Communications to the Editor

STUDIES ON FREE RADICAL SCAVENGING SUBSTANCES FROM MICROORGANISMS

II. NEOCARAZOSTATINS A, B AND C, NOVEL FREE RADICAL SCAVENGERS

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, from the culture of *Streptomyces* sp. strain GP 38, novel substances named neocarazostatins A, B and C. These compounds are structurally similar to carazostatin which we reported recently[5]. Neocarazostatins A, B and C have shown strong inhibitory activities against lipid peroxidation induced by free radicals in rat brain homogenates.

The organism which produced neocarazostatins A, B and C was cultivated on a rotary shaker at 27°C for 5 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of glycerol 3.0%, fish

Fig. 1. Isolation procedures of neocarazostatins A, B and C.

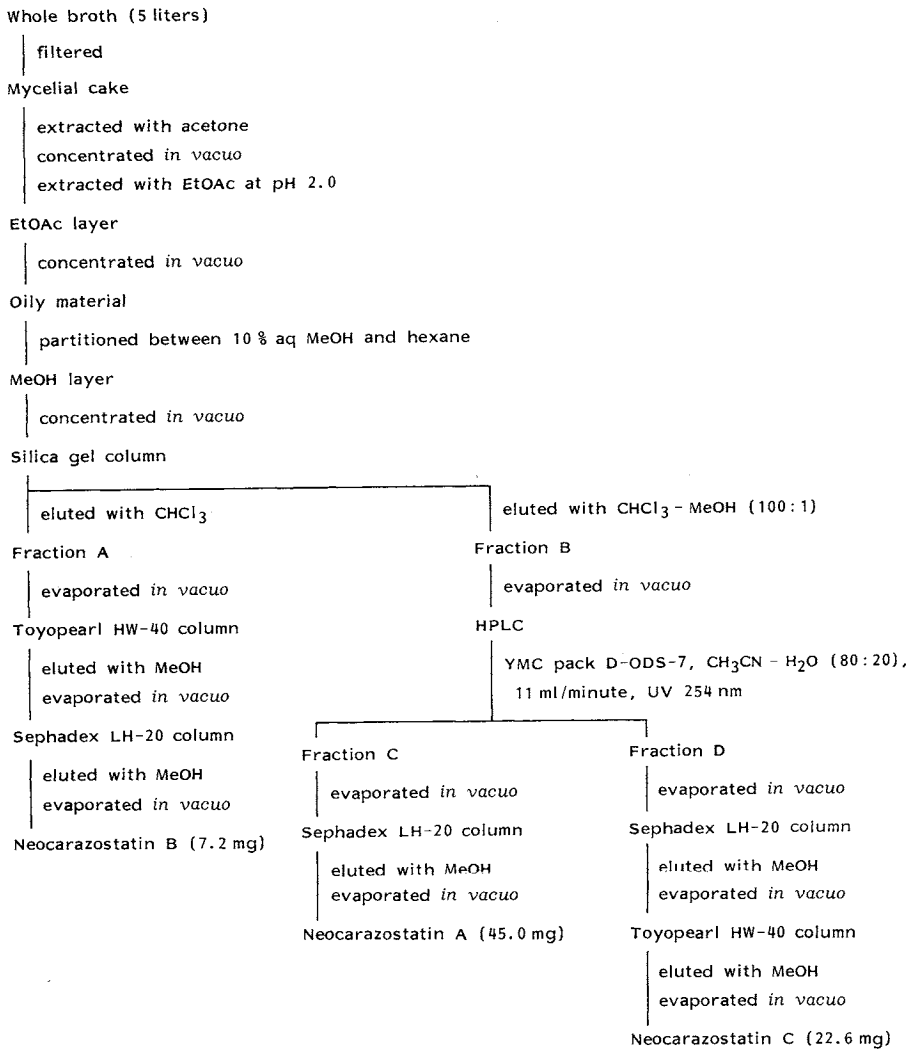


Table 1. Physico-chemical properties of neocarazostatins A, B and C.

