Phytochemistry, Vol. 27, No. 8, pp. 2719–2723, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

10-DEMETHOXYKOPSIDASININE FROM *KOPSIA JASMINIFLORA**

MATTHIAS O. HAMBURGER,† GEOFFREY A. CORDELL, KITTISAK LIKHITWITAYAWUID‡ and NIJSIRI RUANGRUNGSI‡

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, U.S.A.; ‡Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

(*Received* 4 *February* 1988)

IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Kopsia jasminiflora*; Apocynaceae; 10-demethoxykopsidasinine; indole alkaloid; ^{1}H NMR; ^{13}C NMR.

Abstract—10-Demethoxykopsidasinine, a new indole alkaloid, has been isolated from *Kopsia jasminiflora*. The structure elucidation was achieved through the use of extensive ^{1}H NMR (COSY, NOE difference) and ^{13}C NMR (CSCM 1D, APT) studies at variable temperature. Detailed ^{1}H NMR data and revised ^{13}C NMR assignments are reported for the first time for this rare alkaloid skeleton.

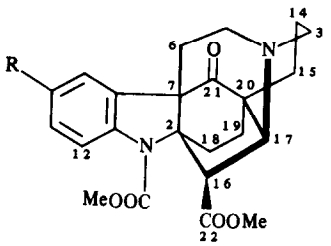
INTRODUCTION

The genus *Kopsia* (Apocynaceae, subfamily Plumerioideae, tribe Rauvolfieae) comprises 30 species distributed over Southeast Asia [2]. Various medicinal uses have been reported, especially from China, where the cultivated species *K. officinalis* is used for the treatment of rheumatoid arthritis, dropsy and tonsilitis. The crude alkaloid mixture has been found to exhibit analgesic properties [3, 4]. A large number of kopsane alkaloids and bisindole alkaloids of the pleimutine type have been isolated from various species of the genus [3–9].

Previous studies by one of our groups of the constituents of *K. jasminiflora* Pitard, a species indigenous to the northern part of Thailand where it is known as 'put dong', led to the isolation of three members of a new indole alkaloid skeleton [10]. We report here on the isolation of 10-demethoxykopsidasinine (**1**), the second member of the rare kopsidasinine series of alkaloids.

RESULTS AND DISCUSSION

Extraction with ethanol, followed by the usual work-up procedures and repeated chromatography over silica gel yielded alkaloid **1**, whose molecular formula $C_{23}H_{26}N_2O_5$ was established by high resolution mass measurement. In the region 1690–1750 cm^{-1}, the IR spectrum displayed three carbonyl bands, one of them indicative for an ester (1745 cm^{-1}). The second band at 1727 cm^{-1} could be assigned with the aid of the ^{13}C NMR spectrum to a keto function (214 ppm); while the absence of an amine proton in the ^{1}H NMR spectrum



1 R = H
2 R = OMe

and the observation of signals at 153 and 52 ppm in the ^{13}C NMR spectrum allowed the assignment of the absorption band at 1697 cm^{-1} to a urethane moiety. The UV spectrum displayed a similarity to a *N*-methoxy carbonyl-2,3-dihydroindole chromophore.

The ^{1}H NMR spectrum recorded at 22° integrated for 26 protons, but displayed three pairs of identical signals (7.91 and 7.48, 4.10 and 3.89, and 1.95 and 1.78 ppm) integrating for one half proton each. Increasing temperature led to a gradual broadening of those signals and to a collapse of the broad singlet buried under the methyl ester signal at 3.74 ppm. This observation suggested that **1** was a 1:1 mixture of two conformers at room temperature in which the *N*-carbomethoxy group was in two energetically available planar conformations. Splitting of all but three of the signals in the ^{13}C NMR spectrum supported that notion. The equilibrium of energetically favorable conformers for indole alkaloids bearing a urethane group has been reported previously [7, 8].

