trol groups) × 100 values or IC_{50} (drug concentration that inhibits colon growth by 50 %) in drug-containing medium relative to colony growth in 0.5 % EtOH medium at the day 5 after drug treetment.

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Study on the Alkaloids of *Melodinus tenuicaudatus*

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Abstract: Fourteen alkaloids were isolated from the stem bark of *Melodinus tenuicaudatus* Tsiang et P. T. Li. Eleven of them were identified as known alkaloids, namely, scandine (**2**), Δ^{14}-eburnamine (**4**), vindolinine N_b-oxide (**5**), 11-methoxytabersonine (**6**), vindolinine (**7**), *epi*-vindolinine N_b-oxide (**8**), hazuntine (**9**), compactinervine (**10**), 11-hydroxytabersonine (**11**), Δ^{14}-vincine (**12**), and normacusine B (**14**). Two alkaloids were new: 10-hydroxyscandine (**1**), and the dimer, tenuicausine (**3**); their structures were elucidated by spectroscopic and chemical methods. One alkaloid (**13**) occurring in trace amounts, could not be identified.

Introduction

Some species of genus *Melodinus* (Apocynaceae) are used in Chinese folk medicine for the treatment of meningitis in children and rheumatic heart diseases (1). Some of them also can "invigorate the circulation of blood", "stimulate sucking and treat fracture" (2). In order to find the active principles from this genus, studies on the stem bark of *M. tenuicaudatus* Tsiang et P. T. Li, a plant collected at Xishuangbanna of the Yunnan province, were carried out. The present paper deals with the isolation and the structural elucidation of two new indole alkaloids along with the identification of eleven known alkaloids.

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Results and Discussion

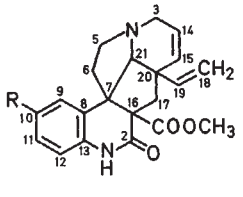
A new alkaloid, 10-hydroxyscandine (**1**), m.p. 180 °C, $[\alpha]_D^{20}$: + 228° (chloroform), was isolated from the crude alkaloid portion II as described in the Experimental section. The molecular formula was determined by HRMS as $C_{21}H_{22}N_2O_4$ (Found: 366.1578; calcd.: 366.1581). By comparing the fragmentation pattern of **1** [MS: m/z = 366 (M^+), 351, 338, 307, 279, 246, 134, 120] with that of **2** [MS: m/z = 350 (M^+), 335, 322, 291, 263, 230, 134, 120], it was found that **1** possessed the same skeleton as **2**, because the main fragments containing the aromatic moiety were all shifted towards the high mass by 16 amu (an oxygen). This was further supported by an absorption due to hydroxyl group (IR: ν = 3440 cm^{-1}), which could be methylated by CH_2N_2. The methylation product **1a** showed one more methoxy group at δ = 3.78 ppm (s, 3H) and three aromatic protons at δ = 6.71, 6.74, and 6.93 ppm, suggesting that one proton of the aromatic ring had been displaced by an OH. According to the peak shape and coupling constants in the ^{1}H-NMR spectrum, this hydroxyl group was likely to be located at C-10 or C-11. Application of the NOE technique when **1a** was irradiated at δ = 8.65 ppm (s, NH-CO), led to an increase in the area by 16 % for the signal at δ = 6.71 ppm (d, J = 8.6 Hz, 1H). It was found in the molecular model that only the C-12 proton has the possibility for an existence of NOE. Similarly, when δ = 3.78 ppm (OMe) was irradiated, the increase by 9 % at δ = 6.74 ppm (dd, 1H, aromatic), and 6 % at δ = 6.93 ppm (d, 1H, aromatic) were also observed. So the structure of **1** was established as 10-hydroxyscandine. The ^{13}C-NMR spectrum of **1** (see Table I) also confirmed this conclusion.

Another new alkaloid, tenuicausine (**3**), m.p. 160 °C, $[\alpha]_D$: + 27° (chloroform), was isolated from the portion I (see Experimental). The molecular formula was afforded by HRMS as $C_{41}H_{46}N_4O_3$ (Found: 642.3558, calcd.: 642.3572). The typical

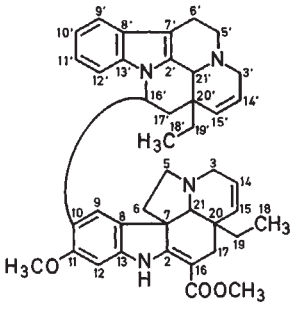
Table I. Comparison of ^{13}C-NMR data of **1** and **2**.

