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Indole alkaloids from *Kopsia jasminiflora*

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ABSTRACT

Seven new indole alkaloids (aspidofractinine type **1**–**3**, kopsine type **5**, strychnos type **6**, and vincamine type **7**, **8**) were isolated from *Kopsia jasminiflora* (Apocynaceae) collected in Thailand. 5-Oxokopsinic acid (**4**) was isolated from nature for the first time. The structures of the new alkaloids were determined by spectroscopic analyses and chemical transformation of a known alkaloid. 5,6-Secokopsinine (**1**) possesses a dialdehyde function that is formed by oxidative cleavage of the C-5–C-6 bond of kopsinine (**9**). New vincamine-type alkaloid **8** showed potent inhibitory activity toward human cancer cell lines (A549, HT29, HCT116).

1. Introduction

Kopsia plants belonging to Apocynaceae are a rich source of monoterpenoid indole alkaloids that often possess unique skeletons and significant bioactivities.[1,2] We have previously reported the isolation of new alkaloids from three *Kopsia* plants distributed in Asia.[3] In our continuing studies of new biologically active alkaloids,[4] seven new indole alkaloids (aspidofractinine type **1**–**3**, kopsine type **5**, strychnos type **6**, and vincamine type **7**, **8**) and 5-oxokopsinic acid (**4**) were isolated from the stem bark of *Kopsia jasminiflora*[5] together with 29 known alkaloids, including **9**–**17** (Fig. 1). Herein, we report the structure elucidation of the new alkaloids by means of spectroscopic analyses and chemical transformation, and the evaluation of the isolated alkaloids regarding their inhibitory activity toward human cancer cell lines.

2. Results and discussion

Alkaloid **1**, named 5,6-secokopsinine, was found to have the molecular formula $C_{21}H_{24}N_2O_4$ from HRESIMS (*m*/*z* 391.16298 $[M+Na]^+$) (Fig. 2). The ^1H and ^{13}C NMR spectra (Table 1) showed signals assignable to four aromatic protons of an indoline system [δ_H 7.78 (dd, H-9), 7.13 (ddd, H-11), 6.87 (ddd, H-10), 6.72 (br d, H-12)], an aminomethine proton [δ_H 4.58 (s, H-21)], a methoxycarbonyl group [δ_H 3.79 (3H, s, CO_2Me); δ_C 173.1 (CO_2Me), 52.1 (CO_2*Me*)], aminomethylene protons [δ_H 3.81–3.73 (overlapped) and 3.64 (dd) (H_2-3)], and a triplet methine proton [δ_H 2.98 (dd, *J*=9.9, 9.9 Hz, H-16)]. Signals in the high-field region were similar to those of kopsinine (**9**),[6] the major alkaloid in this plant. However, signals due to an aldehyde [δ_H 9.25 and δ_C 195.1] and a formamide function [δ_H 8.21 and δ_C 164.1] were observed instead of signals due to the C-5–C-6 ethane bridge in **9**, and H-9 and H-21 signals were shifted to the lower field compared to those in **9** [**1**, δ_H 7.78 (H-9), 4.58 (H-21); **9**, δ_H 7.20 (H-9), 3.02 (H-21)]. HMBC correlations between the formamide proton and C-3, H-3 and the formamide carbon, the aldehyde proton and C-7, and H-21 and the aldehyde carbon revealed that one aldehyde group each was attached to N_b and C-7 positions, respectively. The lower-field shifted H-9 might be a result of the anisotropy effect of the formamide and/or aldehyde group. Coupling constants between H-16 and H_2-17 ($J_{16,17\alpha}=J_{16,17\beta}=9.9$ Hz) revealed that 16-CO_2Me was β-oriented. The positive Cotton effects at 249 and 212 nm in the CD spectrum were similar to those of kopsinilam (**10**),[6c,7] a lactam derivative of kopsinine (**9**), suggesting that the absolute configuration of **1** is the same as that of **10**. From these data, the structure of 5,6-secokopsinine was deduced to be that shown as formula **1**.

