Note

A New Series of Fluorine-containing Derivatives of Ascorbic Acid with Phage-inactivating Activity[†]Akira MURATA, Hidetoshi TASHIRO,
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The phage-inactivating activity of L-ascorbic acid has been well documented.^{1~3)} In an attempt to obtain ascorbic acid derivatives with greater activity, we synthesized a series of ascorbic acid derivatives and tested them as to their inactivating activity against a wide variety of phages. In 117 tests involving 13 derivatives vs. 9 phages, only 2 (2-*O*-octanoyl-L-ascorbic acid vs. phage MS2 and 2,6-di-*O*-benzyl-L-ascorbic acid vs. phage T5) showed greater activity than ascorbic acid.⁴⁾

Fluorine is the most electronegative and the most reactive of all the elements. Chemicals containing fluorine exhibit a variety of biological activities and are the subject of attention in many fields.⁵⁾

We have therefore designed and synthesized a new series of fluorine-containing derivatives of ascorbic acid and tested them as to their inactivating activity against a wide variety of phages.

Table I lists the 8 compounds synthesized. The com-

pounds were synthesized according to the procedures of Tanaka *et al.*⁶⁾ and Nomura *et al.*⁷⁾ but polyfluorocarboxylic acids or polyfluoroacyl chlorides were used instead of carboxylic acids or acyl chlorides, respectively.

7 was prepared by the reaction of ascorbic acid with an equimolar amount of perfluorooctanoic acid in sulfuric acid. mp 150~153°C. IR $\nu_{\max}$ (KBr disk) cm^{-1} : 3350, 3250, 1775, 1760, 1670, 1210, 1140. **8** was prepared by the reaction of ascorbic acid with perfluorooctanoyl chloride (2 equiv.) in acetonitrile. mp 162~165°C. IR $\nu_{\max}$ (KBr disk) cm^{-1} : 3400, 1785, 1760, 1675, 1240, 1205, 1145. **9** was prepared by the reaction of 5,6-*O*-isopropylidene-L-ascorbic acid with an equimolar amount of perfluorooctanoyl chloride in acetonitrile, followed by hydrolysis in the presence of hydrochloric acid. mp 155~158°C. IR $\nu_{\max}$ (KBr disk) cm^{-1} : 3550, 3350, 1780, 1680, 1660, 1200, 1140.

11 was prepared by the same procedure as that for **9**, but 7, H-dodecafluoroheptanoyl chloride was used. mp 118~119°C. IR $\nu_{\max}$ (KBr disk) cm^{-1} : 3550, 3300, 1780, 1675, 1660, 1205, 1145. **12** was prepared by the same procedure as that for **8**, but 7, H-dodecafluoroheptanoyl chloride was used. mp 122~124°C. IR $\nu_{\max}$ (KBr disk) cm^{-1} : 3400, 1770, 1750, 1670, 1195, 1140. **18** was prepared by the same procedure as that for **7**, but 7, H-dodecafluoroheptanoic acid was used. mp 111~113°C. IR $\nu_{\max}$ (KBr disk) cm^{-1} : 3400, 3250, 1775, 1755, 1675, 1200, 1140.

The IR data were consistent with the structures, and the data obtained on elemental analysis were within 0.5% of the calculated values. The details of the syntheses and physicochemical properties of the compounds will be published elsewhere.

The phages used were double-stranded DNA phages (J1 of *Lactobacillus casei*, T3, T4 and T5 of *Escherichia coli*, and M2 of *Bacillus subtilis*), single-stranded DNA phages (ϕ X174 and δ A of *Escherichia coli*), a single-stranded RNA phage (MS2 of *Escherichia coli*) and a double-stranded RNA phage (ϕ 6 of *Pseudomonas*

TABLE I. FLUORINE-CONTAINING DERIVATIVES OF ASCORBIC ACID

Compound No.	Compound name
7	6- <i>O</i> -Perfluorooctanoyl-L-ascorbic acid
8	2,6-Bis- <i>O</i> -perfluorooctanoyl-L-ascorbic acid
9	2- <i>O</i> -Perfluorooctanoyl-L-ascorbic acid
10	2,3-Bis- <i>O</i> -(1,1,2,3,3,3-hexafluoropropyl)-L-ascorbic acid
11	2- <i>O</i> -(7, H-Dodecafluoroheptanoyl)-L-ascorbic acid
12	2,6-Bis- <i>O</i> -(7, H-dodecafluoroheptanoyl)-L-ascorbic acid
18	6- <i>O</i> -(7, H-Dodecafluoroheptanoyl)-L-ascorbic acid
23	3- <i>O</i> -(2-Perfluoroheptyl)ethyl-5,6- <i>O</i> -isopropylidene-L-ascorbic acid