C-Atom	1	2	C-Atom	1	2
C-2	170.05	170.2	C-13	128.99	134.1
C-3	44.98	47.6	C-14	121.80	122.7
C-5	52.54	53.2	C-15	132.33	131.2
C-6	36.58	39.8	C-16	62.47	63.6
C-7	58.68	57.7	C-17	43.84	44.0
C-8	127.21	126.7	C-18	113.25	114.4
C-9	114.62	126.7	C-19	142.75	142.0
C-10	152.76	123.4	C-20	46.42	46.5
C-11	114.37	127.2	C-21	82.21	83.5
C-12	116.26	115.5	C=O	168.41	169.3
			OCH_3	52.69	52.4

UV (λ_{max} 232, 331 nm) and IR (ν_{max} 1680, 1620 cm^{-1}) absorptions suggested an beta-anilinoacrylate chromophore in the molecule. The ^{1}H-NMR indicated the presence of six methoxy protons at δ = 3.76 (s, 3H), 3.86 ppm (s, 3H), four olefinic protons at δ = 5.68 ppm (m, 4H), and six aromatic protons at δ = 6.55 (s, 1H), 7.02 (s, 1H), 6.25 (d, J = 7.3 Hz, 1H), 6.69 (dd, J = 7.6 Hz, 1H), 6.93 (dd, J = 7.3 Hz, 1H), and 7.40 ppm (d, J = 7.6 Hz, 1H). According to the peak shape and coupling constants, it can be deduced that two of the aromatic protons (6.55, 7.02) must be in the *para*-position. Study of the mass spectrum showed that **3** possessed the diagnostic fragments of tabersonine (m/z = 107, 121, 122, 135) and Δ^{14}-vincamine (m/z = 249, 250). So it was concluded that **3** might consist of tabersonine and Δ^{14}-eburnamine moieties. The structure of tenuicausine was tentatively assigned as **3**. The latter was substantiated by ^{13}C-NMR chemical shifts (Table II) and eventually confirmed by partial synthesis with 11-methoxytabersonine and Δ^{14}-eburnamine. The coupling product was consistent with **3** in the mass spectrum, R_f, and color reaction with CAS.



1 R = OH
2 R = H



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Materials and Methods

Melting points (uncorrected) were determined on a Fisher-Johns apparatus. IR spectra were measured in KBr on a Perkin-Elmer Model 599B instrument. UV spectra were measured in MeOH on a Shimadzu Model 3000 spectrophotometer. NMR spectra were recorded in $CDCl_3$ on Bruker AM-400 and AC-100 instruments. Mass spectra were recorded on MAT-711 and MAT-44S mass spectrometers. Alumina used in column chromatography came from the Shanghai 54 Chemical Reagents Plant. Silica gel used in column chromatography and silica gel GF_{254} used in TLC were from Qingdao Marine Chemical Factory. CAS reagent was prepared with 1 % ceric ammonium sulfate in phosphoric acid. Voucher specimens of the plant have been deposited in Yunnan Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Extraction of the alkaloids

The air-dried, ground stem bark of *M. tenuicaudatus* Tsian et P. T. Li (3 kg) was extracted with EtOH, the extract was concentrated under reduced pressure to yield a brown gummy material which was then dissolved in 5 % citric acid, filtered, and adjusted to pH 5 and 9 with NH_4OH, and extracted with chloroform separately. The solutions were dried with Na_2SO_4, filtered, and evaporated to dryness. Two crude alkaloidal portions I (15 g) and II (4 g) were obtained.

Isolation and characterization of the alkaloids

Work-up of portion II: Portion II (pH 9) was subjected to column chromatography on neutral Al_2O_3; diethyl ether, diethyl ether-chloroform (4 : 1, 1 : 1, 1 : 4) were used as eluents. Four main fractions were collected and evaporated separately under reduced pressure.

Fraction I was purified with PTLC (SiO_2 GF_{254}) to give **4**. This product gave a yellow-green coloration with CAS and was identified as Δ^{14}-eburnamine (3) by comparison of the R_f in TLC, UV, and mass spectra with an authentic sample.

Fraction II was subjected to rechromatography on a SiO_2 column. Alkaloids **13** and **14** were obtained by successive elution with cyclohexane-EtOAc (3 : 7) and EtOAc-methanol (9 : 1). Due to the small amount of **13**, it will be investigated later; **14**, m.p. 224 °C, was identified as normacusine B (4) by comparison of the MS and NMR spectra with literature data.

Fraction III gave a new alkaloid, 10-hydroxyscandine (**1**): m.p. 180 °C, $[\alpha]_D$: + 228° (chloroform), $C_{21}H_{22}N_2O_4$ (HRMS: m/z 366.1578, calcd. 366.1581), MS: m/z 366 (M^+), 351, 338, 307, 279, 246, 134, 120; UV: λ_{max}^{MeOH} nm (log ε) 268 (3.85), 305 (3.42); IR: ν_{max}^{KBr} cm^{-1} 3210, 1760, 1680; ^{1}H-NMR: δ = 3.53 (s, 1H), 6.36 (dd, J = 8.34 Hz, 2.04 Hz, 1H), 6.48 (d, J = 8.36 Hz, 1H), 6.92 (d, J = 1.88 Hz, 1H), 8.66 (s, 1H).

