Communications to the Editor

STUDIES ON FREE RADICAL SCAVENGING SUBSTANCES FROM MICROORGANISMS

I. CARAZOSTATIN, A NEW FREE RADICAL SCAVENGER PRODUCED BY *STREPTOMYCES CHROMOFUSCUS* DC 118

Sir:

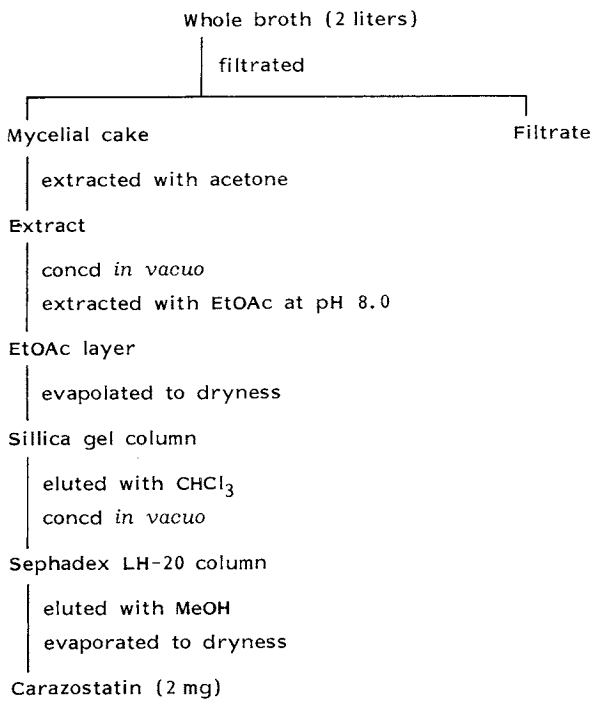
In the course of our screening program for free radical scavenging substances from microorganisms, which are expected to be useful as therapeutic reagents for myocardial and cerebral ischemia[1,2], atherosclerosis[3] and inflammation[4], we have isolated a novel substance named carazostatin from the culture of streptomycete. Carazostatin has shown a strong inhibitory activity against lipid peroxidation induced by free radicals in rat brain homogenate.

The carazostatin producing organism, identified as a *Streptomyces chromofuscus*, was cultivated on a rotary shaker at 30°C for 6 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of glycerol 3.0%, fish meal 2.0% and calcium carbonate 0.2%. The active material was isolated according to the scheme shown in Fig. 1. Further purification was achieved by semi-preparative HPLC (ODS) with MeOH-1% triethylamine in aqueous phosphoric acid at pH 2.0 (80:20) by UV detection at 254 nm, if necessary. In HPLC analysis, carazostatin was eluted as a main peak (more than 60% of total peak area), and several small peaks also existed. We estimated them as closely related compounds to carazostatin because they had the same UV spectrum as carazostatin.

Carazostatin is a pale yellowish powder: Molecular formula $C_{20}H_{25}NO$; field desorption (FD)-MS m/z 295 (M^+); mp 149~152°C (dec); UV λ_{max}^{MeOH} nm (ε) 218 (153,000), 235 (141,000), 254 (76,000), 266 (60,000), 303 (83,000), 342 (20,000); IR ν_{max} (KBr) cm^{-1} 3460, 3360, 2910, 2840, 1590, 1500, 1460, 1430, 1310, 1230, 1140, 1060, 830, 770, 740.

The UV spectrum of carazostatin was very similar to that of carbazomycin B[5,6], therefore the existence of carbazole nucleus was suggested as a chro-

Fig. 1. Isolation and purification of carazostatin.



mophore. In the ^1H NMR spectrum of carazostatin, the signals are classified into the three groups as follows: The four aromatic protons δ_H 7.91 (1H, dd, J=8.0 and 1.5 Hz, 5-H), 7.17 (1H, ddd, J=8.0, 8.0 and 1.5 Hz, 6-H), 7.37 (1H, ddd, J=8.0, 8.0 and 1.5 Hz, 7-H) and 7.42 (1H, dd, J=8.0 and 1.5 Hz, 8-H) due to the same ring of the carbazole nucleus; one aromatic proton δ_H 7.31 (1H, s, 4-H), one methyl group δ_H 2.38 (3H, s, 17-H) and the normal-type heptyl side chain δ_H 2.87 (2H, t, J=7.9 Hz, 10-H), 1.65 (2H, m, 11-H), 1.46~1.30 (8H, m, 12-H~15-H) and 0.91 (3H, t, J=7.0 Hz, 16-H) due to the other ring of this nucleus; and two exchangeable protons δ_H 4.72 (1H, br s) and 7.77 (1H, br s) ascribed to phenolic OH and NH on the carbazole nucleus, respectively. The chemical shifts due to the five aromatic protons of carazostatin are closely related to those of deoxycarbazomycin B[6].

For an elucidation of the structure of carazostatin, *N*,*O*-dimethylcarazostatin was prepared by dimethylation of carazostatin with MeI in the presence of NaH in DMF. In the ^1H NMR spectrum of this compound, two new signals at δ_H 4.05 (3H, s) and 3.95 (3H, s) appeared which could be assigned to *N*-methyl and *O*-methyl groups, respectively. As the nuclear Overhauser effects (NOE's) were remarkably observed as shown in Fig. 2, the structure of carazostatin has been determined to 1-*n*-heptyl-3-hydroxy-2-methylcarbazole (Fig. 3).

