Pergamon

PII: S0031–9422(97)00349–X

Phytochemistry, Vol. 46, No. 4, pp. 789–792, 1997

Printed in Great Britain
0031–9422/97 $17.00+0.00

ASPIDOFRACTININE ALKALOIDS FROM *KOPSIA TEOI*

TOH-SEOK KAM*, K. YOGANATHAN and SHIUEH-LIAN MOK†

Department of Chemistry and †Department of Pharmacology University of Malaya, 59100 Kuala Lumpur, Malaysia

(*Received in revised form* 14 *March* 1997)

Key Word Index—*Kopsia species*; Apocynaceae; leaves; aspidofractinine-type indole alkaloids.

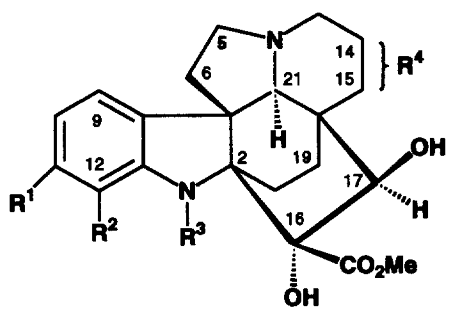
Abstract—Four new alkaloids of the aspidofractinine-type, *viz*., 11-hydroxykopsingine, 11-methoxykopsingine, 11-methoxy-12-hydroxy-kopsinol and 14,15-β-epoxykopsingine, were isolated from the leaf extract of *Kopsia teoi*. © 1997 Elsevier Science Ltd

INTRODUCTION

Kopsia teoi L. Allorge is one of *ca* 17 *Kopsia* species that occur in Malaysia [1, 2]. The chemistry of the Malaysia representatives of this genus has received considerable attention in recent years, which has resulted in the discovery of many novel structures, as well as useful bioactivities [3–5]. We have previously reported the presence of new aspidofractinine-type alkaloids from the stem-bark extract of *Kopsia teoi*. We now wish to report the presence of four new aspidofractinines from the leaf extract of the same species. A preliminary examination of the alkaloidal fraction has shown antihypertensive activity when tested in rats [6].

RESULTS AND DISCUSSION

The ethanol extract of the stem-bark of *Kopsia teoi* furnished the following alkaloids [7, 8]: kopsingine (**1**) [7, 9], kopsaporine (**2**) [7, 9], 11,12-methylenedioxykopsaporine (**3**) [10], kopsinol (**4**), kopsinganol (**5**) kopsinginine (**6**), 17-α-hydroxy-$\Delta^{14,15}$-kopsinine (**7**), kopsinginol (**8**), rhazinilam (**9**) [11, 12], rhazimol (deacetylakuammiline) (**10**) [13], 16-*epi*-deacetylakuammiline (*epi*-rhazimol) (**11**) [14], akuammiline (**12**) [15], lonicerine (**13**) [16] and aspidodasycarpine (**14**) [17]. Two separate extractions were carried out on the leaves. In the first investigation, the following alkaloids were isolated: **1**, **5**, kopsidine A (**15**), kopsidine B (**16**), kopsinitarine A (**19**), kopsinitarine B (**20**), kopsinitarine C (**21**), mersingine A (**23**) and mersingine B (**24**) [18, 19]. In the second study, involving a different collection of plant material, the following alkaloids were obtained [20–22]: **1**, **5**, **15**, **16**, **17** (kopsidine C), **18** (kopsidine D), **19**,



1 R^1 = H, R^2 = OMe, R^3 = CO_2Me, R^4 = $\Delta^{14,15}$

4 R^1 = R^2 = R^3 = H, R^4 = $\Delta^{14,15}$

26 R^1 = OH, R^2 = OMe, R^3 = CO_2Me, R^4 = $\Delta^{14,15}$

27 R^1 = R^2 = OMe, R^3 = CO_2Me, R^4 = $\Delta^{14,15}$

28 R^1 = OMe, R^2 = OH, R^3 = H, R^4 = $\Delta^{14,15}$

29 R^1 = H, R^2 = OMe, R^3 = CO_2Me, R^4 = β–O

20, **22** (kopsinitarine D), **23**, the bisindole, nitaphylline (**25**), 11-hydroxykopsingine (**26**), 11-methoxykopsingine (**27**), 11-methoxy-12-hydroxy-kopsinol (**28**) and 14,15-β-epoxykopsingine (**29**). The last four are new alkaloids. The results also show a seasonal variation of the alkaloidal composition for this species [10], a feature which we have also documented previously in the case of *Uncaria callophylla* [23].

