Henrycinols A and B, Two Novel Indole Alkaloids Isolated from *Melodinus henryi* CRAIB

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Henrycinols A (**1**) and B (**2**), two novel indole alkaloids, together with three known compounds, (+)-Δ^{14}-vincamine (**3**), (+)-16-epi-Δ^{14}-vincamine (**4**), and (+)-isoeburnamine (**5**), were isolated from the roots of *Melodinus henryi* CRAIB. Their structures were established on the basis of 1D- and 2D-NMR spectroscopic analysis. The relative configuration of henrycinols A and B was determined by NOESY analysis.

1. Introduction. – The family Apocynaceae comprises several species distributed throughout the tropical regions of China. *Melodinus henryi* CRAIB is one species of Apocynaceae existing in China, and its fruits are used in traditional medicine for the treatment of children meningitis and bone fracture [1]. Phytochemical studies on the fruits of *M. henryi* led to the isolation of indole alkaloids [1–3]. However, further studies on the roots of *M. henryi* have not yet been reported. Recently, our phytochemical investigation of the roots of *M. henryi* has led to the isolation of two new indole alkaloids henrycinol A (**1**) and B (**2**), named according to the plant name, together with three known alkaloids (+)-Δ^{14}-vincamine (**3**), (+)-16-epi-Δ^{14}-vincamine (**4**), and (+)-isoeburnamine (**5**). Herein, we report the isolation and structure elucidation of these two new compounds.

2. Results and Discussion. – Compound **1** was isolated as a white amorphous solid in a very small amount. The molecular formula was determined to be $C_{27}H_{28}N_2O_4$ by HR-FAB-MS analysis of the protonated-molecule peak $[M+H]^+$ at m/z 445.2121 ($\Delta=+0.3$ amu) in addition to ^{13}C-NMR. The UV spectrum showed absorptions at 232, 278, and 290 nm, typical of an indolic chromophore. The IR spectrum showed absorption bands at 3500, 3030, 1735, and 1620 cm^{-1}, indicating the presence of OH, aromatic, ester, and olefinic moieties. Compared to the ^{1}H-NMR data of the known indole alkaloid Δ^{14}-vincamine (**3**), the ^{1}H-NMR spectrum of **1** showed five aromatic H-atoms at δ 7.36 (*m*, 3 H) and 7.45 ppm (*m*, 2 H), in addition to the four aromatic H-atoms of the vincamine's A-ring at δ 7.51 (*d*), 7.14 (*t*), 7.21 (*m*), and 7.28 ppm (*d*) (*Table*). A coupling constant of $J=16.2$ Hz between the H-atom at δ 6.45 (*d*) and another H-atom

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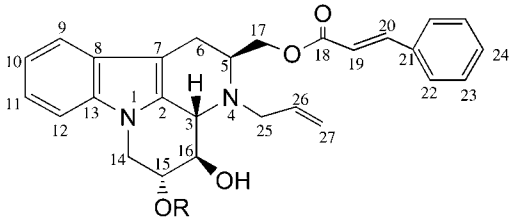
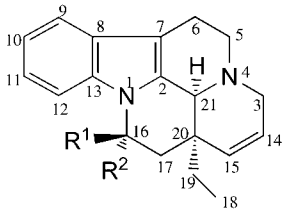
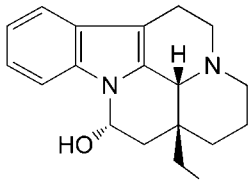