The ^{1}H NMR data were further analysed with the aid of resolution enhanced COSY spectra at 22° and 55° (see Fig 1a and b, Table 1). In addition to the *N*-carbomethoxy and methyl ester groups, five isolated spin systems were observed. Four aromatic protons with a coupling pattern typical for an *o*-disubstituted aromatic ring were

*Part 11 in the series 'Traditional Medicinal Plants of Thailand'. For part 10, see ref. [1].

†Present address: Institut de Pharmacognosie et Phytochemie, Université de Lausanne, CH-1005, Lausanne, Switzerland.

observed in the region 7.0–7.9 ppm, and a two-proton spin system was comprised of a split doublet at 4.10 and 3.89 ppm, coupled to a doublet at 3.52 ppm ($J = 9.4$ Hz). Two pairs of methylene protons, one pair of which, because of their chemical shifts (2.97 and 2.75 ppm) had to be vicinal to a heteroatom, and a second pair were observed at 1.53 and 3.54 ppm. The large chemical shift difference ($\Delta\delta$ 2.01) between these two protons indicated a strong anisotropic effect of a spacially close carbonyl. Another spin system consisted of three pairs of methylene protons (2.92 and 2.79, 2.38 and 2.49 and 1.21 and 1.33 ppm), the first of which was also adjacent to nitrogen. Finally, the signals for another two pairs of methylene protons were observed at 1.95 (1.78) and 1.43, and 1.35 and 1.58 ppm, respectively.

The APT spectrum of **1** displayed resonances for four aromatic CH and two aromatic quaternary carbons, three non-aromatic quaternary, two methine and seven methylene carbons together with signals attributable to the *N*-carbomethoxy, methyl ester and keto functionalities. The latter signal, due to its unusually high chemical shift (214 ppm) was reminiscent of kopsidasinine **2** [7], and a tentative assignment of the other carbon resonances showed good agreement with a kopsidasinine type skeleton.

Positive proof of the nature of the nucleus was obtained through extensive NOE difference experiments at 22° and 55°, which linked all of the discrete spin systems together and completely established the relative stereochemistry and the preferred conformation. Furthermore, the spectral simplification permitted the determination of the coupling constants of otherwise substantially overlapping signals in the region 1.2–1.7 ppm.

The results of the NOE difference experiments are schematically represented in Fig. 2, which also shows the preferred conformation of **1** in solution. On irradiation of the *N*-carbomethoxy resonance, a weak enhancement of the H-12 signals was observed. The fact that the H-12 resonances of both conformers were enhanced can be explained by consideration of an equilibrium state where conformational exchange is much faster than the rather slow NOE build-up and decay. NOE's from H-16 to H-18a, from H-17 to H-19a, H-15a and H-3a, and between H-14b and H-5b defined the relative stereochemistry around the quaternary carbons C-2, C-7 and C-20. Irradiation of H-6b led to an enhancement of H-9, thereby establishing a link to the indole nucleus. The stereochemistry around the urethane moiety was defined by the anisotropic effect of the *N*-carbomethoxy carbonyl group on H-12, H-16 and H-18b, and their spatial proximity was confirmed by a molecular model. The stereochemistry of the C-16 ester group was established from the coupling constant $J_{16,17} = 9.4$ Hz, indicating a small dihedral angle for the H-C-16 to H-C-17 bond. The ester