Methylation of **1**

10 mg of **1** was dissolved in a small amount of acetone-diethyl ether (ca. 1 : 1). To this solution, 20 mg of silica gel was added and then followed by an excess of CH_2N_2 in diethyl ether. After standing overnight at room temperature, the reaction mixture was filtered and evaporated to dryness under reduced pressure. The residue thus obtained was purified by using PTLC (SiO_2 GF_{254}, 7 : 12 : 1 cyclohexane-EtOAc-methanol). The methyl ether (**1a**) was isolated and crystallized from diethyl ether, m.p. 204 °C, MS: m/z (rel. int. %) 380 (M^+, 60), 365 (20), 351 (20), 321 (100), 293 (20), 266 (25), 260 (25), 229 (40), 215 (40), 200 (15), 160 (30), 134 (40), 120 (40), 105 (30). UV: λ_{max} nm (log ε) 267 (4.02), 305 (3.24). IR: ν_{max} cm^{-1} 3200, 1750, 1670. ^{1}H-NMR: δ = 3.57 (s, 3H), 3.78 (s, 3H), 6.71 (d, J = 8.6 Hz, 1H), 6.74 (dd, J = 8.6 Hz, 2.4 Hz, 1H), 6.93 (d, J = 1.9 Hz, 1H).

Fraction IV was further purified by means of PTLC (SiO_2 GF_{254}), developed with chloroform-methanol-acetone (18 : 5 : 5) in a jar saturated with moist NH_3; **10** (m.p. 120 °C, blue coloration with CAS) was isolated and identified as compactinervine by comparison of the UV, MS, and NMR with literature data (5).

Work-up of portion I: Portion I (pH5) was subjected to column chromatography on neutral alumina, using hexane, hexane-diethyl ether (1 : 1 and 1 : 2), and diethyl ether as eluents. Four main fractions were collected and concentrated under reduced pressure separately.

Fraction I was further purified with PTLC (SiO_2 GF_{254}); **6** and **9** were isolated and identified as 11-methoxytabersonine (6) and hazuntine (7), respectively, by comparison with authentic samples.

Fraction II was subjected to column chromatography (SiO_2, 1 : 1, hexane : diethyl ether) to give a new alkaloid, tenuicausine (**3**): m.p. 160 °C, $[\alpha]_D$: − 27° (chloroform), $C_{41}H_{46}N_4O_3$ (HRMS: Found 642.3558, calcd. 642.3572); MS: m/z (rel. int. %) 642 (100, M^+), 613 (5), 557 (3), 535 (10), 520 (2), 506 (3), 391 (9), 307 (2), 294 (6), 285 (6), 284 (6), 250 (34), 249 (29), 135 (90), 122 (20), 121 (24), 107 (32): UV λ_{max}^{MeOH} nm (log ε) 232 (3.58), 331 (3.28); IR: ν_{max} cm^{-1} 3370, 1680, 1620.

Table II. ^{13}C-NMR data of tenuicausine (**3**).

C-Atom	δ	C-Atom	δ	C-Atom	δ	C-Atom	δ
C-2	167.43	C-14	124.77	C-2′	128.49	C-14′	126.03
C-3	49.67	C-15	132.99	C-3′	42.50	C-15′	127.69
C-5	50.28	C-16	92.94	C-5′	50.78	C-16′	30.78
C-6	44.49	C-17	26.33	C-6′	16.90	C-17′	43.81
C-7	54.45	C-18	7.54	C-7′	104.89	C-18′	8.35
C-8	130.25	C-19	28.80	C-8′	121.31	C-19′	34.14
C-9	119.01	C-20	41.35	C-9′	117.43	C-20′	37.38
C-10	137.04	C-21	68.52	C-10′	119.01	C-21′	58.04
C-11	156.50	C = O	168.95	C-11′	120.38	C = O	–
C-12	93.94	OCH_3 (Ar)	55.95	C-12′	112.26	OCH_3 (Ar)	–
C-13	143.63	OCH_3 (C = O)	50.99	C-13′	134.20	OCH_3 (C = O)	–

Partial synthesis of tenuicausine (**3**)

A solution of 1.0 mg of 11-methoxytabersonine hydrochloride (**6**-HCl) and 0.7 mg of Δ^{14}-eburnamine (**4**) in 0.3 ml of 5 % HCl/MeOH was heated at 60 °C for 48 h. After dilution with water and basification with NH_4OH, the mixture was extracted with chloroform. Concentration of the chloroform extracts yielded a residue which was purified by means of PTLC to give **3**. MS: *m/z* (rel. int. %) 642 (85), 613 (8), 535 (30), 520 (4), 506 (17), 391 (20), 284 (26), 250 (60), 249 (53), 135 (100), 122 (16), 121 (36), 107 (50). The coupling product showed color reaction with CAS and gave identical R_f value and MS fragments to those of tenuicausine from plant origin.

After **3** was collected from Fraction II, continuous elution of the column with the same solvent system yielded successively alkaloids **7** and **5**. They were identified as vindolinine and vindolinine N_b-oxide, respectively, by comparison of their UV, IR, MS, and NMR with literature data (8, 9).

Work-up of Fraction III gave **2**, the major alkaloid of this plant, which structure was assigned to be scandine (10) based on the comparison with an authentic sample.

Rechromatography of Fraction IV on a SiO_2 column yielded **11** and **12**; **11** m.p. 173 °C, was identified as 11-hydroxytabersonine by comparison of the UV, MS, and NMR data with literature data (11); **12** was identified as Δ^{14}-vincine by comparison with an authentic specimen (12).

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