	Neocarazostatin A	Neocarazostatin B	Neocarazostatin C
$[\alpha]_D^{25}$ (c 0.1 MeOH)	−36°	−24°	−92°
MP (°C, dec)	141~143	55~57	80~82
Molecular formula	$C_{22}H_{27}NO_4$	$C_{22}H_{27}NO_3$	$C_{23}H_{29}NO_4$
MS m/z (M^+)	369	353	383
UV λ_{max}^{MeOH} nm (ε)	230 (43,700), 250 (65,900), 271 (23,800), 292 (26,800), 331 (6,900), 345 (8,400)	228 (43,700), 249 (65,900), 270 (23,700), 292 (26,800), 331 (6,900), 344 (8,400)	231 (43,800), 250 (65,800), 271 (23,700), 292 (26,900), 331 (6,900), 345 (8,400)
IR (KBr) ν_{max} cm^{-1}	3440, 2970, 2940, 1620, 1590, 1500, 1480, 1450, 1420, 1310, 1120, 1050, 810	3350, 2930, 2880, 1605, 1590, 1495, 1470, 1440, 1400, 1300, 1110, 990, 800	3460, 2920, 1610, 1590, 1500, 1480, 1440, 1410, 1310, 1130, 990, 810

Table 2. ^1H chemical shifts of neocarazostatins A, B and C in CD_3OD.

Carbon No.[a]	Neocarazostatin A	Neocarazostatin B	Neocarazostatin C
5	7.97 (1H, d, J=1.2)	7.97 (1H, d, J=1.3)	8.00 (1H, d, J=1.2)
7	7.09 (1H, dd, J=8.5, 1.2)	7.08 (1H, dd, J=8.5, 1.3)	7.10 (1H, dd, J=8.5, 1.2)
8	7.28 (1H, d, J=8.5)	7.28 (1H, d, J=8.5)	7.30 (1H, d, J=8.5)
10	4.92 (1H, d, J=8.0)	2.93 (1H, dd, J=14.5, 6.7), 3.03 (1H, dd, J=14.5, 6.5)	4.53 (1H, d, J=7.9)
11	4.22 (1H, dq, J=8.0, 6.4)	4.08 (1H, ddq, J=6.7, 6.5, 6.2)	4.26 (1H, dq, J=7.9, 6.4)
12	1.03 (3H, d, J=6.4)	1.21 (3H, d, J=6.2)	1.01 (3H, d, J=6.4)
13	2.42 (3H, s)	2.40 (3H, s)	2.43 (3H, s)
14	3.74 (3H, s)	3.77 (3H, s)	3.76 (3H, s)
15	3.45 (2H, d, J=7.3)	3.45 (2H, d, J=7.3)	3.46 (2H, d, J=7.3)
16	5.41 (1H, t, J=7.3)	5.41 (1H, t, J=7.3)	5.41 (1H, t, J=7.3)
18	1.76 (3H, s)	1.76 (3H, s)	1.76 (3H, s)
19	1.78 (3H, s)	1.78 (3H, s)	1.78 (3H, s)
20	—	—	3.25 (3H, s)

[a] Referred to Fig. 4.
J=Hz.

meal 2.0% and calcium carbonate 0.2%. The active materials were isolated according to the scheme shown in Fig. 1.

Physico-chemical properties of neocarazostatins A, B and C are shown in Table 1.

Since the UV absorption spectra of neocarazostatins are very similar to that of carazostatin[5] and those of carbazomycins[6,7], the existence of a carbazole nucleus was suggested in each compound. The ^1H and ^{13}C NMR spectra of neocarazostatins are shown in Tables 2 and 3, respectively.

