HETEROCYCLES, Vol. 60, No. 6, 2003, pp. 1303 - 1306
Received, 3rd March, 2003, Accepted, 4th April, 2003, Published online, 7th April, 2003

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 μM.

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 ^{1}H 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 (2*R*,3*R*,6*R*) or (2*S*,3*S*,6*S*), 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 ^{1}H 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 (2*R*,3*R*,6*R*). Thus, **1** was identified as a novel alkaloid, (2*R*,3*R*,6*R*)-(+)-3-benzoyloxy-2-methyl-6-(11"-oxododecyl)piperidine (spectamine A).

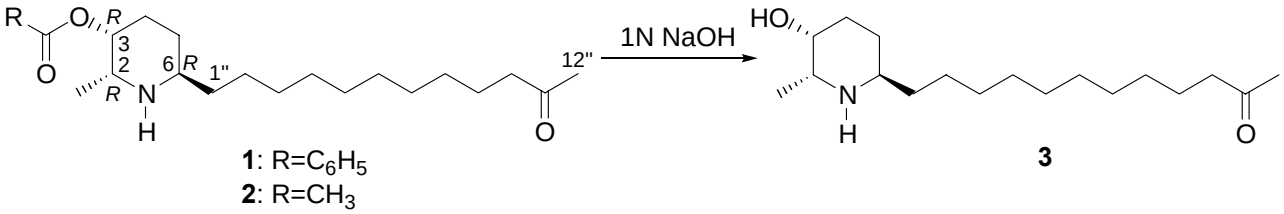

Figure 1. Chemical conversion of **1** and **2** to **3**

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

	Spectamine A (**1**)		Spectamine B (**2**)	
position	^{13}C	^{1}H	^{13}C	^{1}H
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)

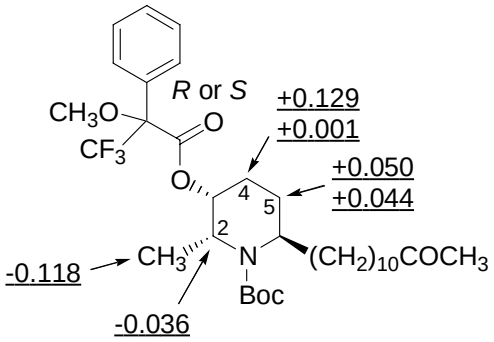

Figure 2. Differences in proton chemical shift (ppm) between the (*S*)- and (*R*)-MTPA esters of Boc-**3**. Underlined values: (δ value from ^{1}H NMR spectrum of the (*S*)-MTPA ester of Boc-**3**) - (δ value from ^{1}H NMR spectrum of the (*R*)-MTPA ester of Boc-**3**)

Table 2. The $[\alpha]_D$ values and the absolute configurations of natural and synthesized **3**

Absolute configuration	$[\alpha]_D$	Method used for determination
(2*R*,3*R*,6*R*)	-3.3° (*c* 0.26, $CHCl_3$)	Horeau's process
(2*R*,3*R*,6*R*)	+1.5° (*c* 1.22, $CHCl_3$)	the modified Mosher's method
(2*S*,3*S*,6*S*)	-1.5° (*c* 1.50, $CHCl_3$)	asymmetric synthesis

The ^{1}H 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 (C=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 $C_{20}H_{37}NO_3$, supported the structure. Hydrolysis of **2** gave **3** whose optical rotation value, $[\alpha]^{30}_D$ +1.4° (*c* 1.97, $CHCl_3$), was almost equal to that of **3** prepared from **1**, $[\alpha]^{30}_D$ +1.5° (*c* 1.22, $CHCl_3$), indicating that the absolute configuration of **2** was also (2*R*,3*R*,6*R*). These observations proved that **2** was a novel alkaloid, (2*R*,3*R*,6*R*)-(+)-3-acetoxy-2-methyl-6-(11"-oxododecyl)piperidine (spectamine B).

Christofidis *et al.* reported the isolation and identification of **3**, $[\alpha]_D^{25}$ -3.3° (*c* 0.26, $CHCl_3$), from *C. spectabilis*, and determined its absolute configuration to be (2*R*,3*R*,6*R*) by Horeau's process (Table 2).[4] The asymmetric synthesis of (2*S*,3*S*,6*S*)-**3**, which corresponded to the enantiomer of the natural **3**, was achieved by Toyooka *et al*.[5] The $[\alpha]_D^{25}$ value of the synthesized (2*S*,3*S*,6*S*)-**3** was -1.5° (*c* 1.50, $CHCl_3$), which should have been +3.3° 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 (2*R*,3*R*,6*R*) using the modified Mosher's method. The $[\alpha]_D$ values and the absolute configuration we described were well correlated with those of Toyooka *et al.*,[5] suggesting that the $[\alpha]_D$ 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

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

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

ACKNOWLEDGEMENTS

We thank Ms. M. Mizu and Ms. K. Hashimoto for providing the NMR spectra.

REFERENCES AND NOTES

1. N. Kaul and H. J. Forman, 'Toxicology of the Human Environment,' ed. by C. J. Rhodes, Taylor & Francis Ltd., London, 2000, pp. 311-335.
2. A. Kaul and K. L. Khanduja, *Nutr Cancer.*, 1999, **35**, 207; V. A. Kostyuk and A. I. Potapovich, *Arch. Biochem. Biophys.*, 1998, **355**, 43.
3. A. Murakami, Y. Nakamura, K. Torikai, T. Tanaka, T. Koshiba, K. Koshimizu, S. Kuwahara, Y. Takahashi, K. Ogawa, M. Yano, H, Tokuda, H, Nishino, Y. Mimaki, Y. Sashida, S. Kitanaka, and H. Ohigashi, *Cancer Res.*, 2000, **60**, 5059; O. K. Kim, A. Murakami, Y. Nakamura, N. Takeda, H. Yoshizumi, and H. Ohigashi, *J. Agric. Food Chem.*, 2000, **48**, 1557.
4. I. Christofidis, A. Welter, and J. Jadot, *Tetrahedron*, 1977, **33**, 977.
5. N. Toyooka, Y. Yoshida, Y. Yotsui, and T. Momose, *J. Org. Chem.*, 1999, **64**, 4914.
6. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.
7. The XOD method was partly owing to the report by H. Imai, M. Hashimoto, and Y. Nakabayashi, *Bunseki Kagaku*, 1994, **43**, 51 (in Japanese). The bioassays will be described in detail elsewhere.