Alkaloid **2** was found to have the molecular formula $C_{21}H_{26}N_2O_3$ from HRESIMS (*m*/*z* 355.20321 $[M+H]^+$), which indicated that **2** has an extra oxygen atom compared to kopsinine (**9**) (Fig. 3). Its UV

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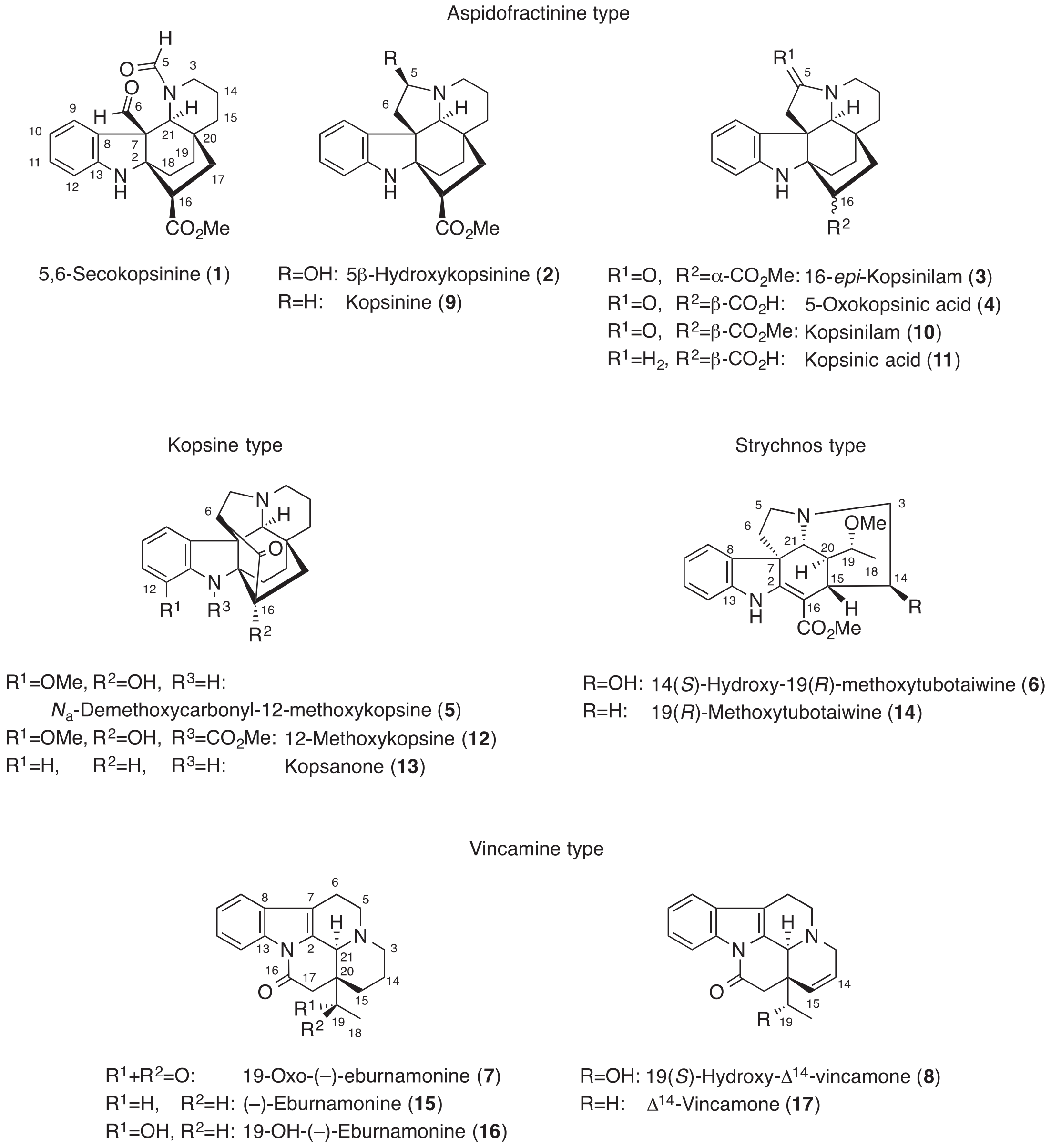