Compound **26**, showed a $[M]^+$ at m/z 472 ($C_{24}H_{28}N_2O_8$). The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) were generally similar to those of **1** [7], except for the following changes. Only two aromatic hydrogens are present which are deduced to be in an *ortho*-arrangement from their appearance as a pair of AB-doublets, indicating additional aromatic sub-

* Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for compounds **26–29** (270 MHz, $CDCl_3$)*

H	26	27	28	29
3	3.32 *br d* (16)	3.18 *dt* (15.5, 2)	3.15 *br d* (16)	2.94 *br d* (12.5)
	3.47 *ddd* (16, 4.5, 2)	3.48 *ddd* (15.5, 4.5, 2)	3.47 *ddd* (16, 4.5, 2)	3.47 *dd*, (12.5, 5)
5	2.63 *ddd* (12, 8.5, 4)	2.63 *ddd* (12, 8.5, 4)	2.58 *ddd* (12, 8.5, 4)	2.47 *ddd* (13, 8.5, 4)
	2.96 *dd* (8.5, 6)	2.91 *dd* (8.5, 6)	2.90 *dd* (8.5, 6)	2.77 *dd* (8.5, 6)
6	1.69 *dd* (13, 4)	1.67 *dd* (13, 4)	1.61 *dd* (13, 4)	1.61 *dd* (13, 4)
	3.13 *m*	3.16 *m*	3.12 *m*	3.08 *td* (13, 6)
9	6.76 *d* (8)	6.59 *d* (8)	6.59 *d* (8)	6.71 *d* (7)
10	6.64 *d* (8)	6.64 *d* (8)	6.34 *d* (8)	7.01 *dd* (8, 7)
11	—	—	—	6.82 *d* (8)
14	5.97 *ddd* (10, 4.5, 2)	5.98 *ddd* (10, 4.5, 2)	5.95 *ddd* (10, 4.5, 2)	3.89 *dd* (5,4)
15	5.67 *m*	5.68 *dt* (10, 2)	5.66 *dt* (10, 2)	3.16 *d* (4)
17	*ca.* 3.80	*ca* 3.80	3.60 *br s*	3.99 *br s*
18	1.51 *m*	1.52 *m*	1.70 *m*	1.40 *m*
	2.11 *br t* (13)	2.00 *br t* (13)	1.82 *br t* (13)	2.60 *m*
19	1.16 *br t* (13)	1.17 *br t* (13)	1.20 *br t* (13)	1.43 *m*
	1.76 *m*	1.72 *m*	1.65 *m*	1.68 *m*
21	2.72 *d* (2)	2.75 *d* (2)	2.76 *d* (2)	2.84 *d* (2)
17-OH	8.16 *br s*	8.31 *br s*	8.24 *br s*	8.23 *br s*
16-OH	5.68 *br s*	—	—	5.79 *s*
11-OH	6.05 *br s*	—	—	—
NCO_2Me	3.82 *s*	3.83 *s*	—	3.79 *s*
CO_2Me	3.77 *s*	3.80 *s*	3.81 *s*	3.79 *s*
11-OMe	—	3.87 *s*	3.84 *s*	—
12-OMe	3.60 *s*	3.87 *s*	—	3.82 *s*

* Assignments based on COSY, HETCOR and NOE.

Table 2. ^{13}C NMR spectral data for compounds **1–4** (67.5 MHz, $CDCl_3$)*

C	26	27	28	29
2	76.9	77.0	71.1	76.4
3	49.9	49.9	50.1	48.5
5	48.5	48.2	48.9	48.5
6	40.8	40.3	39.8	39.9
7	55.5	55.4	55.5	56.5
8	135.9	136.0	135.4	143.7
9	116.1	110.8	111.1	113.3
10	110.3	107.8	102.5	125.1
11	149.2	149.4	146.8	112.0
12	137.0	136.7	133.2	149.4
13	131.2	126.2	135.9	128.3
14	128.8	128.9	128.7	53.6
15	131.2	130.8	131.4	57.1
16	80.0	80.8	81.1	80.0
17	81.6	82.3	83.6	81.9
18	27.3	27.8	27.7	26.7
19	25.8	26.0	26.5	23.4
20	39.1	38.6	39.4	38.4
21	68.5	68.3	68.9	62.7
11-OMe	—	56.4	56.2	—
12-OMe	59.2	56.4	—	56.1
NCO_2Me	53.3	53.7	—	53.1
CO_2Me	52.0	51.9	51.9	52.0
$N\underline{C}O_2Me$	155.1	153.5	—	155.6
$\underline{C}O_2Me$	171.8	171.8	171.0	171.8

* Assignments based on HETCOR and HMBC.

stitution. There is a slight upfield shift involving the 12-OMe signal and the additional presence of a broad singlet (exchangeable with D_2O) attributable to OH, which must be on the aromatic ring. The observed NOE interaction between the doublet at δ 6.76 and H-21 (2.72) allows the assignment of this signal to H-9. The hydroxyl group must therefore be at C-11, which is further confirmed by the observed NOE interaction between H-10 and the phenolic 11-OH. Compound **26** is therefore 11-hydroxykopsingine, which is also in agreement with the ^{13}C NMR spectral data (Table 2), as well as the observed *m/z* 457 fragment in the mass spectrum due to loss of a methyl radical [24].

