

SPECTAMINES A AND B, POSSIBLE INHIBITORS OF SUPEROXIDE ANION PRODUCTION OF MACROPHAGES FROM *CASSIA SPECTABILIS*

Tsunashi Kamo,* Kentaro Maehara, Kazuya Sato, and Mitsuru Hirota

Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan. e-mail: kamo274@gipmc.shinshu-u.ac.jp

Abstract – Two novel piperidine alkaloids were isolated from an African legume, *Cassia spectabilis*, and identified as the *O*-benzoyl (**1**, named spectamine A) and *O*-acetyl (**2**, named spectamine B) derivatives of (+)-iso-6-cassine (**3**). The absolute configurations of **1-3** were established to be (2*R*,3*R*,6*R*) using the modified Mosher's method. Compound (**1**) inhibited the superoxide anion production of macrophages, while it did not quench the superoxide anion which is produced by xanthine oxidase at a concentration of 25 μ M.

Superoxide anion (O_2^-) is one of active oxygens which are produced in human body. The over-production of O_2^- causes several diseases, such as inflammation, cancer, and hypertension.¹ Scavenging of the over-produced superoxide or depression of the superoxide production would be useful for maintaining a healthy human body condition. There are many reports that radical scavengers such as polyphenols are able to quench the superoxide anion,² however, there are a few reports on the inhibitors of superoxide production.³ We searched for an inhibitor of the superoxide anion production from African plants using the macrophage test and found that the methanolic extract of *Cassia spectabilis*, a Leguminosae plant, suppressed the superoxide production of macrophages. The bioassay-guided isolation from the methanolic extract of this plant by silica gel column chromatography led to two novel piperidine alkaloids, called spectamines A (**1**) and B (**2**).

The 1H NMR spectrum of **1** was similar to that of (+)-iso-6-cassine (**3**),⁴ except for the presence of signals assignable to a phenyl group (5H, δ 7.45-8.06 ppm; Table 1). In the ^{13}C NMR spectrum of **1**, signals were observed at δ 128.4, 129.6, 130.6 and 132.9 ppm (C_6H_5) and 165.9 ppm (C=O) in addition to those

of **3**. These spectra suggested that **1** was an *O*-benzoyl derivative of **3**, which was supported by a molecular ion peak at m/z 401.2963 ($C_{25}H_{39}NO_3$) in the HREIMS of **1**. Compound (**1**) was converted to **3** by hydrolysis, confirming the assumption (Figure 1). The absolute configuration of **1**, either (*2R,3R,6R*) or (*2S,3S,6S*), could not be established by the optical rotation value, since the absolute configuration and the optical rotation values had been contradictory in past studies on **3**.^{4,5} We determined the absolute configuration of **3** using the modified Mosher's method.⁶ The (*R*)- and (*S*)-MTPA esters of Boc-**3** were prepared from **3** and then their chemical shift values in the 1H NMR spectra were compared (Figure 2). The δ values at C-2, C-2-Me, C-4 and C-5 revealed that the absolute configuration at C-3 was (*R*), meaning that the absolute configuration of **3** was (*2R,3R,6R*). Thus, **1** was identified as a novel alkaloid, (*2R,3R,6R*)-(+)-3-benzoyloxy-2-methyl-6-(11''-oxododecyl)piperidine (spectamine A).

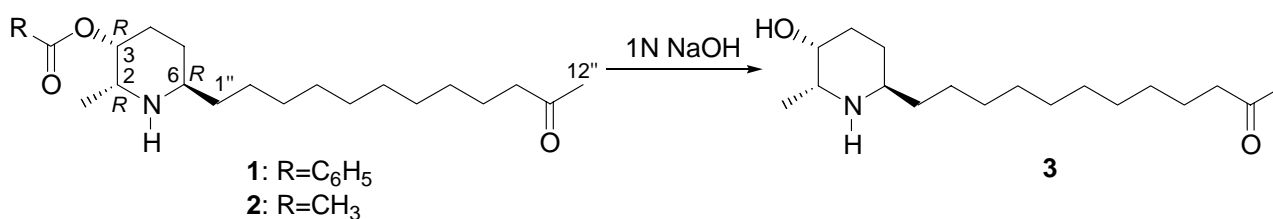


Figure 1. Chemical conversion of **1** and **2** to **3**

Table 1. 1H and ^{13}C NMR spectral data for **1** and **2** (500 MHz for 1H and 125 MHz for ^{13}C , $CDCl_3$)

