JUGLORIN, A NEW SPERMIDINE SYNTHASE INHIBITOR

Sir:

It is well known that polyamines are closely related to the promotion, proliferation and metastasis of cancer cells[1~3]. For this reason, much effort has been made to develop specific inhibitors of polyamine synthesis[4,5], especially spermidine synthase[6]. Thus, 5'-methylthioadenosine (MTA)[6], dicyclohexylamine[7] and *N*-chlorosulfonyl-dicyclohexylamine (CSD)[8] were found to be potent inhibitors of spermidine synthase. CSD showed an anti-proliferative effect against Ehrlich ascites carcinoma and an anti-metastatic effect against Lewis lung cancer[8].

In order to find new inhibitors of spermidine synthase, we employed an assay system consisting of putrescine, decarboxylated *S*-adenosylmethionine (DCSAM) and enzyme protein as described by SAMEJIMA *et al.*[9].

Spermidine synthase used in our experiments was partially purified from bovine brain by ammonium sulfate fractionation[9]. DCSAM was prepared by treatment of SAM with SAM decarboxylase of *Escherichia coli*[10] followed by purification by HPLC[11]. The reaction was traced by determining the amount of spermidine formed by HPLC and monitoring the fluorescence at 540 nm[9].

As a result of our screening using the above mentioned system, an active compound, juglorin, was isolated from the culture filtrate of *Streptomyces malachiticus* strain No. S-1998.

Fermentation was carried out at 27°C for 3 days in two 360-liter jar fermentors containing the following medium; glycerol 3.0%, corn steep liquor 1.0%, dry yeast 0.3%, NaCl 0.5% and $CaCO_3$ 0.35%.

The fermentation broth (430 liters) was filtered and adsorbed on a Diaion HP-20 (20 liters) column, which was, after being washed with water (200 liters) and 30% MeOH, eluted with 100% MeOH. The eluate was concentrated *in vacuo* to a small volume and extracted with EtOAc (50 liters). The organic layer was concentrated *in vacuo* to 8 liters, washed with 0.01 N HCl (8 liters) and water, and finally extracted with saturated $NaHCO_3$ solution. The aqueous extract was adjusted to pH 2.0 with 1 N HCl and extracted again with EtOAc. The organic layer was concentrated *in vacuo* and the residual dry material was subjected to silica gel column chromatography. The active fraction eluted with chloroform - acetone (3 : 1) was evaporated *in vacuo* to give a crude material, which was further purified by Sephadex LH-20 column chromatography developed with MeOH. The active eluate was evaporated to dryness to give 200 mg of juglorin.

The UV and IR spectral data suggest the presence of a substituted 5-hydroxy-1,4-benzoquinone moiety (juglone[12], λ_{max} 249 and 425 nm) and a carboxyl function (1755 cm^{-1}) in juglorin. The molecular formula of juglorin was established to be $C_{20}H_{20}O_7$ based on high resolution electron ion (HREI) mass spectral data (found: *m/z* 372.1224, calcd: 372.1209). The ^1H NMR

Fig. 1. Partial structures of juglorin.

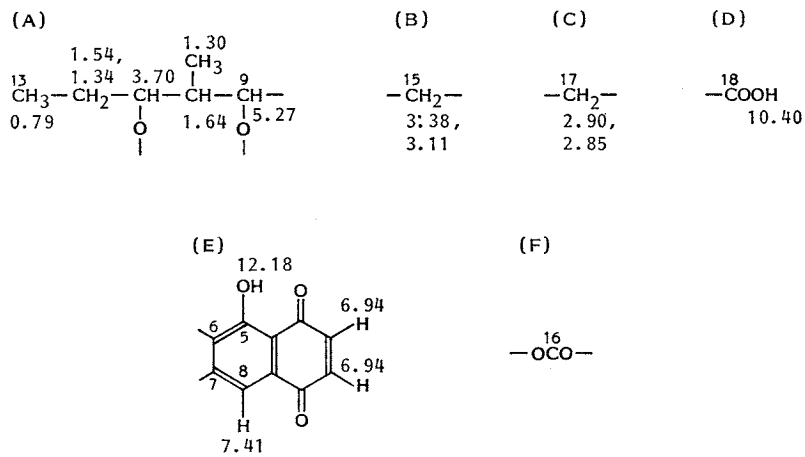

Table 1. ^{13}C NMR spectral data of juglorin (100 MHz, $CDCl_3$).