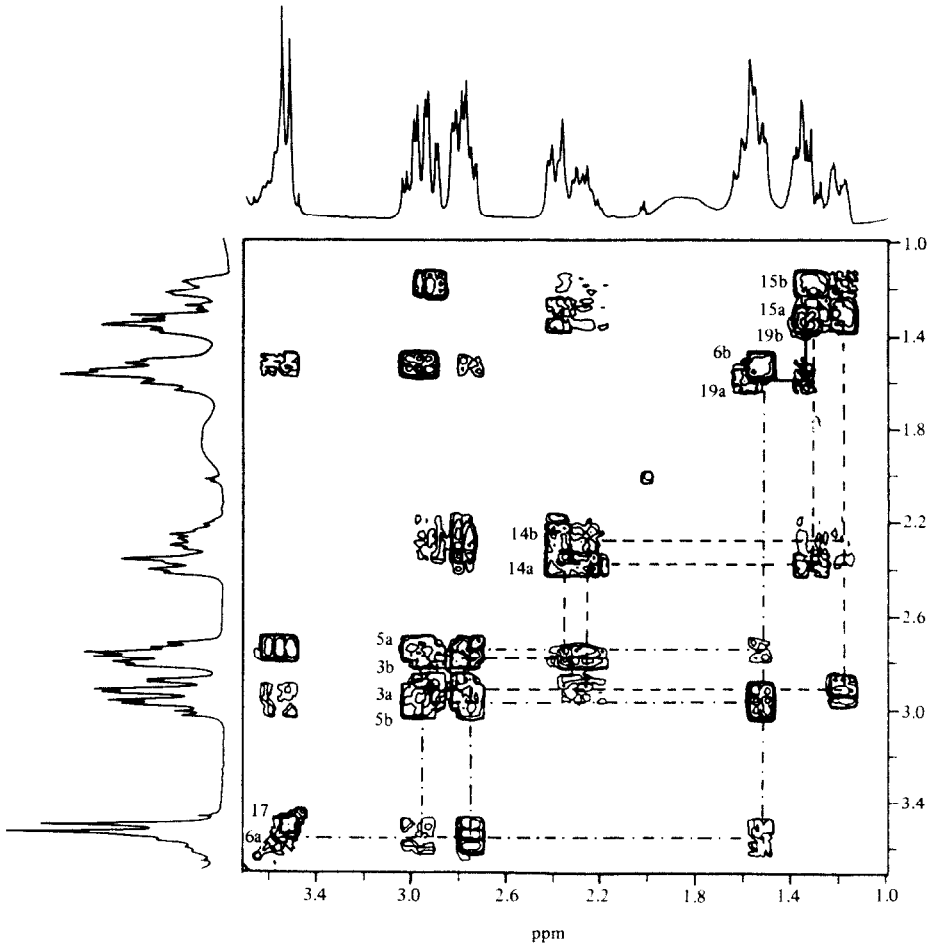


Fig. 1. Upfield region of the COSY spectra of 10-demethoxykopsidasinine (**1**); (a) recorded at 22°, (b) recorded at 55°.