Compound **27** showed a $[M]^+$ at *m/z* 486 ($C_{25}H_{30}N_2O_8$), differing from **1** by 30 mu. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) showed that, as in compound **26**, there has been additional aromatic substitution by a methoxyl group at C-11. Compound **27** is therefore 11-methoxykopsingine.

Compound **28** showed a $[M]^+$ at *m/z* 414 ($C_{22}H_{26}N_2O_6$). The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) were generally similar to **4** (the N_1-decarbomethoxy derivative of (**2**) [8], except for the aromatic region, which indicated substitution by hydroxyl and methoxyl groups. The position of the aromatic methoxyl substituent is established to be at C-11 from NOE difference experiments, which showed that irradiation of H-10 resulted in enhancement of the aromatic methoxyl signal and *vice* *versa*. Compound **28** is therefore 11-methoxy-12-hydroxykopsinol.

Compound **29** showed a $[M]^+$ at m/z 472 ($C_{24}H_{28}N_2O_8$), differing from **1** by the addition, of an oxygen atom. The mass spectrum is also similar to that of **1**, except for the presence of an $[M-16]^+$ fragment, indicative of epoxide or *N*-oxide functions. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) indicate that **29** is similar to **1**, except for the 14,15 double bond, which is now absent, being replaced instead by signals due to an epoxide function (δ_H3.89, *dd*, $J = 5$, 4 Hz, 3.16, *d*, $J = 4$ Hz; δ_C 53.6, 57.1). The stereochemistry of the epoxide function is deduced to be β from the observed NOE interaction between H-21 and H-14α. Compound **29** is therefore 14,15-β-epoxykopsingine.

The observation of *W*-coupling between H-17α and H-21 in all four compounds confirm that the stereochemistry of the 17-OH is β in all cases. The configuration at the remaining stereocentres is assumed to be similar to that in **1**, as well as the other aspidofractinine compounds occurring in the species, from the similarity of the NMR spectral data and assuming a common biogenetic origin.

EXPERIMENTAL

Plant material. Details of collection, deposition of voucher specimens, etc., have been reported earlier [7].

Extraction and isolation. Extraction of alkaloids was carried out in the usual manner, as described in detail elsewhere [25]. Alkaloids were isolated by CC, prep. TLC and centrifugal TLC on silica gel. Solvent systems used for CC were $CHCl_3$–MeOH and Et_2O–EtOAc. Solvent systems used for prep. TLC and centrifugal TLC were Et_2O, Et_2O–hexane, Et_2O–EtOAc and MeOH–$CHCl_3$. The yields (g kg^{-1}) of the alkaloids from the stem extract were: **1** (1.7), **2** (0.08), **3** (0.027), **4** (0.005), **5** (0.268), **6** (0.0064), **7** (0.0096), **8** (0.003), **9** (0.017), **10** (0.013), **11** (0.014) **12** (0.011), **13** (0.066) and **14** (0.05). The yields (g kg^{-1}) of the alkaloids from the leaf extract (first extraction) were: **1** (6.7), **5** (0.1), **15** (0.035), **16** (0.026), **19** (0.004), **20** (0.003), **21** (0.003), **23** (0.007) and **24** (0.003). The yields of the alkaloids from the second extraction were: **1** (6), **5** (0.07), **15** (0.022), **16** (0.019), **17** (0.0015), **18** (0.0017), **19** (0.002), **20** (0.001), **22** (0.019), **23** (0.002), **25** (0.0475), **26** (0.0082), **27** (0.005), **28** (0.012) and **29** (0.0087).

11-Hydroxykopsingine (**26**). $[\alpha]_D + 28°$ ($CHCl_3$, *c* 0.33). UV (EtOH), λ_{max} (log ε): 220 (4.43), 252 (3.87), 289 (3.27). EIMS, m/z (rel. int.): 472 [M^+, $C_{24}H_{28}N_2O_8$] (100), 457 (12), 444 (15), 413 (10), 395 (15), 385 (18), 369 (20), 354 (30), 122 (12), 107 (15). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

11-Methoxykopsingine (**27**). $[\alpha]_D - 24°$ ($CHCl_3$, *c* 0.18). UV (EtOH), λ_{max} (log ε): 220 (4.37), 251 (3.77), 280 (3.08), 286 (3.05). EIMS, m/z (rel. int.): 486 [M^+, $C_{25}H_{30}N_2O_8$] (100), 472 (30), 457 (15), 428 (70), 414 (60), 399 (20), 386 (25), 368 (35), 353 (25), 309 (28), 122 (22), 107 (25). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

11-Methoxy-12-hydroxykopsinol (**28**) $[\alpha]_D + 7°$ ($CHCl_3$, *c* 0.26). UV (EtOH), λ_{max} (log ε): 214 (4.51), 248 (3.72), 282 (3.16). EIMS (probe) 70 eV, m/z (rel. int.): 414 [M^+, $C_{22}H_{26}N_2O_6$] (100), 386 (20), 355 (10), 326 (25), 297 (30), 122 (12), 107 (10), ^1H NMR and ^{13}C NMR: Tables 1 and 2.