1 R=H, Henrycinol A
2 R=Me_2CHCO, Henrycinol B



3 R^1=OH, R^2=MeOCO
4 R^1=MeOCO, R^2=OH



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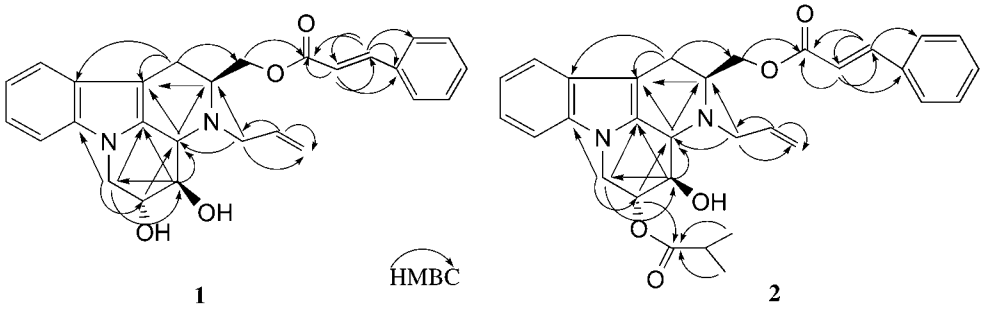
at δ 7.61 ppm (*d*) typical for an (*E*)-configured double bond, and two C=C bond C-atom resonances at 117.74 and 145.31 ppm were observed in ^{13}C-NMR, indicating the presence of a cinnamoyl group, which was further confirmed by HMBC (*Fig. 1*) correlations of H–C(19) to C(18), C(20), and C(21), and H–C(20) to C(18), C(19), C(21), and C(22). Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of **1**, along with its ^{1}H,^{1}H-COSY and HMQC, revealed two high-field H-atoms at δ 2.95 (*dd*, $J = 16.2$, 4.8 Hz) and 2.65 ppm (*d*, $J = 16.2$ Hz) assigned to C(6), one H-atom at δ 3.71 ppm (*m*) assigned to C(5), two H-atoms at δ 4.25 (*dd*, $J = 10.8$, 7.8 Hz) and 4.67 ppm (*dd*, $J =$ 10.8, 6.0 Hz) assigned to C(17), two H-atoms at δ 3.62 (*t*, $J = 10.8$ Hz) and 4.56 ppm (*dd*, $J = 10.8$, 6.0 Hz) assigned to C(14), as well as five H-atoms at δ 3.07 (*dd*, $J = 13.8$, 8.4 Hz), 3.52 (br. *d*), 6.10 (*m*), 5.20 (*d*, $J = 10.2$ Hz), and 5.28 ppm (*d*, $J = 17.4$ Hz) assigned to C(25), C(26), and C(27), respectively, of the allyl group connected at N(4). These assignments were evidenced by their HMBC correlations (*Fig. 1*).



1 **2**

Fig. 1. *The key ^{1}H,^{13}C long-range correlations* (HMBC) *of* **1** *and* **2**

Table 1. *^{1}H- and ^{13}C-NMR, and 2D-NMR Data of Henrycinol A* (**1**) (500 MHz, $CDCl_3$, δ in ppm)

Position	^{1}H-NMR	J [Hz]	^{1}H,^{1}H COSY	^{13}C-NMR	HMQC
N(1)	–	–	–	–	–
2				130.07	
3	4.20 (*d*)	9.6	H–C(16)	55.30	H–C(3)
N(4)	–	–	–	–	–
5	3.71 (*m*)		H_β–C(6), H_a–C(17), H_b–C(17)	54.56	H–C(5)
6α	2.95 (*dd*)	16.2, 4.8	H–C(5), H_α–C(6)	18.18	H–C(6), H_β–C(6)
6β	2.65 (*d*)	16.2	H_α–C(6)		
7				104.45	
8				128.87	
9	7.51 (*d*)	7.2	H–C(10)	118.45	H–C(9)
10	7.14 (*t*)	7.2	H–C(9), H–C(11)	119.75	H–C(10)
11	7.21 (*m*)		H–C(9), H–C(10), H–C(12)	121.65	H–C(11)
12	7.28 (*d*)	7.8	H–C(11)	109.66	H–C(12)
13				136.64	
14α	3.62 (*t*)	10.8	H_β–C(14), H–C(15)	46.88	H_α–C(14), H_β–C(14)
14β	4.56 (*dd*)	10.8, 6.0	H_α–C(14), H–C(15)		
15	4.29 (*ddd*)	10.8, 9.6, 6.0	H_α–C(14), H_β–C(14), H–C(16)	70.86	H–C(15)
15-OH	4.75 (br. *s*)				
16	3.92 (*t*)	9.6	H–C(3), H–C(15)	71.82	H–C(16)
16-OH	1.76 (*s*)				
17a	4.67 (*dd*)	10.8, 6.0	H–C(5), H_b–C(17)	65.55	H_a–C(17), H_b–C(17)
17b	4.25 (*dd*)	10.8, 7.8	H–C(5), H_a–C(17)		
18 (C=O)				166.87	
19	6.45 (*d*)	16.2	H–C(20)	117.74	H–C(19)
20	7.61 (*d*)	16.2	H–C(19)	145.31	H–C(20)
21				134.24	
22	7.45 (*m*)		H–C(23), H–C(24)	128.11 (C_o)	H–C(22)
23/24	7.36 (*m*)		H–C(22)	128.87 (C_m)	H–C(23)
				130.37 (C_p)	H–C(24)
25a	3.52 (br. *d*)	13.8	H_b–C(25), H_a–C(25), H–C(26)	51.08	H_a–C(25), H_b–C(25)
25b	3.07 (*dd*)	13.8, 8.4	H_a–C(25), H–C(26)		
26	6.10 (*m*)		H_a–C(25), (*E*/*Z*)-H–C(27)	136.12	H–C(26)
27 (*Z*)	5.20 (*d*)	10.2	H–C(26)	118.09	(*E*/*Z*)-H–C(27)
27 (*E*)	5.28 (*d*)	17.4	H–C(26)		