Carbon†			ppm
C=O region:	–CO–	1	189.5
		4	183.5
	–COOH	18	171.2
sp^2 region:	–C=	5	156.5
		7	143.3
		6	131.6
		8a	129.8
		4a	112.6
	–CH=	2	139.2
		3	138.3
		8	117.7
sp^3 region:	OCO	16	95.7
	CHO	11	72.6
		9	72.3
	CH	10	34.0
	CH_2	17	46.6
		15	37.0
		12	25.6
	CH_3	14	11.8
		13	9.8

† The assignments were performed by C-H correlation and INEPT analysis.

spectrum suggested the presence of the following partial structures (Fig. 1); (A) $CH_3CH_2CHCH(CH_3)CH$–, (B) $-CH_2-$, (C) $-CH_2-$, (D) –COOH and (E) a juglone moiety. The ^{13}C NMR spectrum (Table 1) revealed the presence of an additional function (F) –OCO–. The assignments of ^1H and ^{13}C NMR spectra were established by the aid of ^1H-^1H and ^{13}C-^1H shift correlated spectroscopies. These moieties were connected by long range selective proton decoupling (LSPD) experiments. Long range couplings were observed between the 9-H methine proton in (A) and C-5 and C-7 carbons in (E), and between 15-H methylene protons in (B) and C-6, C-7 and C-8 in (E), C-16 in (F) and C-17 in (C) carbons (see Fig. 1). The assignments of C-5, C-6, C-7 and C-8 were ensured by comparison with that of the ^{13}C NMR spectrum of juglone[13]. Thus, the structure of juglorin was determined as shown in Fig. 2.

Irradiation of the 3.73 ppm methine signal (11-H) resulted in a nuclear Overhauser effect at the 15a-H signal (3.11 ppm) suggesting the close stereochemical relationship between these protons (Fig. 3). The rest of the relative configuration of juglorin was determined by consideration of the possible structure of the molecular model as shown in Fig. 3.

Fig. 2. The structure of juglorin.

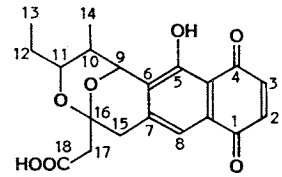

Fig. 3. The relative stereochemistry of juglorin.

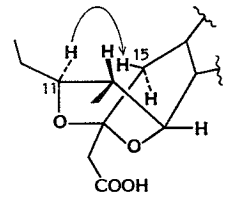

Table 2. Inhibition of spermidine synthase.

	Inhibition (%)		
	1 mM	0.5 mM	0.1 mM
Juglorin	80	60	30
5′-Methylthioadenosine	95	70	50
Dicyclohexylamine	100	97	95

The structure of juglorin is unique among known antibiotics containing a juglone moiety such as griseusin[14], nanaomycin[15] and juglomycin[16] (side chains or ring systems connected with the quinone ring), in that juglorin has a ring system on the aromatic ring.

The inhibitory activity of juglorin in our assay system on spermidine synthesis is compared with the known inhibitors mentioned above[6,7] in Table 2. Juglorin inhibited spermidine synthase almost as strongly as 5′-methylthioadenosine, but its activity was weaker than that of dicyclohexylamine.

