Syntheses and Anti-inflammatory Activities of *O*-Acyloximes. II

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Novel *O*-acyloximes having an acetyl group or *N*-protected amino acid as an *O*-acyl group were synthesized by reaction with acetyl chloride or by a mixed anhydride method. 4′-Morpholinoacetophenone oxime (oxime-2) was determined to be the (*E*) isomer by X-ray crystal structure analysis. The anti-inflammatory activities of the test compounds were assessed in terms of the inhibitory effect on increased vascular permeability induced by histamine, and several compounds were assessed together by means of the carrageenan-induced paw edema assay. In general, acetyl oximes and *tert*-butyloxycarbonylphenylalanyl oximes showed inhibitory action on increased vascular permeability. Particularly important for the appearance of anti-inflammatory activity was direct attachment of the acetyl group to the oxime. Of the two isoforms of cyclooxygenase (COX-1 and COX-2), COX-1 activity was inhibited by oxime-2 and 4′-piperidinoacetophenone oxime (oxime-3) with IC_{50} values of 50 and 130 μM, respectively, while COX-2 activity was not inhibited. The *in vitro* inhibitory effect of oxime-2 and oxime-3 on COX-1 activity decreased with *O*-acylation of the oximes.

Key words oxime; anti-inflammatory activity; cyclooxygenase inhibition; acetyl oxime; *O*-acyloxime

We have previously synthesized several novel oximes and *O*-acyloximes in work on the development of non-steroidal anti-inflammatory drugs.[1] We found that 4′-morpholinoacetophenone oxime (oxime-2), *o*-methoxyphenylacetone oxime (oxime-5), and a group of propiophenone oxime esters (esters of oxime-1) showed higher anti-inflammatory potency than aspirin in carrageenan-induced rat paw edema assay.

In this paper, we report the extension of these studies to the anti-inflammatory activity of oxime derivatives, that is, acetyl oximes and *N*-protected amino acid-linked oximes. The structures of the oximes in this study were selected on the basis that their skeletons had been found to be effective or interesting in previous studies. The amino acids as acyl groups of the oximes were chosen for the ease with which their structures could be varied at the amino acid-side chain, that is, Phe and Tyr were chosen for their aromatic ring, and Gly and Ala for their small side chain. Since aspirin inhibits cyclooxygenase (COX) activity by acetylation of Ser-530 of the enzyme, the acetyl oximes and *N*- or *O*-acetyl-protected amino acid-linked oximes were presumed to acetylate the hydroxyl group of the serine residue in COX. The COX enzyme is well known to initiate the synthesis of pro-inflammatory prostaglandins from arachidonic acid. Recent investigations have revealed the occurrence of two isoforms of the enzyme (COX-1 and COX-2).[2]

In the synthesis of the acetyl oximes, the corresponding oximes were allowed to react with acetyl chloride in the presence of triethylamine. The *N*-protected amino acid-linked oximes were synthesized from the oximes with an *N*-protected (Ac, Boc, or Fmoc) amino acid (Phe, Tyr(Ac), Ala, or Gly) by a mixed anhydride method using isobutyl chloroformate (IBCF) in the presence of *N*-methylmorpholine (NMM). The coupling reaction was modified for preparation of the Fmoc amino acid-binding oximes, *i.e.*, NMM was added to the reaction mixture after addition of IBCF. The crude product was purified to homogeneity by silica-gel column chromatography and/or recrystallization from appropriate solvents. The structures of the *O*-acyloximes were confirmed by elemental analyses, fast atom bombardment mass spectrometry (FAB-MS) and/or infrared (IR) spectroscopy. The configuration of oxime-2 was obtained by an X-ray crystal structure analysis, which showed that it was the *E*-isomer. The steric structure is shown in Fig. 1 and interatomic distances, angles and fractional coordinates of non-H atoms are presented in Tables 1 and 2.

The structural formulas of the oximes (**1**—**6**) used are shown in Table 3, together with the yield, melting point, spectral and elemental analysis data of the synthetic *O*-acyloximes, as well as the anti-inflammatory activities.