The assignment of ^{13}C chemical shifts was confirmed as shown in Table 1 by the analyses of ^1H-^{13}C correlation spectroscopy and heteronuclear multiple bond correlation spectra.

Fig. 2. The NOE's in the structure of *N*,*O*-dimethylcarazostatin (arrows).

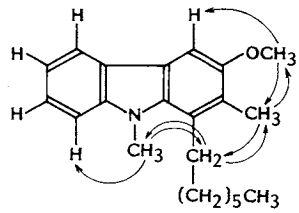
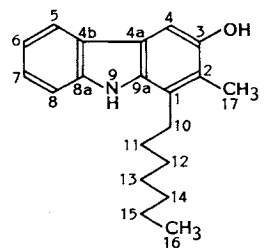


Fig. 3. Structure of carazostatin.



As far as we know, carazostatin is the first carbazole derivative having a long alkylated chain to be isolated in nature. In addition, it is interesting to note that the strain which produces carazostatin does not produce any type of carbazomycins under the condition described above.

The inhibitory effect of carazostatin on the lipid peroxidation induced by free radicals generated in the presence of Fe^{2+} (10 μM) and ascorbic acid (100 μM) in rat brain homogenate is shown in Table 2. IC_{50} value of carazostatin (0.172 μM) indicates

Table 1. ^{13}C chemical shifts of carazostatin in $CHCl_3$-d_1.

Carbon	δ_C
1	124.1
2	121.4
3	148.2
4	103.0
4a	120.9
4b	123.7
5	120.0
6	118.9
7	125.2
8	110.6
8a	139.8
9a	134.0
10	28.8
11	29.5
12	30.0[a]
13	29.3[a]
14	31.7[a]
15	22.7[a]
16	14.1
17	12.0

[a] Tentatively assigned.

Table 2. Inhibitory effects of carazostatin and other compounds on lipid peroxidation in rat brain homogenate in the presence of Fe^{2+} and ascorbic acid.

Drug	Conc (μM)	Inhibition (%)
Carazostatin	1.0	100
	0.3	97.5
	0.1	7.8
Flunarizine·2HCl	100	62.7
	30	38.1
	10	8.8
BHT	10	99.4
	3	18.5
	1	0

The inhibitory effects of each drug were measured according to the method of KUBO *et al.*[7] in the presence of Fe^{2+} (10 μM) and ascorbic acid (100 μM).

that the compound is much more active than flunarizine (55.0 μM) which is a brain protective reagent with free radical scavenging activity[7], and that of butylated hydroxytoluene (BHT; 4.90 μM) which is a well known antioxidant. These results suggest that carazostatin may be useful for the alleviation of tissue damage due to generation of free radicals such as superoxide radical and subsequent peroxidative disintegration of cell membranes.

Further detailed results will be reported in future.

SHINICHIRO KATO
HIROYUKI KAWAI
TOMIKO KAWASAKI
YOICHIRO TODA
TETSURO URATA
YOICHI HAYAKAWA†

Pharmaceutical Laboratory,
Kirin Brewery Co., Ltd.,
Takasaki, Gunma 370-12, Japan

(Received August 23, 1989)

† Present address: Institute of Applied Microbiology, the University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

References

1) HESS, M. L.; G. T. ROWE, M. CAPLAN, J. L. ROMSON & B. LUCCHESI: Identification of hydrogen peroxide and hydroxyl radicals as mediators of leukocyte-induced myocardial dysfunction. Limitation of infarct size with neutrophil inhibition and depletion. Adv. Myocardiol. 5: 159~175, 1985
2) FLAMM, E. S.; H. B. DEMOPOULOS, M. L. SELIGMAN, R. G. POSER & J. RANSOHOFF: Free radicals in cerebral ischemia. Stroke 9: 445~447, 1978
3) PALINSKI, W.; M. E. ROSENFELD, H. S. YLA, G. C. GURTNER, S. S. SOCHER, S. W. BUTLER, S. PARTHASARATHY, T. E. CAREW, D. STEINBERG & J. L. WITZTUM: Low density lipoprotein undergoes oxidative modification *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 86: 1372~1376, 1989
4) CHEESEMAN, K. H. & L. G. FORNI: An investigation of the novel antiinflamatory agents ONO-3144 and MK-447. Studies on their potential antioxidant activity. Biochem. Pharmacol. 37: 4225~4233, 1988
5) SAKANO, K.; K. ISHIMARU & S. NAKAMURA: New antibiotics, carbazomycins A and B. I. Fermentation, extraction, purification and physico-chemical and biological properties. J. Antibiotics 33: 683~689, 1980
6) SAKANO, K. & S. NAKAMURA: New antibiotics, carbazomycins A and B. II. Structural elucidation. J. Antibiotics 33: 961~966, 1980
7) KUBO, K.; I. YOSHITAKE, Y. KUMADA, K. SHUTO & N. NAKAMIZO: Radical scavenging action of flunarizine in rat brain *in vitro*. Arch. Int. Pharmacodyn. Ther. 272: 283~295, 1984