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References

1) SCALABRIO, G. & M. E. FERIOLI: Polyamines in mammalian tumors. Part I. Adv. Cancer Res. 35: 151~268, 1981
2) SCALABRIO, G. & M. E. FERIOLI: Polyamines in mammalian tumors. Part II. Adv. Cancer Res. 36: 1~102, 1982
3) TABOR, C. W. & H. TABOR: Polyamines. Ann. Rev. Biochem. 53: 749~790, 1984
4) METCALF, B. W.; P. BEY, C. DANZIN, M. J. JUNG, P. CASARA & J. P. VEVERT: Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogues. J. Am. Chem. Soc. 100: 2551~2553, 1978
5) WILLIAMS-ASHMAN, H. G. & A. SCHENONE: Methyl glyoxal bis(guanylhydrazone) as a potent inhibitor of mammalian and yeast *S*-adenosylmethionine decarboxylases. Biochem. Biophys. Res. Commun. 46: 288~295, 1972
6) HIBASAMI, H.; R. T. BORCHART, S. Y. CHEM, J. K. COWARD & A. E. PEGG: Studies of inhibition of rat spermidine synthase and spermine synthase. Biochem. J. 187: 418~428, 1980
7) HIBASAMI, H.; M. TANAKA, J. NAGAI & T. IKEDA: Dicyclohexylamine, a potent inhibitor of spermidine synthase in mammalian cells. FEBS Lett. 116: 99~101, 1980
8) HIBASAMI, H.; T. TSUKUDA & K. NAKASHIMA: Inhibition of tumor growth and metastasis by a spermidine synthase inhibitor, *N*-chlorosulfonyl-dicyclihexylamine. *In* Polyamines; Basic and Clinical Aspects. *Ed.*, K. IMAHORI *et al.*, pp. 243~250, VNU Science Press, Amsterdam, 1985
9) SAMEJIMA, K.; A. RAINA, B. YAMAHAMA & T. ELORANTA: Purification of putrescine aminopropyltransferase (spermidine synthase) from eukaryotic tissues. *In* Methods in Enzymology. Vol. 94., *Eds.*, H. TABOR & C. W. TABOR, pp. 270~276, Academic Press Inc., New York, 1983
10) PEGG, A. E. & R. A. BENNET: Determination of cellular decarboxylated *S*-adenosylmethionine content. *In* Methods in Enzymology. Vol. 94., *Eds.*, H. TABOR & C. W. TABOR, pp. 69~72, Academic Press Inc., New York, 1983
11) MARKHAM, G. D.; C. W. TABOR & H. TABOR: *S*-adenosylmethionine decarboxylase (*Escherichia coli*). *In* Methods in Enzymology. Vol. 94., *Eds.*, H. TABOR & C. W. TABOR, pp. 228~230, Academic Press Inc., New York, 1983
12) MORTON, R. A. & W. T. EARLAM: Absorption spectra in relation to quinones: 1,4-naphthoquinone, anthraquinone, and their derivatives. J. Chem. Soc. 1941: 159~169, 1941
13) CASEY, M.L.; R.C. PAULICK & H.W. WHITLOCK, Jr.: A carbon-13 nuclear magnetic resonance study of mollisin and its biosynthesis. J. Am. Chem. Soc. 98: 2636~2640, 1976
14) TSUJI, N.; M. KOBAYASHI, Y. WAKISAKA, Y. KAWAMURA, M. MAYAMA & K. MATSUMOTO: New antibiotics, griseusins A and B, isolation and characterization. J. Antibiotics 29: 7~9, 1976
15) TANAKA, H.; Y. KOYAMA, T. NAGAI, H. MARUMO & S. ŌMURA: Nanaomycins, new antibiotics produced by a strain of *Streptomyces*. II. Structure and biosynthesis. J. Antibiotics 28: 868~875, 1975
16) TANAKA, N.; H. OGATA, K. USHIYAMA & H. ONO: New antibiotics, juglomycins. II. The structure of juglomycins A and B. Jpn. J. Antibiotics 24: 222~224, 1971