As seen from Table 3, the Ac- and BF-oximes showed inhibitory action on increased vascular permeability by

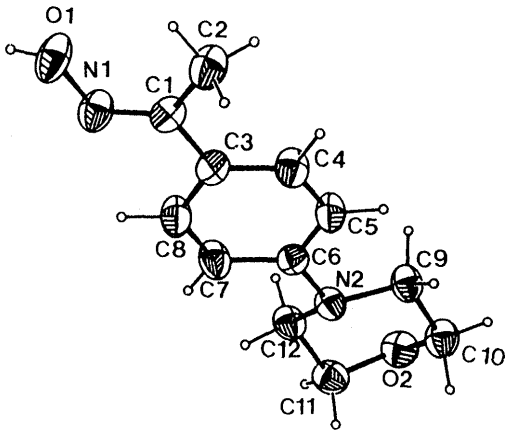

Fig. 1. X-Ray Structure of Oxime-2

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Table 1. Selected Bond Distances (Å) and Angles (deg.) for Oxime-2

Bond distances			
C(1)–N(1)	1.274 (3)	C(1)–C(2)	1.500 (3)
C(1)–C(3)	1.483 (3)	N(1)–O(1)	1.412 (2)
C(3)–C(4)	1.386 (3)	C(3)–C(8)	1.392 (3)
C(4)–C(5)	1.380 (3)	C(5)–C(6)	1.395 (3)
C(6)–C(7)	1.401 (3)	C(7)–C(8)	1.370 (3)
N(2)–C(6)	1.399 (2)	N(2)–C(9)	1.465 (2)
N(2)–C(12)	1.463 (2)	C(9)–C(10)	1.508 (3)
O(2)–C(10)	1.430 (3)	O(2)–C(11)	1.426 (3)
C(11)–C(12)	1.503 (3)		

Bond angles			
O(1)–N(1)–C(1)	113.4 (2)	N(1)–C(1)–C(2)	124.4 (2)
C(2)–C(1)–C(3)	121.1 (2)	N(1)–C(1)–C(3)	114.5 (2)
C(1)–C(3)–C(4)	122.8 (2)	C(1)–C(3)–C(8)	121.2 (3)
C(4)–C(3)–C(8)	116.0 (2)	C(3)–C(4)–C(5)	122.3 (2)
C(3)–C(8)–C(7)	122.3 (2)	C(4)–C(5)–C(6)	121.7 (2)
C(5)–C(6)–C(7)	115.8 (2)	C(6)–C(7)–C(8)	121.9 (2)
N(2)–C(6)–C(5)	122.4 (2)	N(2)–C(6)–C(7)	121.8 (2)
C(6)–N(2)–C(9)	116.7 (1)	C(6)–N(2)–C(12)	116.4 (1)
C(9)–N(2)–C(12)	112.3 (1)	N(2)–C(9)–C(10)	111.6 (2)
C(9)–C(10)–O(2)	112.2 (2)	C(10)–O(2)–C(11)	108.5 (2)
O(2)–C(11)–C(12)	111.7 (2)	N(2)–C(12)–C(11)	111.6 (2)

Table 2. Fractional Coordinates of Non-H Atoms and Equivalent Isotropic Temperature Factors with e.s.d.'s in Parentheses

Atom	x	y	z	B_{eq} (Å^2)
O(1)	−0.3096 (2)	−0.0707 (3)	0.47095 (6)	4.25 (5)
O(2)	0.0898 (2)	0.1432 (2)	0.05708 (5)	3.35 (4)
N(1)	−0.2496 (2)	−0.0783 (3)	0.41540 (7)	3.44 (5)
N(2)	0.0806 (2)	0.0726 (3)	0.17726 (6)	2.65 (4)
C(1)	−0.1405 (2)	0.0741 (3)	0.40599 (8)	2.84 (5)
C(2)	−0.0810 (3)	0.2485 (4)	0.44865 (9)	3.84 (6)
C(3)	−0.0778 (2)	0.0730 (3)	0.34754 (8)	2.66 (5)
C(4)	0.0171 (3)	0.2480 (4)	0.32620 (8)	3.07 (5)
C(5)	0.0682 (3)	0.2501 (3)	0.27093 (8)	3.11 (5)
C(6)	0.0273 (2)	0.0748 (3)	0.23314 (8)	2.45 (4)
C(7)	−0.0675 (3)	−0.1028 (4)	0.25498 (9)	3.40 (5)
C(8)	−0.1163 (3)	−0.1037 (4)	0.31008 (9)	3.51 (6)
C(9)	0.1316 (3)	0.2897 (3)	0.15353 (8)	3.01 (5)
C(10)	0.2034 (3)	0.2616 (4)	0.09568 (8)	3.38 (5)
C(11)	0.0594 (3)	−0.0756 (4)	0.07981 (9)	3.36 (5)
C(12)	−0.0213 (3)	−0.0623 (4)	0.13632 (8)	3.06 (5)