In the ^{13}C NMR spectrum, twelve signals due to the carbazole nucleus in neocarazostatin A were almost identical to those of the analogous carbons in the spectrum of carbazomycin B, except for the signals assigned to C-1 and C-6. Furthermore, the signals due to 6-H and 10-CH_3 in the ^1H NMR spectrum of carbazomycin B could not be found in that of neocarazostatin A. These results suggested that the chromophore of neocarazostatin A could be of the 1,6-disubstituted carbazomycin B type. The ^1H, ^{13}C NMR and ^1H-^1H COSY of neocarazostatin A indicated the existence of partial structures of **1**, **2** and two methyl groups in it as shown in Fig. 2. The heteronuclear multiple-bond correlation (HMBC) spectrum of neocarazostatin A showed long range couplings of 10-H (CH) to C-1, 2 and 9a, and 15-H (CH_2) to C-5, 6 and 7. Therefore, the attachments of the partial structure **1** to C-1, and **2** to C-6 were confirmed as shown in Fig. 3. Since the HMBC spectrum also provided long range couplings of the 18-CH_3 and 19-CH_3 to C-17, the partial structure **2** was identified as an isoprenyl side chain. From these results, the structure of neocarazostatin A was determined as shown in Fig. 4.

Since the molecular formula of neocarazostatins A and B were deduced to be $C_{22}H_{27}NO_4$ and $C_{22}H_{27}NO_3$, respectively, neocarazostatin B was

Table 3. ^{13}C chemical shifts of neocarazostatins A, B and C, and carbazomycin B.

Carbon No.[a]	Neocarazostatin			Carbazomycin B[b]
	A	B	C	
1	114.8 (s)	112.0 (s)	111.2 (s)	109.7 (s)
2	127.7 (s)	127.6 (s)	126.1 (s)	127.8 (s)
3	139.5 (s)	139.7 (s)	139.5 (s)	139.3 (s)
4	145.0 (s)	144.6 (s)	146.0 (s)	143.6 (s)
4a	112.6 (s)	111.5 (s)	112.2 (s)	110.6 (s)
4b	124.0 (s)	124.7 (s)	123.8 (s)	124.3 (s)
5	122.7 (d)	122.7 (d)	122.6 (d)	123.1 (d)
6	132.8 (s)	132.8 (s)	132.9 (s)	119.2 (d)
7	126.1 (d)	125.0 (d)	126.0 (d)	124.8 (d)
8	110.9 (d)	110.8 (d)	110.9 (d)	110.0 (d)
8a	139.6 (s)	139.7 (s)	139.5 (s)	140.7 (s)
9a	138.0 (s)	139.3 (s)	137.5 (s)	137.9 (s)
10	76.5 (d)	38.6 (t)	86.6 (d)	13.3 (q; 1-CH_3)
11	71.4 (d)	69.0 (d)	70.5 (d)	—
12	19.9 (q)	23.0 (q)	19.2 (q)	—
13 (2-CH_3)	13.2 (q)	13.1 (q)	13.2 (q)	12.8 (q)
14 (3-OCH_3)	61.4 (q)	61.3 (q)	61.4 (q)	61.3 (q)
15	35.5 (t)	35.5 (t)	35.4 (t)	—
16	126.1 (d)	126.1 (d)	126.1 (d)	—
17	132.1 (s)	132.0 (s)	132.0 (s)	—
18	18.0 (q)	17.9 (q)	17.9 (q)	—
19	26.0 (q)	26.0 (q)	26.0 (q)	—
20	—	—	56.9 (q)	—

The spectra of neocarazostatins and carbazomycin B were run in CD_3OD and $(CD_3)_2CO$ as a solvent, respectively.

[a] Referred to Fig. 4.

[b] Cited from the data by NAID *et al.*[7].

Fig. 2. Partial structures of neocarazostatin A.