The relative configuration of compound **1** was deduced by NOESY studies (*Fig. 2*). A *cis*-relationship (α-orientation) of H–C(3), H–C(15), and CH_2(17) was established by the NOE correlations between H–C(3) and H–C(15), and H–C(3) and CH_2(17). Similarly, the NOE correlations between H–C(5) and H_β–C(6), and H–C(16) and H_β–C(14) indicated a *cis*-relationship (β-orientation) of H–C(5) to H_β–C(6), and H–C(16) to H_β–C(14), respectively.

Compound **2** was obtained as a white amorphous powder. In comparison to the ^{1}H- and ^{13}C-NMR data of **1**, those of **2** indicated an additional isobutyryl group, which was substantiated by the MS of **2** with the molecular ion at *m*/*z* 514. HR-FAB-MS gave a molecular formula of $C_{31}H_{34}N_2O_5$ ($[M + H^+]$ at *m*/*z* at 515.2537). A multiple *singlet* at δ

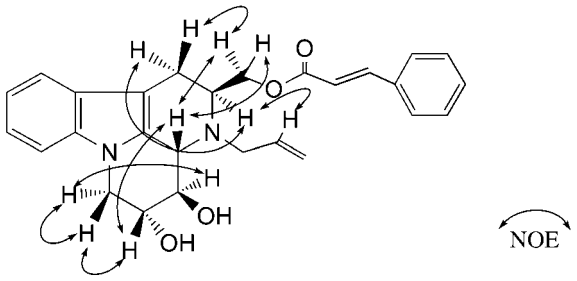


Fig. 2. *The relative configuration of* **1** *deduced from NOESY data* (arrows)

2.65 and two respective double *singlets* at δ 1.22 and 1.20 ppm were assigned to the isobutyryl group by HMBC correlations of a H-atom at δ 2.65 ppm to two Me moieties (δ(C) 18.99 (*q*) and 18.90 ppm (*q*)). These signals, together with HMBC correlations of these two Me moieties and H–C(15) (δ 5.40 ppm (*td*)) to a new C=O group resonance at δ(C) 176.75 ppm, indicated that the isobutyryloxy group was at C(15). Therefore, **2** was determined to be 15-isobutyrylhenrycinol A.

Besides henrycinols A and B, the other three compounds isolated were established to be alkaloids (+)-Δ^{14}-vincamine (**3**) [3][4], (+)-16-epi-Δ^{14}-vincamine (**4**) [3][4], and (+)-Isoeburnamine (**5**) [3–7], respectively.

Experimental Part

General. Column chromatography (CC): *Merck* silica gel *60* (100–200 mesh). Prep. TLC: *Merck* silica gel *60 F254* plates (0.8-mm thickness), detection under UV light or by spraying with 10% H_2SO_4 and then by heating on a hot plate. Optical rotations: *Horiba SEPA-300* digital polarimeter. IR and UV Spectra: *Jasco IR-810* and *Shimadzu UV-1600* spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR, and 2D-NMR: *Varian Unity INOVA-500* or *INOVA-600* spectrometers, in $CDCl_3$ with TMS as an internal standard; chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and in Hz, resp. MS and HR-MS: *Jeol JMS-700* spectrometer by EI or FAB method.