14,15-β-Epoxykopsingine (**29**). $[\alpha]_D + 15°$ ($CHCl_3$, *c* 0.33). UV (EtOH) λ_{max} (log ε): 217 (4.34), 254 (3.83), 283 (3.15), 289 (3.14). EIMS (probe) 70 eV, m/z (rel int.): 472 [M^+, $C_{24}H_{28}N_2O_8$] (100), 456 (10), 444 (12), 413 (30), 385 (55), 355 (85), 122 (16), 107 (22). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

Acknowledgements—We thank the University of Malaya and IRPA for financial support of this work.

REFERENCES

1. Markgraf, F., *Blumea*, 1972, **20**, 416.
2. Sevenet, T., Allorge, L., David, B., Awang, K., Hadi, A. H. A., Kan-Fan, C., Quirion, J. C., Remy, F., Schaller, H. and Teo, L. E., *Journal of Ethnopharmacology*, 1994, **41**, 147.
3. Kam, T. S., Yoganathan, K., Koyano, T. and Komiyama, K., *Tetrahedron Letters*, 1996, **37**, 5765.
4. Kam, T. S., Yoganathan, K. and Chuah, C. H., *Tetrahedron Letters*, 1995, **36**, 759.
5. Awang, K., Sevenet, T., Hadi, A. H. A., David, B. and Pais, M., *Tetrahedron Letters*, 1992, **33**, 2493.
6. Mok, S. L. unpublished observations.
7. Kam, T. S., Yoganathan, K., Chuah, C. H. and Chen Wei., *Phytochemistry*, 1993, **32**, 1343.
8. Kam, T. S. and Yoganathan, K., *Phytochemistry*, 1996, **42**, 539.
9. Thomas, D. W., Bienmann, K., Kiang, A. K. and Amarasingham, R. D., *Journal of the American Chemical Society*, 1967, **89**, 3235.
10. Varea, T., Kan, C., Remy, F., Sevenet, T., Quirion, J. C., Husson, H. P. and Hadi, H. A., *Journal of Natural Products*, 1993, **56**, 2166.
11. Banerji, A., Majumder, P. L. and Chatterjee, A., *Phytochemistry*, 1970, **9**, 1491.
12. Goh, S. H., Ali, A. R. M. and Wong, W. H., *Tetrahedron*, 1989, **45**, 7899.
13. Akinloye, B. A. and Court, W. E., *Phytochemistry*, 1980, **19**, 2741.
14. Kan, C., Deverre, J. R., Sevenet, T., Quirion, J. C. and Husson, H. P., *Natural Product Letters*, 1995, **7**, 275.
15. Dugan, J. J., Hesse, M., Renner, U. and Schmid, H., *Helvetia Chimica Acta*, 1969, **52**, 701.
16. Naranjo, J., Hesse, M. and Schimd, H., *Helvetia Chimica Acta*, 1970, **53**, 749.
17. Joule, J. A., Ohashi, M., Gilbert, B. and Djerassi, C., *Tetrahedron*, 1965, **21**, 1717.

18. Kam, T. S., Yoganathan, K. and Chuah, C. H., *Tetrahedron Letters*, 1993, **34**, 1819.
19. Kam, T. S., Yoganathan, K. and Chuah, C. H., *Tetrahedron Letters*, 1994, **35**, 4447.
20. Kam, T. S., Yoganathan, K. and Chuah, C. H., *Phytochemistry*, 1997, **45**, 623.
21. Kam, T. S., Yoganathan, K. and Chen Wei, *Journal of Natural Products*, 1996, **59**, 1109.
22. Kam, T. S. and Yoganathan, K., *Natural Products Letters*, 1997, **10**, 69.
23. Kam, T. S., Lee, K. H. and Goh, S. H., *Phytochemistry*, 1992, **31**, 2031.
24. Zheng, J. J., Zhou, Y. L. and Huang, Z. H., *Acta Chimica Sinica*, English Edition, 1989, **2**, 168.
25. Kam, T. S. and Tan, P. S., *Phytochemistry*, 1990, **29**, 2321.