Aspidofractinine type

5,6-Secokopsinine (**1**)

R=OH: 5β-Hydroxykopsinine (**2**)
R=H: Kopsinine (**9**)

R^1=O, R^2=α-CO_2Me: 16-*epi*-Kopsinilam (**3**)
R^1=O, R^2=β-CO_2H: 5-Oxokopsinic acid (**4**)
R^1=O, R^2=β-CO_2Me: Kopsinilam (**10**)
R^1=H_2, R^2=β-CO_2H: Kopsinic acid (**11**)

Kopsine type

R^1=OMe, R^2=OH, R^3=H:
N$_a$-Demethoxycarbonyl-12-methoxykopsine (**5**)
R^1=OMe, R^2=OH, R^3=CO_2Me: 12-Methoxykopsine (**12**)
R^1=H, R^2=H, R^3=H: Kopsanone (**13**)

Strychnos type

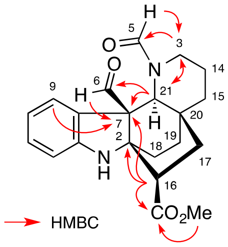
R=OH: 14(*S*)-Hydroxy-19(*R*)-methoxytubotaiwine (**6**)
R=H: 19(*R*)-Methoxytubotaiwine (**14**)

Vincamine type

R^1+R^2=O: 19-Oxo-(−)-eburnamonine (**7**)
R^1=H, R^2=H: (−)-Eburnamonine (**15**)
R^1=OH, R^2=H: 19-OH-(−)-Eburnamonine (**16**)

R=OH: 19(*S*)-Hydroxy-Δ^{14}-vincamone (**8**)
R=H: Δ^{14}-Vincamone (**17**)

Fig. 1. Structures of the isolated alkaloids from *Kopsia jasminiflora*.



1

Fig. 2. Selected HMBC correlations of 5,6-secokopsinine (**1**).

spectrum exhibited absorptions at 293.5, 245.0, and 206.0 nm, which are characteristic of an indoline chromophore. The ^1H and ^{13}C NMR spectra were similar to those of kopsinine (**9**) except for the existence of signals for an aminal function at δ_H 4.78 and δ_C 95.5 instead of the aminomethylene function at C-5 in **9** (δ_H 3.35 and 3.30, δ_C 50.7). The aminal proton was coupled with C-6 methylene protons in the COSY spectrum. In the HMBC spectrum, correlations between H-3 and H-6 and the C-21 aminomethine carbon were observed. NOE correlations from H-5 to H-9 and from H-16 to H-18 and H-19 indicated that H-5 and H-16 are α-oriented. The existence of a 5β-hydroxy group was also supported by the lower-field shifted H-17, which was interpreted to be due to the anisotropy effect of the hydroxy group. From the above data, compound **2** was deduced to be 5β-hydroxykopsinine.

Alkaloid **3** was found to have the same molecular formula $C_{21}H_{24}N_2O_3$ as kopsinilam (**10**) from HRESIMS (*m*/*z* 353.18646 $[M+H]^+$) (Fig. 4). The ^1H and ^{13}C NMR spectra were similar to those of **10** except for the coupling pattern of the methine proton at C-16; the signal for H-16 was observed at δ_H 3.06 as a broad doublet (J=11.0 Hz) in **3**, whereas it was observed at δ_H 2.97 as a double-doublet (J=9.3, 9.3 Hz) in **10**. This suggested that **3** is a C-16 epimer of kopsinilam (**10**). NOE correlation from H-16 to H-6 as well as coupling constants between H-16 and H_2-17 ($J_{16,17\beta}$=11.0 Hz, $J_{16,17\alpha}\approx0$ Hz) indicated that 16-CO_2Me is α-oriented. From these data as well as 2D NMR analysis, compound **3** was deduced to be 16-*epi*-kopsinilam.