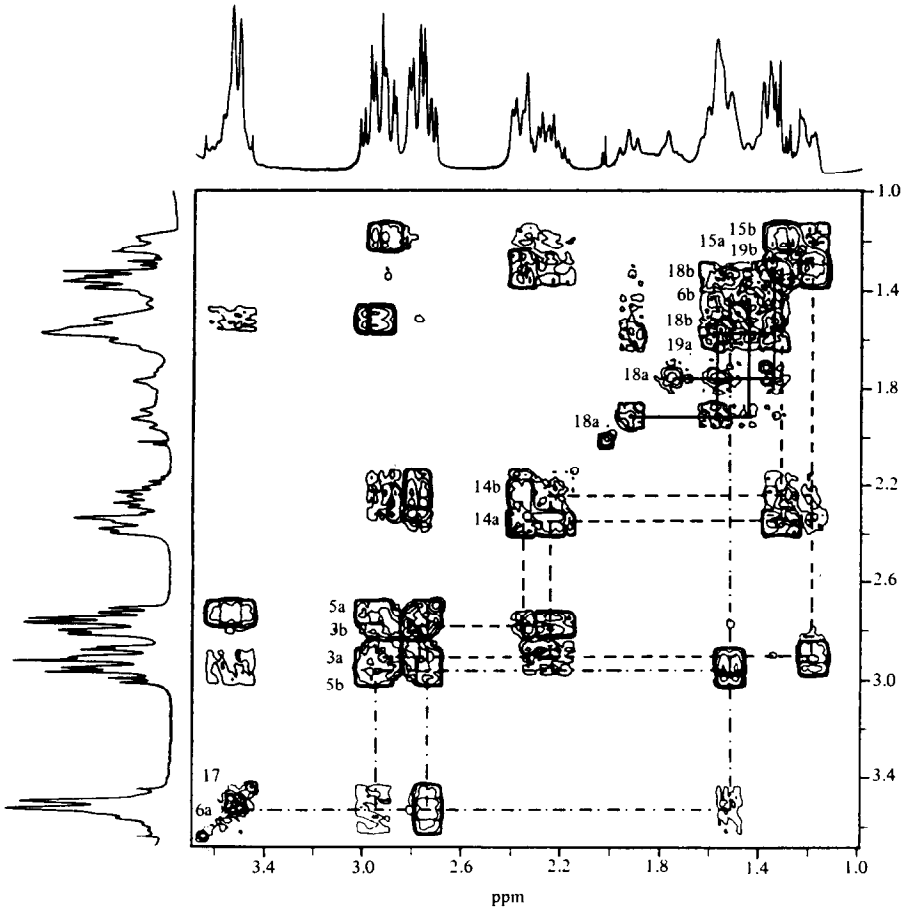


Fig. 1b.

function had therefore to be *endo* relative to the C-18–C-19 bridge. Molecular models revealed a very close spatial relationship between the ester carbonyl and H-6a, thereby explaining the unusually strong deshielding observed for that proton. Additional NOE's that could be observed are also indicated in Fig. 2.

The ^{13}C NMR spectral data were assigned through 1D heteronuclear correlation using the CSCM 1D pulse sequence [11]. Selective population transfer from both the upfield and downfield ^{13}C satellites in the ^{1}H NMR spectrum allowed for the unambiguous assignment of all of the protonated carbons (Table 2). Compared to the previously reported assignments of the closely related kopsidasinine [7], all of the interchangeable assignments have been clarified. The assignment of the C-3 and C-5 resonances had to be inverted, and the resonances of the aromatic carbons C-9, C-10 and C-11, all of them close in chemical shift, could be distinguished based on the well resolved ^{1}H–NMR spectrum. In a similar fashion, the four signals in the region 28–35 ppm were attributed to the aliphatic methylene carbons C-6, C-14, C-18 and C-19.

Based on this spectral evidence, compound **1** was identified as 10-demethoxykopsidasinine. To our knowledge it is only the second indole alkaloid known with this particular skeleton, and since the structure of kopsidasinine **2** was established essentially through chemical correlation with kopsidasine and pleicarpine, the present report represents the first detailed NMR spectral analysis of a compound in this series.

EXPERIMENTAL

General. Mps: uncorr. NMR spectra were measured at 299.94 MHz for proton and at 75.00 MHz for carbon. ^{1}H–^{1}H coupling constants were extracted from a resolution enhanced high resolution spectrum or from the NOE spectra. COSY and NOE spectra were recorded at 22° and 55°. Exponential multiplication (line broadening 0–1 Hz) was applied to the difference FID prior to Fourier transformation. Typically, 200–600 transients were acquired with a presaturation of 3 sec. ^{13}C NMR spectra were recorded on a Nicolet NMC 1280 spectrometer operating at 360.07 MHz for proton and at 90.80 MHz for carbon. CSCM 1D experiments were performed according to [11]: ^{13}C; 32K datapoints, 300–600 acquisitions; 32 × 180° ^{13}C presaturation pulses of 18 sec, spaced by a 44 msec delay (2.2 × ^{1}H 180° pulse). ^{1}H; 180° soft pulse = 20 msec; average $^{1}J_{CH}$ values of 160 Hz for aromatic CH, 150 Hz for nonaromatic CH, and of 130 Hz for CH_2 and CH_3 were used.