position	Spectamine A (1)		Spectamine B (2)	
	^{13}C	1H	^{13}C	1H
2	48.8 (CH)	3.41 (1H, qd, $J = 6.8, 3.9$ Hz)	48.4 (CH)	3.27 (1H, qd, $J = 6.8, 3.5$ Hz)
3	72.8 (CH)	5.11 (1H, ddd, $J = 4.4, 4.1, 3.9$ Hz)	72.1 (CH)	4.85 (1H, ddd, $J = 4.3, 3.8, 3.5$ Hz)
4	24.5 (CH ₂)	1.85 (1H, m)	24.4 (CH ₂)	1.72 (1H, m)
		1.92 (1H, m)		1.80 (1H, m)
5	26.5 (CH ₂)	1.30 (1H, m)	26.6 (CH ₂)	1.30 (1H, m)
		1.35 (1H, m)		1.36 (1H, m)
6	49.0 (CH)	2.88 (1H, m)	49.3 (CH)	2.84 (1H, m)
2-CH ₃	14.8 (CH ₃)	1.21 (3H, d, $J = 6.8$ Hz)	14.9 (CH ₃)	1.10 (3H, d, $J = 6.8$ Hz)
3-O-C(=O)-	165.9 (C)		170.6 (C)	
1'	130.6 (C)		21.3 (CH ₃)	2.07 (3H, s)
2', 6'	129.6 (CH)	8.06 (2H, d, $J = 7.3$ Hz)		
3', 5'	128.4 (CH)	7.45 (2H, dd, $J = 7.4, 7.3$ Hz)		
4'	132.9 (CH)	7.56 (1H, t, $J = 7.4$ Hz)		
1''	34.6 (CH ₂)	1.30 (1H, m)	34.1 (CH ₂)	1.30 (1H, m)
		1.58 (1H, m)		1.44 (1H, m)
2''-8''	29.4-29.8 (CH ₂)	1.27 (14H, m)	29.2-29.7 (CH ₂)	1.27 (14H, m)
9''	23.9 (CH ₂)	1.57 (2H, m)	23.9 (CH ₂)	1.56 (2H, m)
10''	43.8 (CH ₂)	2.41 (2H, t, $J = 7.5$ Hz)	43.8 (CH ₂)	2.41 (2H, t, $J = 7.4$ Hz)
11''	209.4 (C)		209.3 (C)	
12''	29.9 (CH ₃)	2.13 (3H, s)	29.8 (CH ₃)	2.13 (3H, s)

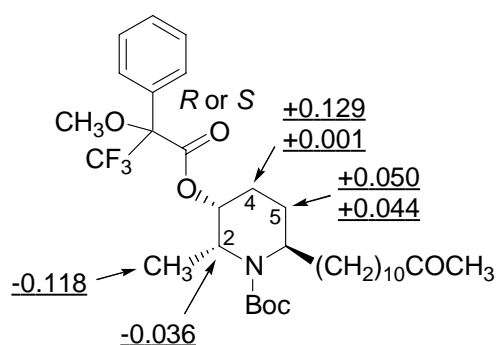


Figure 2. Differences in proton chemical shift (ppm) between the (*S*)- and (*R*)-MTPA esters of Boc-**3**. Underlined values: (δ value from ^1H NMR spectrum of the (*S*)-MTPA ester of Boc-**3**) - (δ value from ^1H NMR spectrum of the (*R*)-MTPA ester of Boc-**3**)

Table 2. The $[\alpha]_{\text{D}}$ values and the absolute configurations of natural and synthesized **3**

Absolute configuration	$[\alpha]_{\text{D}}$	Method used for determination
(<i>2R,3R,6R</i>)	-3.3° (<i>c</i> 0.26, CHCl_3)	Horeau's process
(<i>2R,3R,6R</i>)	$+1.5^\circ$ (<i>c</i> 1.22, CHCl_3)	the modified Mosher's method
(<i>2S,3S,6S</i>)	-1.5° (<i>c</i> 1.50, CHCl_3)	asymmetric synthesis

