UCA1064-B, A NEW ANTITUMOR ANTIBIOTIC ISOLATED FROM *Wallemia sebi*: PRODUCTION, ISOLATION AND STRUCTURAL DETERMINATION

Sir:

From a culture broth of *Wallemia sebi*, we have isolated a new compound, UCA1064-B, and UCA1064-A which is identified with A25822B isolated by CHAMBERLIN *et al.* in 1974.[1] UCA1064-B, which is structurally related to A25822B, exhibited antitumor activity against mouse mammary tumor model. In this communication, we report the production, isolation and structural determination of UCA1064-A and UCA1064-B.

The producing organism (KAC 1341) was isolated from dried potato and was taxonomically classified as *Wallemia sebi*. A seed broth was prepared by inoculating spores of the producing strain into a medium consisting of V8 juice 20% and sucrose 3% (pH 6.0). After inoculation at 28°C for 48 hours, a 5%-vegetative seed culture was inoculated into fermentation medium consisting of sucrose 6%, yeast extract 3%, KH_2PO_4 0.05% and $MgSO_4 \cdot 7H_2O$ 0.05% (pH 6.0). The peak titers were usually reached after 4 days incubation at 28°C in jar fermentor culture.

UCA1064-A and -B were accumulated in both mycelium and culture filtrate. Therefore, propanol (500 liters) was added into the culture broth (1,000 liters) and the mixture was filtered. The filtrate was diluted with deionized water (1,500 liters). After adjustment to pH 8.0 with 2 N NaOH, the filtrate was applied to a column of Diaion HP-20 (50 liters) (Mitsubishi Chemical Industries Limited). The column was washed with deionized water and 30% methanol and then eluted with methanol. After concentration, the eluate was diluted with deionized water and adjusted to pH 10.0 with 6 N NaOH and then extracted with ethyl acetate. The extract was concentrated and the residue was subjected to silica gel (Merck Art. No. 7734) column chromatography using step wise method of the mixture of $CHCl_3$ - MeOH as eluting solvents. The active fractions were combined and evaporated to dryness. The residue was rechromatographed on silica gel (Merck Lichroprep Si 60) with $CHCl_3$ - MeOH, and the active fractions were further purified with HPLC using a packed column (YMC-ODS SH-363-5, 80% MeOH, pH 4.0) to yield 100 mg of UCA1064-A and 100 mg of UCA1064-B.

The physico-chemical properties of UCA1064-A and -B are summarized in Table 1. The data of the UV absorption maxima and the IR spectrum of UCA1064-A and -B show that these compounds closely resemble the 15-azasterol type antibiotics reported by MICHEL *et al.*[2]

The molecular formula of UCA1064-A was determined as $C_{28}H_{45}NO$ (Calcd. 411.3498) by HREI-MS which showed molecular ion at *m/z* 411.3468. A battery of 2D NMR techniques including COSY, C-H COSY, COLOC, HOHAHA and HMQC-HOHAHA experiments were used to determined all proton and carbon assignments of UCA1064-A (Tables 2 and 3). From these results and NOESY experiments, the relative structure of UCA1064-A was determined as shown in Fig. 1 which is identified with A25822B established by X-ray crystallographic analysis.[1]

On the other hand, UCA1064-B was obtained as an optically active white powder, $[\alpha]_D^{24}$ −28.5° in MeOH. The molecular formula of UCA1064-B was determined as $C_{28}H_{47}NO$ (Calcd. 413.3655) by HREI-MS which showed molecular ion at *m/z* 413.3641. ^1H NMR spectra of UCA1064-B are

Table 1. Physico-chemical properties of UCA1064A- and UCA1064-B.

	UCA1064-A	UCA1064-B
Appearance	White powder	White powder
Molecular formula	$C_{28}H_{45}NO$	$C_{28}H_{47}NO$
EI-MS (*m/z*)	411 $(M)^+$	413 $(M)^+$
HR-MS (*m/z*) Calcd:	411.3498	413.3655
Found:	411.3468	413.3641
$[\alpha]_D^{24}$ (*c* 0.5, MeOH)	−8.7°	−28.5°
UV λ_{max} in MeOH acidic	279 nm	279 nm
neutral	241, 270 (sh) nm	241, 270 (sh) nm
basic	241 nm	241 nm
IR ($CHCl_3$) ν cm^{-1}	3400, 2950, 1690, 1610, 1450, 1370, 1010, 890	3400, 2950, 1690, 1610, 1450, 1370, 1070

Table 2. ^{1}H NMR data of UCA1064-A and -B (500 MHz, $CDCl_3$).