Plant material. The leaves of *Kopsia jasminiflora* Pitard were collected from the medicinal plant garden, Faculty of Pharma-

Table 1. ^{1}H NMR spectral data of 10-demethoxykopsidasinine (1)

H	Chemical shift				Coupling constants (Hz)	
	22°		55°			
3a	2.92	*ddd*	2.94	*ddd*	5a, 6b	1.4
3b	2.79	*dd*	2.78	*dd*	5a, 5b	13.0
5a	2.75	*ddd*	2.72	*ddd*	5a, 6a	6.1
5b	2.97	*ddd*	2.96	*ddd*	6a, 6b	13.7
6a	3.54	*ddd*	3.56	*ddd*	6a, 5b	13.0
6b	1.53	*dd*	1.53	*dd*	5b, 6b	5.4
9	7.73	*br d*	7.73	*d*	3a, 3b	13.0
10	7.05	*dd*	7.03	*dd*	3a, 14a	5.2
	7.06	*dd*			3a, 14b	12.9
11	7.20	*dd*	7.21	*dd*	3b, 14a	1.5
	7.24	*dd*			3b, 14b	5.0
12	7.91	*br d*	—†		3b, 15b	1.5
	7.48	*br d*	—†		14a, 15a	5.2
14a	2.39	*dddd*	2.39	*dddd*	14a, 15b	2.0
14b	2.28	*ddddd*	2.28	*ddddd*	14b, 15a	12.0
15a	1.33	*ddd*	1.33	*ddd*	14b, 15b	5.0
15b	1.21	*dddd*	1.19	*dddd*	14a, 14b	12.9
16	4.10	*d*	—†		15a, 15b	13.2
	3.89	*d*	—†		9, 10	7.6
17	3.52	*d*	3.52	*d*	10, 11	7.5
18a	1.95	*ddd*	—†		11, 12	7.5
	1.78	*ddd*	—†		16, 17	9.4
18b	1.43	*dd*	—†		18a, 18b	10.0
18b	1.43	*dd*	—†		18a, 18b	10.0
19a	1.58	*br dd*	1.61	*dd*	18a, 19a	8.8
19b	1.35	*dd*	1.35	*dd*	18b, 19b	3.0
16-CO_2Me	3.74	*s*	3.74	*s*	19a, 19b	10.0
NCO_2Me	3.74	*br s*	3.81	*br s*		
	3.85	*br s*				

*Obtained at 300 MHz, in $CDCl_3$.
†Extremely broadened signals.

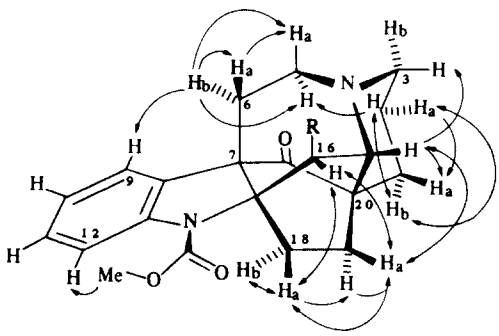


Fig. 2. NOE's observed for 10-demethoxykopsidasinine (1).

ceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Authentication was achieved by comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. A voucher specimen is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction and isolation. The air-dried and powdered leaves (700 g) of *K. jasminiflora* were exhaustively extracted with 95% EtOH at room temp. The EtOH extracts were pooled, the alcohol removed *in vacuo*. The syrupy residue (120 g) was acidified with 2% tartaric acid to pH 2.7, filtered and partitioned against EtOAc. The aqueous phase was rendered alkaline (pH 9) by the addition of $NaHCO_3$. Partitioning with EtOAc yielded a crude alkaloid fraction (4.6 g) which was chromatographed over silica gel. Elution with $CHCl_3$ and increasing amounts of MeOH yielded 10 fractions. Fraction 10 (629 mg) was further purified over silica gel with C_6H_6–EtOAc (3:7) as eluent. Crude **1** was crystallized from EtOH.