$B_{eq}=(8\pi^2/3)\Sigma_i\Sigma_j U_{ij}a_i^*a_j^*\boldsymbol{a}_i\cdot\boldsymbol{a}_j$.

histamine in rat skin, as described in the experimental section. Of particular interest is the finding that the anti-inflammatory activity appeared in all the acetyl oximes. The AcF-, BYAc-, FG-, BA-, and FA-oximes had no inhibitory action, showing the *O*-acyloxime linking of *N*- or *O*-acetyl-protected amino acid, Fmoc amino acid, and alanine derivatives to be inefficient. These results suggest the importance of an acetyl group directly attached to the oxime for anti-inflammatory activity generated by inhibiting the increased vascular permeability induced by histamine in rat skin. The anti-inflammatory activity of BF-2, Ac-2, and oxime-2 was measured as the percentage inhibition of carrageenan-induced rat paw edema. The results are shown in Fig. 2. BF-2 and Ac-2 had almost the same effect as oxime-2 in inhibiting carrageenan-induced paw edema.

Typical non-steroidal anti-inflammatory drugs inhibit the metabolism of arachidonic acid to prostaglandins (PG) *via* the COX pathway. Several of our synthetic compounds were tested for COX-1 and COX-2 inhibition (see Table 4). The percentage inhibition of the COX activity *in vitro* was assayed based on the reaction of each enzyme with [1-^{14}C]arachidonic acid as described in the experimental section. Oxime-2 and oxime-3 inhibited the COX-1 activity with IC_{50} values of 50 and 130 μM, respectively. The concentration-dependence of the effect of oxime-2 on COX-1 activity *in vitro* is shown in Fig. 3. The COX-1 inhibitory action of oxime-2 and oxime-3 decreased when the oximes were *O*-acylated. Contrary to our expectation, these results suggest the importance of the oxime structure rather than oxime derivatives in COX-1 inhibition. Oxime-2 showed almost the same IC_{50} value as that of aspirin. Although it could not be verified from the results of this experiment, COX-2 inhibitory action of oxime-1 and its derivatives can be expected. Oxime-2 and oxime-3 did not exhibit COX-2 inhibition. Further study is needed to find COX-2-specific inhibitors.

Experimental

All melting points were measured with a Yanaco MP-S3 apparatus and are uncorrected. FAB-MS were recorded on a JEOL JMS-DX-303 spectrometer. For the measurement, each sample was dissolved in a matrix of *m*-nitrobenzyl alcohol and the solution was bombarded with a beam of neutral Xe atoms.

General Procedures for Preparation of *O*-Acyloximes Acetyloxime: A solution of CH_3COCl (15 mmol) in anhydrous $CHCl_3$ (60 ml) was added dropwise to a mixture of the oxime (10 mmol), triethylamine (15 mmol), and anhydrous $CHCl_3$ (20 ml) under ice-cooling. After having been stirred overnight at room temperature, the reaction mixture was diluted with $CHCl_3$. The solution was washed with water, 10% $NaHCO_3$, and water, then dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified to homogeneity by column chromatography on silica gel and/or recrystallization from appropriate solvents as shown in Table 3.

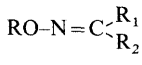
Boc Amino Acid Oxime Ester: IBCF (3 mmol) was added to a solution of Boc amino acid (3 mmol) and NMM (3 mmol) in anhydrous tetrahydrofuran (THF) (5—10 ml) under stirring at −15—−20 °C. After 15 min, a cooled solution of the oxime (3 mmol) in anhydrous THF or dimethylformamide (DMF) (8—15 ml) was added, and the mixture was stirred for 2 h at −15—−20 °C, then overnight at room temperature. After evaporation *in vacuo*, ethyl acetate was added to the residue. The solution was washed with water, 10% $NaHCO_3$, and water, then dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified by the method used for the acetyloxime.