Plant Material. The roots of *Melodinus henryi* CRAIB were collected in the Yunnan province of China in 1999, and a voucher specimen has been deposited in the Tianjin Medical University in China.

Extraction and Isolation. The dried root powder (1.0 kg) was extracted with 95% EtOH under reflux. The EtOH extracts were concentrated, and the residue was treated with 5% AcOH, followed by neutralization with NH_4OH to result in pH 9. The aq. phase was extracted with $CHCl_3$ (3×), and the org. phase was washed with H_2O. $CHCl_3$ was removed under reduced pressure, and the residue was applied to a column of silica gel eluted with petroleum ether/AcOEt (2:1, 1:1, 1:2, and 1:4 (*v*/*v*)) to give four fractions. The second fraction was further subjected to repeated CC and prep. TLC with $CHCl_3$/AcOEt (1:1 (*v*/*v*)) to give compounds **2**–**5**. The third fraction was subjected to repeated CC with $CHCl_3$/AcOEt (1:1 (*v*/*v*)) and prep. TLC with $CHCl_3$/AcOEt (1.2 (*v*/*v*)) finally to afford compound **1** as a white amorphous solid.

Henrycinol A (**1**): 11 mg/kg. $[\alpha]_D^{20} = -43.7$ ($c = 0.4$, $CHCl_3$). UV (EtOH): λ_{max} 232 (2.6), 278 (2.4), 280 (2.8), 290 (2.3). IR (film): 3500, 3030, 2980, 2870, 1735, 1670, 1620, 1465, 1430, 1210, 750. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 444 (9, M^+), 429 (7), 426 (12), 415 (23), 411 (3), 383 (5), 367 (17), 340 (8), 326 (42), 290 (35), 284 (21), 267 (6), 265 (7), 253 (32), 229 (67), 197 (57), 180 (100), 167 (4), 140 (13), 122 (40), 118 (22). HR-FAB-MS: 445.2121 ($C_{27}H_{29}N_2O_4$; calc. 445.2126).

Henrycinol B (**2**): 7 mg/kg. $[\alpha]_D^{20} = -60.7$ ($c = 0.7$, $CHCl_3$). UV (EtOH): λ_{max} 232 (2.4), 278 (2.9), 290 (2.6). IR (film): 3450, 3010, 2980, 2920, 2870, 1740, 1725, 1665, 1620, 1465, 1430, 1340, 1210, 1060, 765. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.62 (*d*, $J = 16$, H–C(20)); 7.52 (*d*, $J = 7.5$, H–C(9)); 7.49–7.46 (*m*, 2 H, Ph); 7.39–7.37 (*m*, 3 H, Ph); 7.26 (*d*, $J = 8.0$, H–C(12)); 7.21 (*td*, $J = 8.0$, 1.0, H–C(11)); 7.14 (*td*, $J = 8.0$, 1.0, H–C(10)); 6.46 (*d*, $J = 16$, H–C(19)); 5.98 (*dddd*, $J = 17.5$, 10.5, 7.5, 5.0, H–C(26)); 5.40 (*td*, $J = 9.0$, 6.0, H–C(15)); 5.29