10-Demethoxykopsidasinine (**1**). Colourless prisms, 60 mg, 0.0086%, mp 194–196°, $[\alpha]_D^{20}$ -159° (MeOH, *c* 1.00); UV λ_{max} (MeOH) nm (ε): 208 (26, 800), 243 (13, 600), 280 (2, 200), 287 (2, 000); IR ν_{max}^{KBr} cm^{-1}: 2800, 2720, 1747, 1727, 1697, 1585, 1570, 1481, 1463, 1444, 1365, 1359, 1197, 1169, 740; EIMS *m/z* (rel. int.) 410 (47, M^+), 379 (37), 378 (100), 351, 350 (16), 323 (8), 322 (7), 215 (19), 201 (10), 182 (8), 180 (8), 168 (9), 167 (8), 156 (9), 154 (9), 122 (15), 109 (14); HR EIMS *m/z* found 410.1842 (M^+), calcd $C_{23}H_{26}N_2O_5$ 410.1841; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

Table 2. ^{13}C NMR spectral assignments of 10-demethoxykopsidasinine (1)*

C	Chemical shift		C	Chemical shift	
2	69.95	*s*	14	29.10	*t*
	69.76	*s*			
3	54.40	*t*	15	14.83	*t*
5	44.41	*t*	16	49.98	*d*
				49.09	*d*
6	34.18	*t*	17	66.90	*d*
				66.81	*d*
7	62.11	*s*	18	29.36	*t*
	61.58	*s*		28.15	*t*
8	131.50	*s*	19	29.04	*t*
	131.26	*s*			
9	125.29	*d*	20	47.85	*s*
	124.88	*d*		47.77	*s*
10	123.49	*d*	21	213.90	*s*
	123.32	*d*		213.53	*s*
11	128.26	*d*	22	170.95	*s*
	128.07	*d*		170.74	*s*
12	115.30	*d*	$N\underline{C}O_2Me$	153.84	*s*
	115.27	*d*		152.69	*s*
13	141.38	*s*	$NCO_2\underline{Me}$	52.70	*q*
	140.36	*s*		52.05	*q*
			$CO_2\underline{Me}$	51.76	*q*

*Obtained at 90.8 MHz, in $CDCl_3$.

Acknowledgements—This work was supported, in part, by a grant from the Division of Cancer Treatment, National Cancer Institute. We thank the Research Resources Center of the University of Illinois at Chicago for the provision of spectroscopic facilities.

REFERENCES

1. Hamburger, M.O., Cordell, G.A., Ruangrungsi, N. and Tantivatana, P. (1988) *J. Org. Chem.* (in press).
2. Engler, A. (1964) *Syllabus der Pflanzen-familien* 12th Edn, Vol. 2, p. 413. Gebr. Borntrager, Berlin.
3. Feng, X. Z., Kan, C., Husson, H. -P., Potier, P., Kan, S.-K., and Lounasmaa, M. (1984) *J. Nat. Prod.* **47**, 117.
4. Feng, X. Z., Kan, C., Potier, P., Kaḥ, S. -K., and Lounasmaa, M. (1983) *Planta Med.* **48**, 280.
5. Zhu, J., Guggisberg, A. and Hesse, M. (1986) *Planta Med.* **51**, 63.
6. Kan-Fan, C., Sevenet, T., Husson, H. -P. and Chan, K. C. (1985) *J. Nat. Prod.* **48**, 124.
7. Homberger, K. and Hesse, M. (1982) *Helv. Chim. Acta* **65**, 2548.
8. Guggisberg, A., Hesse, M., von Philipsborn, W., Nagarajan, K. and Schmid, H. (1966) *Helv. Chim. Acta* **49**, 2321.
9. Wei-shin, C., Sao-han, L., Kirfel, A., Will, G. and Breitmaier, E. (1981) *Liebigs Ann. Chem.* 1886.
10. Ruangrungsi, N., Likhitwitayawuid, K., Jongbunprasert, V., Ponglux, D., Aimi, N., Ogata, K., Yasuaoka, M., Haginiwa, J. and Sakai, S. (1987) *Tetrahedron Letters* **28**, 3679.
11. Sarkar, S. K. and Bax, A. (1985) *J. Magn. Reson.* **62**, 109.