Fmoc Amino Acid Oxime Ester: NMM (3 mmol) was added to a solution of Fmoc amino acid (3 mmol) and IBCF (3 mmol) in anhydrous THF or DMF (5—10 ml) under stirring at −15—−20 °C. After 15 min, a cooled solution of the oxime (2.7 mmol) in anhydrous THF or DMF (8—15 ml) was added, and the mixture was stirred for 2 h at −15—−20 °C, then overnight at room temperature. The remaining procedure was the same as that for the Boc amino acid oxime ester.

X-Ray Crystal Structure Analysis of Oxime-2 Measurement was performed on a Rigaku AFC 5R instrument with CuK_α radiation (graphite monochromator). Crystals of the oxime-2 were grown in chloroform solution. Data for the X-ray diffraction study of the oxime-2 are briefly outlined below. The crystal of oxime-2 belonged to monoclinic space group $P2_1/n$ with cell constants: $a=7.894(1)$ Å, $b=5.919(1)$ Å, $c=23.5374(9)$ Å, and $\beta=92.629(7)°$; $V=1098.6(2)$ Å^3; $Z=4$; $\mu=0.744$ cm^{-1} (CuK_α); 1744 measured reflections ($2\theta_{max}=125°$); 1646 independent reflections ($R_{sym}=0.015$); 1460 observed reflections ($I>2\sigma(I)$); final R value: 4.5%; and 195 parameters.

Anti-inflammatory Activity Inhibitory Effect on Increased Vascular Permeability Induced by Histamine: Groups of five male Sprague–Dawley rats (Japan SLC) weighing 120—150 g were used. The backs of the rats were shaved on the day before the experiment. The rats were

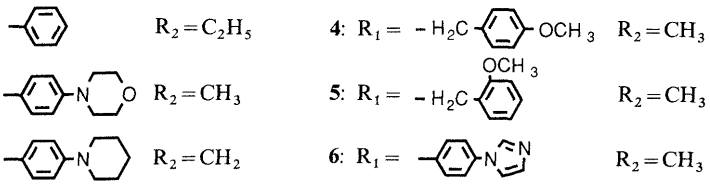
Table 3. Anti-inflammatory Activity (AIA) and Physical Data



O-acyloxime

acyl group (R): Ac (acetyl–), BF (Boc–Phe–), AcF (acetyl–Phe–), FF (Fmoc–Phe–), BYAc (Boc–Tyr(Ac)–), BA (Boc–Ala–), FA (Fmoc–Ala–), BG (Boc–Gly–), FG (Fmoc–Gly–)
Boc, *tert*-butyloxycarbonyl; Fmoc, 9-fluorenylmethyloxycarbonyl

oxime (R=H)