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TABLE II. INACTIVATING ACTIVITY OF FLUORINE-CONTAINING DERIVATIVES OF ASCORBIC ACID TOWARD A WIDE VARIETY OF PHAGES

Compound No.	Concn. (M)	Survival (%)								
		Phage								
		J1	T5	T4	M2	T3	$\phi \times 174$	δA	MS2	$\phi 6$
1 ^a	1×10^{-3}	0~1	40~50	70~80	5~10	0~1	0~1	30~40	0~1	40~50
	3×10^{-4}	10~15	60~80	90~100	30~50	5~10	3~6	60~80	10~20	60~80
	1×10^{-4}	20~30	90~100	100	60~80	20~30	15~20	90~100	40~60	90~100
7	1×10^{-3}	0	30~40	40~50	2~4	0	0	20~30	0~1	20~30
	3×10^{-4}	5~10	50~60	80~90	10~20	2~4	1~2	40~60	3~6	50~60
	1×10^{-4}	15~20	70~90	90~100	40~60	10~20	10~15	70~90	15~30	70~90
8	1×10^{-3}	0	20~30	30~40	0~1	0	0	10~20	0	10~20
	3×10^{-4}	0~1	40~50	60~80	5~10	0~1	0~1	30~40	0~1	40~50
	1×10^{-4}	10~15	60~80	90~100	30~50	5~10	5~10	60~80	10~20	60~80
9	1×10^{-3}	0~1	40~50	70~80	5~10	0~1	0~1	30~40	0~1	40~50
	3×10^{-4}	10~15	60~80	90~100	30~50	5~10	3~6	60~80	10~20	60~80
	1×10^{-4}	20~30	90~100	100	60~80	20~30	15~20	90~100	40~60	90~100
10	1×10^{-3}	90~100	90~100	90~100	90~100	90~100	90~100	90~100	90~100	90~100
11	1×10^{-3}	0~1	30~40	60~70	3~6	0~1	0~1	25~40	0~1	30~40
	3×10^{-4}	5~10	50~70	80~90	20~40	3~6	2~4	50~60	5~10	50~70
	1×10^{-4}	15~20	80~90	90~100	50~70	15~30	10~20	70~90	20~40	80~90
12	1×10^{-3}	0	20~30	30~40	0~1	0	0	10~20	0	10~20
	3×10^{-4}	0~1	40~50	60~80	5~10	1~2	0~1	30~40	0~1	40~50
	1×10^{-4}	10~15	60~80	90~100	30~50	10~15	5~10	60~80	10~20	60~80
18	1×10^{-3}	0	30~40	40~50	2~4	0	0	20~30	0~1	20~30
	3×10^{-4}	5~10	50~70	80~90	10~20	2~4	1~2	40~60	3~6	50~60
	1×10^{-4}	15~20	80~90	90~100	40~60	15~30	10~20	70~90	15~30	70~80
23	1×10^{-3}	90~100	90~100	90~100	90~100	90~100	90~100	90~100	90~100	90~100

Phages ($1 \sim 4 \times 10^7$ PFU/ml) were incubated with different concentrations of each compound (dissolved in ethanol) in 0.02 M Tris-HCl buffer (pH 7.4) for 30 min at 37°C. The reaction mixture contained 5% ethanol, but this concentration of ethanol had no effect on the phages except $\phi 6$. Phage $\phi 6$ was incubated for 20 min at 25°C in the buffer containing 2% ethanol. The survival of the control without a compound is taken as 100%.

^a L-Ascorbic acid, for reference.

phageolicola).

These 8 compounds were tested as to their inactivating activity against the 9 phages. The results are shown in Table II. Compound 8 and 12, C(2) and C(6)-disubstituted derivatives, exhibited about 3-fold greater activity than ascorbic acid toward all of the phages. Compounds 7 and 18, C(6)-monosubstituted derivatives, exhibited about 2-fold greater activity toward all of the phages. Compounds 9 and 11, C(2)-monosubstituted derivatives, had essentially the same activity as ascorbic acid. On the other hand, 10, a C(2) and C(3)-disubstituted derivative, and 23, a C(3)-monosubstituted derivative, showed no inactivating activity at all toward any of the phages.

These results indicate that the introduction of substituents containing fluorine at C(2) and C(6) or C(6) of ascorbic acid increases the inactivating activity toward a wide variety of phages. The increase in activity can be attributed to the presence of highly reactive fluorine on the substituents, since the introduction of substituents con-

taining no fluorine, in general, does not lead to an increase in the activity.⁴⁾

This series of fluorine-containing compounds appears to be suitable for further development.

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