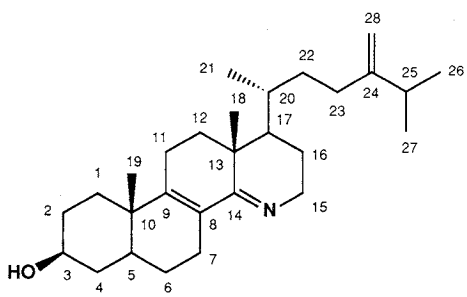
	UCA1064-A	UCA1064-B		UCA1064-A	UCA1064-B
1-H	1.86 (eq)	1.85 (eq)	15-H	3.97 (ax)	3.97 (ax)
	1.26 (ax)	1.29 (ax)		3.50 (eq)	3.49 (eq)
2-H	1.88 (eq)	1.88 (eq)	16-H	1.55	1.54
	1.49 (ax)	1.55 (ax)		1.55	1.54
3-H	3.63 (ax)	3.63 (ax)	17-H	1.19 (ax)	1.19 (ax)
4-H	1.70 (eq)	1.70 (eq)	18-H	0.95	0.95
	1.37 (ax)	1.37 (ax)	19-H	1.03	1.03
5-H	1.48 (ax)	1.49 (ax)	20-H	1.62	1.55
6-H	1.46	1.49	21-H	0.96	0.93
	1.46	1.49	22-H	1.61	1.51
7-H	2.45	2.46		1.05	0.81
	2.12	2.13	23-H	2.13	1.45
11-H	2.28	2.29	25-H	2.21	1.55
	2.28	2.29	26-H	1.02	0.85
12-H	2.06 (eq)	2.03 (eq)	27-H	1.03	0.79
	1.32 (ax)	1.36 (ax)	28-H	4.73	0.77
				4.66	

eq: equatorial, ax: axial.

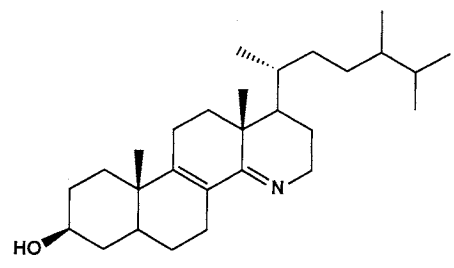
Table 3. ^{13}C NMR data of UCA1064-A and -B (125 MHz, $CDCl_3$).

	UCA1064-A	UCA1064-B		UCA1064-A	UCA1064-B
C-1	34.9	34.9	C-15	51.2	51.2
C-2	31.5	31.5	C-16	19.3	19.2
C-3	70.8	70.9	C-17	18.3	48.3
C-4	38.2	38.2	C-18	16.8	16.8
C-5	40.7	40.7	C-19	18.6	18.5
C-6	25.5	25.5	C-20	30.7	31.6
C-7	27.2	27.2	C-21	21.5	21.7
C-8	127.5	127.5	C-22	31.6	30.5
C-9	147.3	147.3	C-23	33.3	33.4
C-10	37.2	37.2	C-24	156.4	39.0
C-11	20.7	20.7	C-25	33.8	31.7
C-12	33.4	33.4	C-26	22.0	20.4
C-13	37.7	37.7	C-27	21.8	17.8
C-14	172.5	172.7	C-28	106.4	15.5

Fig. 1. Structures of UCA1064-A (=A25822B) and UCA1064-B.



UCA1064-A (=A25822B)



UCA1064-B

similar to those of UCA1064-A except that signals assigned as exomethylene observed at 4.73, 4.66 ppm for UCA1064-A are replaced by signals assigned as methyl observed at 0.77 ppm (3H, d, $J=6.8$ Hz) for UCA1064-B (Table 2). ^{13}C NMR spectra of UCA1064-B are similar to those of UCA1064-A except that 156.4 (s), 106.4 (t) ppm assigned as exomethylene in UCA1064-A are replaced by 15.5 ppm (q) and 39.0 ppm (d) assigned as methyl and methine, respectively, in UCA1064-B (Table 3). Based on these results and NOESY experiments, the relative structure of UCA1064-B excepting C-24 configuration was determined as shown in Fig. 1. The structure differs from UCA1064-A only in that exomethylene at C-24 is reduced. UCA1064-B as well as UCA1064-A (=A25822B) possess a unique structure, 15-azahomosterol skelton, which has been reported the capability of Δ^{14}-sterol reductase inhibition.[3]

UCA1064-B showed weaker antifungal activity against *Saccharomyces cerevisiae* (MTU 09001) than UCA1064-A (=A25822B) (MIC; 0.39 μg/ml, 0.05 μg/ml, respectively, by agar dilution method). Both compounds exhibited weak antimicrobial activities against Gram-positive bacteria at 40 μg/ml, but not against Gram-negative bacteria. They showed the antiproliferative activities against HeLa S3 cells (IC_{50} value; A, 12.7 μM and B, 14.8 μM, respectively). In addition, UCA1064-B was effective against mouse mammary tumor SC-4 inoculated into nude mice by the treatment iv at the maximum tolerated dose for 4 consecutive days (T/C=0.49 at 15 mg/kg × 4), while UCA1064-A was less effective under the same conditions (T/C=0.51).

The results of our work show that UCA1064-B is a new azasteroid antibiotic with antimicrobial and antitumor potency. Further detailed studies on biological activity, antitumor spectra and toxicity of UCA1064-B are in progress and will be reported in due course.

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