Compd.[a]	AIA[b] (% inhibition)	Yield (%)	mp (°C)	Recrystn. solvent[c]	FAB-MS[d] (*m/z*)	Formula	Elemental analysis (%) Calcd (Found)		
							C	H	N
1	14±3								
Ac-**1**	19±3**	20	44—46	Et–H	192 $(M+H)^+$	$C_{11}H_{13}NO_2$	69.09	6.85	7.32
							(69.08	7.09	7.45)
BF-**1**	—	78	75—79	E–W	397 $(M+H)^+$	$C_{23}H_{28}N_2O_4$	69.68	7.12	7.07
							(69.54	7.24	7.08)
AcF-**1**	—	57	61—63	Et–P	339 $(M+H)^+$	$C_{20}H_{22}N_2O_3$	70.98	6.55	8.28
							(71.03	6.50	8.33)
FF-**1**	15±2	76	152—155	C–P	519 $(M+H)^+$	$C_{33}H_{30}N_2O_4$	75.55	5.89	5.34
						$\cdot 1/3H_2O$	(75.55	5.78	5.49)
BYAc-**1**	—	69	110—111	A–P	455 $(M+H)^+$	$C_{25}H_{30}N_2O_6$	65.54	6.69	6.11
						$\cdot 1/5H_2O$	(65.72	6.75	6.23)
BA-**1**	—	47	63—65	H	321 $(M+H)^+$	$C_{17}H_{24}N_2O_4$	63.73	7.55	8.74
							(63.57	7.81	8.73)
FA-**1**	—	53	146—148	C–H	443 $(M+H)^+$	$C_{27}H_{26}N_2O_4$	73.29	5.92	6.33
							(73.01	5.84	6.53)
BG-**1**	12±1*	52	70—72	Et–Pn	307 $(M+H)^+$	$C_{16}H_{22}N_2O_4$	62.73	7.24	9.14
							(62.58	7.34	9.23)
FG-**1**	—	32	155—157	C–P	429 $(M+H)^+$	$C_{26}H_{24}N_2O_4$	72.27	5.69	6.48
						$\cdot 1/5H_2O$	(72.29	5.64	6.56)
2	11±4								
Ac-**2**	13±5*	80	107—108	C–Et	262 $(M)^{+\cdot}$	$C_{14}H_{18}N_2O_3$	64.11	6.92	10.68
							(63.98	7.10	10.75)
BF-**2**	23±4**	53	148—150	M	467 $(M)^{+\cdot}$	$C_{26}H_{33}N_3O_5$	66.79	7.11	8.99
							(66.66	7.30	8.98)
AcF-**2**	—	46	175—176	C–M–Et	409 $(M)^{+\cdot}$	$C_{23}H_{27}N_3O_4$	67.46	6.65	10.26
							(67.36	6.76	10.22)
FF-**2**	—	91	160—165	C–Et	589 $(M)^{+\cdot}$	$C_{36}H_{35}N_3O_5$	72.22	6.06	7.02
						$\cdot 1/2H_2O$	(72.44	5.95	7.10)
BYAc-**2**	—	57	191—192	C–M	525 $(M)^{+\cdot}$	$C_{28}H_{35}N_3O_7$	63.99	6.71	7.99
							(63.79	6.79	8.05)
BA-**2**	—	82	155—157	C–A	391 $(M)^{+\cdot}$	$C_{20}H_{29}N_3O_5$	61.36	7.47	10.73
							(61.24	7.58	10.73)
FA-**2**	—	79	168—172	C–M	513 $(M)^{+\cdot}$	$C_{30}H_{31}N_3O_5$	69.55	6.13	8.11
						$\cdot 1/4H_2O$	(69.50	6.11	8.21)
BG-**2**	—	80	119—120	C–Pn	377 $(M)^{+\cdot}$	$C_{19}H_{27}N_3O_5$	60.46	7.21	11.13
							(60.19	7.27	11.02)
FG-**2**	—	77	158—160	C–M	499 $(M)^{+\cdot}$	$C_{29}H_{29}N_3O_5$	69.72	5.85	8.41
							(69.58	5.84	8.37)
3	18±3**								
Ac-**3**	23±2**	65	86—87	Et	260 $(M)^{+\cdot}$	$C_{15}H_{20}N_2O_2$	69.20	7.74	10.76
							(69.09	7.92	10.75)
BF-**3**	11±2	83	137—139	A–H	465 $(M)^{+\cdot}$	$C_{27}H_{35}N_3O_4$	69.65	7.58	9.03
							(69.57	7.67	9.07)
AcF-**3**	—	31	140—142	A	407 $(M)^{+\cdot}$	$C_{24}H_{29}N_3O_3$	70.74	7.17	10.31
							(70.56	7.27	10.28)
FF-**3**	—	91	151—153	Ac–H	587 $(M)^{+\cdot}$	$C_{37}H_{37}N_3O_4$	74.47	6.42	7.04
						$\cdot 1/2H_2O$	(74.20	6.29	7.00)
BYAc-**3**	—	43	170—172	C–M	523 $(M)^{+\cdot}$	$C_{29}H_{37}N_3O_6$	65.95	7.16	7.96
						$\cdot 1/4H_2O$	(66.03	7.23	8.15)

Table 3. (continued)