The ^1H NMR spectrum of **2** was similar to that of **1**, except for another singlet (3H, δ 2.07 ppm; Table 1) assignable to an acetyl group instead of the benzoyl group. The ^{13}C NMR spectrum of **2** showed signals at δ 21.3 ppm (CH_3) and 170.6 ppm ($\text{C}=\text{O}$) in addition to those of **3**, suggesting that **2** was an *O*-acetyl derivative of **3**. A molecular ion peak at m/z 399.2762 in the HREIMS of **2**, suggesting a molecular formula of $\text{C}_{20}\text{H}_{37}\text{NO}_3$, supported the structure. Hydrolysis of **2** gave **3** whose optical rotation value, $[\alpha]_{\text{D}}^{30} +1.4^\circ$ (*c* 1.97, CHCl_3), was almost equal to that of **3** prepared from **1**, $[\alpha]_{\text{D}}^{30} +1.5^\circ$ (*c* 1.22, CHCl_3), indicating that the absolute configuration of **2** was also (*2R,3R,6R*). These observations proved that **2** was a novel alkaloid, (*2R,3R,6R*)-(+)-3-acetoxy-2-methyl-6-(11"-oxododecyl)piperidine (spectamine B). Christofidis *et al.* reported the isolation and identification of **3**, $[\alpha]_{\text{D}}^{25} -3.3^\circ$ (*c* 0.26, CHCl_3), from *C. spectabilis*, and determined its absolute configuration to be (*2R,3R,6R*) by Horeau's process (Table 2).⁴ The asymmetric synthesis of (*2S,3S,6S*)-**3**, which corresponded to the enantiomer of the natural **3**, was achieved by Toyooka *et al.*⁵ The $[\alpha]_{\text{D}}^{25}$ value of the synthesized (*2S,3S,6S*)-**3** was -1.5° (*c* 1.50, CHCl_3), which should have been $+3.3^\circ$ if that of natural **3** was correct. In the present paper, we described the isolation of **3** as the *O*-benzoyl and *O*-acetyl derivatives (**1** and **2**, respectively), and the identification of its absolute configuration as (*2R,3R,6R*) using the modified Mosher's method. The $[\alpha]_{\text{D}}$ values and the absolute configuration we described were well correlated with those of Toyooka *et al.*,⁵ suggesting that the $[\alpha]_{\text{D}}$ value of the previously reported natural **3** was incorrect.⁴ A trace of impurity might have produced a bad effect on the optical rotation value of **3** in their experiment.

The inhibitory activities of the superoxide production of **1-3** were evaluated using both the xanthine oxidase (XOD) test and the macrophage test.⁷ As shown in Table 3, a radical scavenger, quercetin,

quenched 30.9% and 72.3% of the superoxide which was produced by XOD, at 25 and 125 μM , respectively, while **1-3** showed slight activities at 25 and 125 μM during the XOD test. For the macrophage test, **1** showed the strongest activity and suppressed 46.7% of the superoxide production at 25 μM , although its activity to quench the superoxide anion was weak. Compound (**1**) might be a specific inhibitor of the superoxide production of macrophages.

Table 3. Inhibitory activities of **1-3** against the superoxide production by xanthine oxidase (XOD) and macrophage

Tested compound	% Inhibitory activity (\pm Standard deviation)		
	XOD test		Macrophage test
	25 μM	125 μM	25 μM
spectamine A (1)	8.7 (\pm 3.4)	15.1 (\pm 6.0)	46.7 (\pm 13.2)
spectamine B (2)	2.5 (\pm 4.7)	-1.9 (\pm 7.4)	5.0 (\pm 9.9)
(+)-iso-6-cassine (3)	-0.7 (\pm 3.5)	4.8 (\pm 15.5)	7.0 (\pm 5.4)
quercetin*	30.9 (\pm 8.4)	72.3 (\pm 1.6)	19.0 (\pm 7.5)

*A positive control in the XOD and the macrophage tests.

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