ for the observed reflections, $w = [\sigma^2(F_o^2) + (0.0563P)^2]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$. Hydrogen atoms were generated geometrically and were allowed to ride on their respective parent atoms. The atomic coordinates for the non-hydrogen atoms and their equivalent isotropic displacement parameters, calculated coordinates for the hydrogen atoms, anisotropic displacement parameters for the non-hydrogen atoms, a full list of bond distances and angles, and the structure factor table are deposited as supplementary material at the Cambridge Crystallographic Data Centre.

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References

Chen, W.S., Li, S.H., Kirfel, A., Will, G., & Breitmaier, E. (1981). *Liebigs Ann. Chem*., 1886.

Cordell, G. A. (1977). In R. H. F. Manske, *The Alkaloids*, *Vol. XVI* (p. 435). New York: Academic Press.

Crow, W. D., & Michael, M. (1962). *Australian Journal of Chemistry*, *15*, 130.

Dickinson, E. M., & Jones, G. (1969). *Tetrahedron*, *25*, 1523.

Fan, H. F., Yao, J. X., Zheng, C. D., Gu, Y. X., & Qian, J. Z. (1991). *SAPI-91, a computer program for automatic solution of crystal structures from X-ray diffraction data*. Beijing, P. R. China: Institute of Physics, Chinese Academy of Sciences.

Feng, X. Z., Kan, C., Potier, P., Kan, S. K., & Lounasmaa, M. (1983). *Planta Medica*, *48*, 280.

Gilbert, B. (1965). In R. H. F. Manske, *The Alkaloids*, *Vol. VIII* (p. 439). New York: Academic Press.

Homberger, K., & Hesse, M. (1982). *Helvetica Chimica Acta*, *65*, 2548.

Homberger, K., & Hesse, M. (1984). *Helvetica Chimica Acta*, *67*, 237.

Kam, T. S., & Sim, K. M. (1998). *Phytochemistry*, *47*, 145.

Kam, T. S., & Subramaniam, G. (1998). *Natural Product Letters*, *11*, 131.

Kam, T. S., & Tan, P. S. (1990). *Phytochemistry*, *29*, 2321.

Kam, T. S., Lim, T. M., Choo, Y. M., & Subramaniam, G. (1998). *Tetrahedron Letters*, *39*, 5823.

Kam, T. S., Lim, T. M., & Choo, Y. M. (1999). *Tetrahedron*, *55*, 1457.

Kam, T. S., Subramaniam, G., & Chen, W. (1999). *Phytochemistry*, in press.

Kam, T. S., Tan, P. S., Hoong, P. Y., & Chuah, C. H. (1993). *Phytochemistry*, *32*, 489.

Kam, T. S., Yoganathan, K., & Chen, W. (1997). *Journal of Natural Products*, *60*, 673.

Kan, C., Sevenet, T., Hadi, A. H. A., Bonin, M., Quirion, J. C., & Husson, H. P. (1995). *Natural Product Letters*, *7*, 283.

Streeter, M., Adolphen, G., & Appel, H. H. (1969). *Chemistry and Industry (London)*, 1631.

Zheng, J. J., Zhou, Y. L., & Huang, Z. H. (1989). *Acta Chimica Sinica*, *2*, 168 (English Edn.).