Compd.[a]	AIA[b] (% inhibition)	Yield (%)	mp (°C)	Recrystn. solvent[c]	FAB-MS[d] (*m/z*)	Formula	Elemental analysis (%) Calcd (Found)		
							C	H	N
BA-3	—	50	150—152	C–Pn	389 $(M)^{+\cdot}$	$C_{21}H_{31}N_3O_4 \cdot 1/4H_2O$	64.02 (64.02	8.06 8.21	10.67 10.69)
FA-3	—	72	155—156	C–M	511 $(M)^{+\cdot}$	$C_{31}H_{33}N_3O_4 \cdot 1/3H_2O$	71.93 (71.80	6.56 6.52	8.12 8.16)
BG-3	—	66	125–130	Et	375 $(M)^{+\cdot}$	$C_{20}H_{29}N_3O_4$	63.98 (63.80	7.79 8.02	11.19 11.18)
FG-3	—	64	144—146	C–M	497 $(M)^{+\cdot}$	$C_{30}H_{31}N_3O_4$	72.41 (72.22	6.28 6.29	8.44 8.51)
4	—								
Ac-**4**	20±4**	83	Oil	C[e]	221 $(M)^{+\cdot}$	$C_{12}H_{15}NO_3$		*f*)	
BF-**4**	15±3**	81	75—80	P	427 $(M+H)^+$	$C_{24}H_{30}N_2O_5$	67.59 (67.34	7.09 7.17	6.57 6.57)
AcF-**4**	—	64	112—115	A–P	369 $(M+H)^+$	$C_{21}H_{24}N_2O_4$	68.46 (68.43	6.57 6.66	7.60 7.59)
FF-**4**	—	63	119—122	D–P	549 $(M+H)^+$	$C_{34}H_{32}N_2O_5$	74.43 (74.16	5.88 5.89	5.11 5.21)
BYAc-**4**	—	55	119—121	A–P	485 $(M+H)^+$	$C_{26}H_{32}N_2O_7$	64.45 (64.36	6.66 6.80	5.78 5.94)
BA-**4**	—	18	47—50	H–Pn	351 $(M+H)^+$	$C_{18}H_{26}N_2O_5$	61.70 (61.79	7.48 7.62	7.99 8.21)
FA-**4**	—[g]	77	Oil	C[e]	473 $(M+H)^+$	$C_{28}H_{28}N_2O_5$		*f*)	
BG-**4**	—	46	82—84	Et–P	336 $(M)^{+\cdot}$	$C_{17}H_{24}N_2O_5$	60.70 (60.60	7.19 7.41	8.33 8.33)
FG-**4**	—[g]	78	Oil	C[e]	459 $(M+H)^+$	$C_{27}H_{26}N_2O_5$		*f*)	
5	—								
Ac-**5**	13±4*	61	Oil	C[e]	222 $(M+H)^+$	$C_{12}H_{15}NO_3$		*f*)	
BF-**5**	—	93	Oil	C[e]	427 $(M+H)^+$	$C_{24}H_{30}N_2O_5$		*f*)	
AcF-**5**	—	48	79—82	Et	369 $(M+H)^+$	$C_{21}H_{24}N_2O_4$	68.46 (68.39	6.57 6.69	7.60 7.62)
BYAc-**5**	—	63	96—97	Et–P	485 $(M+H)^+$	$C_{26}H_{32}N_2O_7$	64.45 (64.25	6.66 6.68	5.78 5.89)
6	27±3**								
Ac-**6**	26±3**	46	98—100	C–P	244 $(M+H)^+$	$C_{13}H_{13}N_3O_2$	64.19 (64.21	5.39 5.36	17.27 17.25)
Aspirin	23±4**								
Indomethacin	22±3*,[g]								

a) The abbreviations of the *O*-acyloximes consist of the abbreviation of the acyl group and the oxime number. *b*) Anti-inflammatory activity was measured as the percentage inhibition of increased vascular permeability induced by histamine in rat skin. Histamine (100 μg/0.05 ml/site) was injected intradermally 1 h after drug (200 mg/kg, *p.o.*, 0.5% CMC-Na suspension) administration, and after 30 min the blueing area was measured (*g*): 20 mg/kg, *p.o.*, 0.5% CMC-Na suspension). Each value represents the mean±S.E. of 5 animals. Percent inhibition of 10% or less was regarded as inactive (—). ** $p<0.01$ and * $p<0.05$, as compared with the control group. *c*) Solvents: A (ethyl acetate), Ac (acetone), B (benzene), C (chloroform), D (dichloromethane), E (ethanol), Et (diethyl ether), H (*n*-hexane), M (methanol), P (petroleum ether), Pn (*n*-pentane), W (water). *d*) Matrix: *m*-nitrobenzyl alcohol. *e*) The oily compounds were purified by silica-gel column chromatography. Eluting solvent: C, chloroform (100%). *f*) The oily compounds were not measured. IR ($CHCl_3$): Ac-**4**, 1748 (C=O, ester), 1638 (C=N, oxime); FA-**4**, 1764 (C=O, ester), 1717 (C=O, carbamate), 1638 (C=N, oxime); FG-**4**, 1763 (C=O, ester), 1722 (C=O, carbamate), 1638 (C=N, oxime); Ac-**5**, 1748 (C=O, ester), 1636 (C=N, oxime); BF-**5**, 1756 (C=O, ester), 1708 (C=O, carbamate), 1636 (C=N, oxime) cm^{-1}.