(*dd*, *J* = 17.5, 1.0, (*E*)-H–C(27)); 5.19 (*dd*, *J* = 10.5, 1.0, (*Z*)-H–C(27)); 4.59 (*dd*, *J* = 11.5, 6.0, H_α–C(14)); 4.53 (*dd*, *J* = 11.0, 6.5, H_α–C(17)); 4.30 (*dd*, *J* = 11.0, 7.5, H_β–C(17)); 4.23 (br. *dt*, H–C(3)); 4.04 (*t*, *J* = 9.0, H–C(16)); 3.71 (*m*, H–C(5)); 3.65 (*dd*, *J* = 11.5, 9.0, H_β–C(14)); 3.52 (*m*, H_b–C(25)); 3.21 (*dd*, *J* = 13.5, 7.5, H_a–C(25)); 2.96 (*ddd*, *J* = 16.0, 6.0, 2.0, H_a–C(6)); 2.67 (br. *s*, OH); 2.65 (*m*, Me_2CH); 2.63 (*dd*, *J* = 16.0, 2.0, H_β–C(6)); 1.22, 1.20 (2*d*, *J* = 7, 2 Me). ^{13}C-NMR (125 MHz, $CDCl_3$): 176.75 (C=O); 166.89 (C(18)); 145.15 (C(20)); 136.84 (C(13)); 136.48 (C(26)); 134.27 (C(21)); 130.31 (C(24)); 128.84 (C(23)); 128.24 (C(2)); 128.11 (C(22)); 128.10 (C(8)); 121.67 (C(11)); 119.86 (C(10)); 118.52 (C(9)); 117.78 (C(27)); 117.53 (C(19)); 109.48 (C(12)); 105.38 (C(7)); 72.30 (C(16)); 70.26 (C(15)); 64.77 (C(17)); 55.80 (C(3)); 54.47 (C(5)); 51.39 (C(25)); 44.71 (C(14)); 33.96 (*d*, Me_2CH); 18.99 (*q*, Me_2CH), 18.90 (*q*, Me_2CH); 18.82 (C(6)). EI-MS: 514 (3, M^+), 513 (5), 537 (3), 528 (11), 497 (13), 489 (6), 465 (34), 448 (21), 431 (4), 389 (6), 357 (31), 331 (23), 315 (2), 289 (8), 284 (9), 250 (34), 235 (47), 221 (14), 180 (100), 171 (56), 144 (25), 117 (17). HR-FAB-MS: 515.2537 ($[M+1]^+$, $C_{31}H_{35}N_2O_5$; calc. 515.2544).

(+)-Δ^{14}-Vincamine (**3**): 23 mg/kg. $[\alpha]_D^{20} = +114.7$ (*c* = 0.9, $CHCl_3$). UV (EtOH): λ_{max} 232 (2.6), 278 (2.8), 290 (2.1). IR (KBr): 3430, 3040, 2950, 2924, 1740, 1610, 1450, 1210, 745. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.45 (*d*, *J* = 7.8, 1 H); 7.19 (*d*, *J* = 8.1, 1 H); 7.15 (*m*, 1 H); 7.13 (*m*, 1 H); 5.80 (*d*, *J* = 10, 1 H); 5.61 (*m*, 1 H); 4.09 (*s*, 1 H); 3.87 (*s*, 3 H); 3.20–3.27 (*m*, 2 H); 3.10–3.15 (*m*, 2 H); 2.91 (*m*, 1 H); 2.48 (*m*, 1 H); 2.37 (*d*, *J* = 15.1, 1 H); 2.30 (*d*, *J* = 15.1, 1 H); 1.92 (*m*, 1 H); 1.72 (*s*, OH); 1.61 (*m*, 1 H); 0.97 (*t*, *J* = 7.4, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 168.95; 134.56; 128.41; 127.42; 126.98; 126.78; 120.10; 119.03; 117.41; 112.21; 104.56; 76.57; 58.07; 51.01; 50.99; 43.87; 42.65; 37.61; 33.52; 16.78; 8.49. EI-MS: 352 (3, M^+), 337 (7), 323 (9), 319 (3), 305 (23), 293 (2), 284 (11), 263 (45), 250 (42), 249 (41), 235 (23), 224 (15), 221 (11), 206 (3), 180 (100), 170 (78), 169 (73), 144 (31), 132 (12), 117 (17).