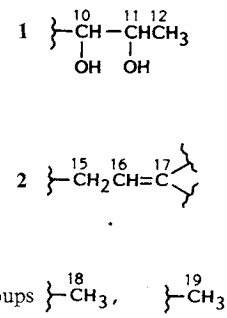
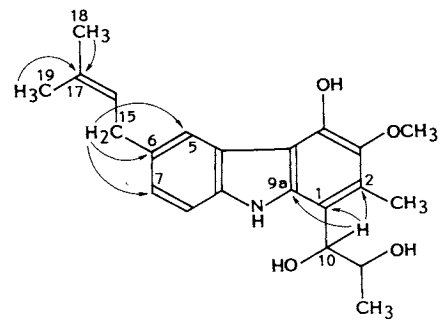


Fig. 3. HMBC experiment of neocarazostatin A (arrows).

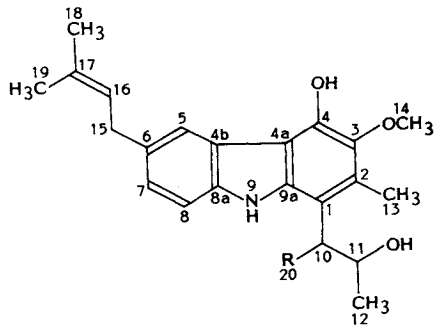


proposed to be a deoxy-derivative of neocarazostatin A. In the ^1H and ^{13}C NMR spectra of neocarazostatins A and B, the signals due to 10-H (δ_H 4.92, vs. 2.93 and 3.03, respectively) and C-10 (δ_C 76.5 vs. 38.6, respectively) were different between these two compounds. Accordingly neocarazostatin B is 10-deoxyneocarazostatin A.

The ^1H and ^{13}C NMR spectra of neocarazostatin C were very similar to those of neocarazostatin A except for the existence of one methoxy group (δ_H 3.25, δ_C 56.9). The HMBC spectrum of neocarazostatin C revealed the long range coupling of the *O*-methyl protons to C-10. Therefore, neocarazostatin C is 10-OCH_3 derivative of neocarazostatin A and may be an artifact derived from neocarazostatin A.

The structures of the neocarazostatins A, B and C, as determined, are shown in Fig. 4; the relative

Fig. 4. Structures of neocarazostatins A, B and C.



Neocarazostatin A R=OH
Neocarazostatin B R=H
Neocarazostatin C R=OCH_3

Table 4. Inhibitory effects of neocarazostatins A, B and C and other compounds on lipid peroxidation in rat brain homogenate in the presence of Fe^{2+} and ascorbic acid.

Drug	Conc (μM)	Inhibition (%)
Neocarazostatin A	1.0	99.2
	0.3	12.7
	0.1	0.0
Neocarazostatin B	1.0	100.0
	0.3	40.1
	0.1	2.4
Neocarazostatin C	1.0	100.0
	0.3	36.2
	0.1	0.0
Flunarizine·2HCl	100	62.7
	30	38.1
	10	8.8
BHT	10	99.4
	3	18.5
	1	0.0

The inhibitory effect of each drug was measured according to the method of KUBO *et al.*[8] in the presence of Fe^{2+} (10 μM) and ascorbic acid (100 μM).

stereochemistry is under study.

The inhibitory effects of neocarazostatins A, B and C 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 was shown in Table 4. IC_{50} values of neocarazostatin A (0.509 μM), neocarazostatin B (0.370 μM) and neocarazostatin C (0.390 μM) were much lower than that of flunarizine (55.0 μM) which is a brain protective reagent with free radical scavenging activity[8], and that of butylated hydroxytoluene (BHT; 4.90 μM) which is a well known antioxidant. They are similar to that of carazostatin (0.172 μM)[5].

Besides carazostatin and neocarazostatins, antiostatins[9], which have a carbazole nucleus in each structure, were reported as free radical scavenging substances.

Our results suggest that neocarazostatins A, B and C 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.

SHINICHIRO KATO
KAZUTOSHI SHINDO
YOKO KATAOKA
YUJI YAMAGISHI
JUNICHIRO MOCHIZUKI

Pharmaceutical Laboratory,
Kirin Brewery Co., Ltd.,
3 Miyahara-cho, Takasaki-shi,
Gunma 370-12, Japan

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