(+)-16-Epi-Δ^{14}-vincamine (**4**): 15 mg/kg. $[\alpha]_D^{20} = +29.8$ (*c* = 0.4, $CHCl_3$), M.p. 181–183°. UV (EtOH): λ_{max} 232 (3.2), 278 (2.4), 290 (2.8). IR (KBr): 3420, 3040, 2970, 2920, 1730, 1620, 1455, 1320, 910, 750. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.43 (*d*, *J* = 7.4, 1 H); 7.40 (*d*, *J* = 8.4, 1 H); 7.15 (*m*, 1 H); 7.11 (*m*, 1 H); 5.46 (*m*, 1 H); 5.27 (*d*, *J* = 10.0, 1 H); 3.77 (*s*, 1 H); 3.48 (*s*, 3 H); 3.21–3.26 (*m*, 2 H); 3.02–3.08 (*m*, 2 H); 2.87 (*m*, 1 H); 2.63 (*d*, *J* = 15.3, 1 H); 2.46 (*m*, 1 H); 2.01 (*d*, *J* = 15.3, 1 H); 1.81 (*q*, *J* = 7.4, 2 H); 0.93 (*t*, *J* = 7.4, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 169.13; 132.47; 127.46; 127.21; 126.63; 126.59; 120.72; 120.01; 116.25; 111.39; 104.27; 78.24; 59.21; 52.27; 49.87; 42.16; 42.13; 36.49; 34.64; 16.54; 9.07. EI-MS: 352 (2, M^+), 351 (6), 323 (7), 305 (17), 293 (11), 284 (2), 263 (7), 250 (32), 249 (27), 235 (12), 221 (42), 206 (17), 185 (56), 167 (100), 149 (58), 144 (14), 132 (9), 121 (21).

(+)-Isoeburnamine (**5**): 13 mg/kg. $[\alpha]_D^{20} = +112.4$ (*c* = 0.3, $CHCl_3$). UV (EtOH): λ_{max} 230 (2.1), 276 (2.3), 292 (2.9). IR (KBr): 3340, 3035, 2945, 2860, 1660, 1460, 1270, 745. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.73 (*d*, *J* = 1.6, 1 H); 7.46 (*d*, *J* = 8.1, 1 H); 7.17 (*m*, 1 H); 7.13 (*m*, 1 H); 5.54 (*dd*, *J* = 11.5, 4.8, 1 H); 3.52 (*s*, 1 H); 3.21–3.29 (*m*, 2 H); 2.91 (*m*, 1 H); 2.49 (*m*, 1 H); 2.47 (*m*, 1 H); 2.46 (*m*, 1 H); 2.19 (*m*, 1 H); 2.17 (*dd*, *J* = 15.4, 4.8, 1 H); 2.0 (*m*, 1 H); 1.86–1.90 (*m*, 2 H); 1.54 (*dd*, *J* = 15.4, 11.5, 1 H); 1.40 (*m*, 1 H); 1.37 (*m*, 1 H); 0.97 (*t*, *J* = 7.4, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 136.21; 132.10; 128.72; 121.68; 120.54; 119.40; 111.67; 104.27; 75.62; 56.11; 50.13; 44.62; 42.15; 34.78; 30.21; 25.89; 21.34; 15.94; 7.20. EI-MS: 296 (3, M^+), 295 (5), 278 (6), 268 (11), 250 (16), 248 (4), 220 (8), 208 (32), 206 (36), 193 (78), 167 (100), 139 (42), 112 (23), 97 (19).

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REFERENCES

[1] C. M. Li, S. G. Wu, G. D. Tao, J. Y. Zhong, C. You, Y. L. Zhou, L. Y. Dong, *Zhong Chao Yao* **1987**, *2*, 1.
[2] J. Bruneton, A. Cave, A. D. Cave, *Tetrahedron* **1973**, *29*, 1131.
[3] C. M. Li, G. D. Tao, S. G. Wu, J. Y. Zhong, Y. L. Zhou, *Acta Botanica Sinica* **1989**, *31*, 792.
[4] W. G. Kump, M. B. Patel, J. M. Rowson, H. Schmid, *Helv. Chim. Acta* **1964**, *47*, 1497.
[5] K. Awang, M. Pais, T. Sevenet, H. Schaller, A. M. Nasir, A. Hamid, A. Haid, *Phytochemistry* **1991**, *30*, 3164.
[6] T. S. Kam, P. S. Tan, C. H. Chuah, *Phytochemistry* **1992**, *31*, 2936.
[7] T. S. Kam, P. S. Tan, C. Wei, *Phytochemistry* **1993**, *33*, 921.

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