starved overnight before the experiment, and then dosed orally with the test compounds (200 mg/kg) suspended in a 0.5% sodium carboxymethylcellulose (CMC-Na) solution. After 1 h, 1% Evans blue solution was injected i.v., then histamine (100 μg/0.05 ml/site) was immediately injected intradermally at four sites on the back of each rat. After 30 min, the rats were killed with diethyl ether. After abrasion of the rat skin, the blueing area of the skin was measured. The mean of the four blueing areas was used as the result for each animal, and the area of the test group was compared with that of the control group to calculate the percent inhibition.

Inhibitory Effect of Carrageenan-Induced Rat Paw Edema: Groups of six male Sprague–Dawley rats (Japan SLC) weighing 120—150 g were used. The rats were starved overnight before the experiment, and then dosed orally with the test compounds (150 mg/kg) suspended in a 0.5% CMC-Na solution. After 30 min, edema was produced in the right hind paw of rats by subplantar injection of 0.1 ml of a 1% λ-carrageenan suspension in sterile saline. Paw volume was determined with a plethysmometer (TK-101, Unicom, Chiba) by a previously described procedure.[1]

Statistical Analysis: The data were expressed as mean±S.E. Statistical analyses were performed by analysis of variance, followed by Fisher's PLSD test. Asterisks indicate significant difference from the control group (** $p<0.01$, * $p<0.05$).

Inhibition of COX Enzyme Preparation: Sheep vesicular gland COX-1 was prepared from lyophilized powder of sheep seminal vesicle microsomes which was purchased from Eldan Technologies (Jerusalem). The lyophilized powder was dissolved in 20 mM phosphate buffer (pH 7.4) containing 5 mM tryptophan. MC3T3-E1 cells were provided by Dr. Kodama of Ohu University. For COX-2 expression the cells were cultured for 9 h after addition of both 0.1 μM phorbol 12-myristate 13-acetate and 0.1 μM calcium ionophore A23187 as described previously.[3] The cells were subjected to sonic disruption[3] and centrifuged at $1.8\times10^5\times g$ for 50 min. The resultant pellet was collected as the microsomal fraction and suspended in 20 mM Tris–HCl (pH 7.4) containing 5 mM tryptophan. Protein concentration was determined by the method of Lowry *et al.*[4] with bovine serum albumin as the standard.

COX Assay: The reaction mixture (190 μl) containing each enzyme, 100 mM Tris–HCl (pH 8.0), 2 μM hematin, and 5 mM tryptophan was

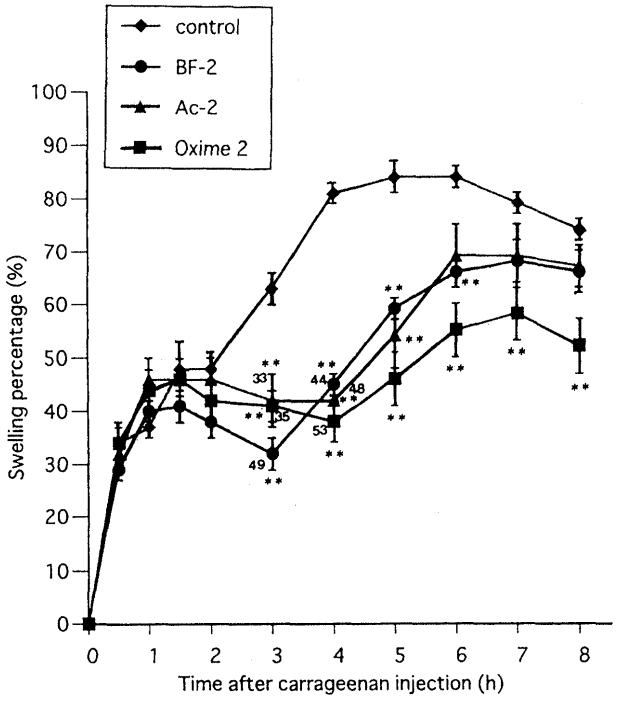


Fig. 2. Inhibitory Effect of BF-2, Ac-2, and Oxime-2 on the Swelling of Rat Hind Paw Induced by Carrageenan (1%, 0.1 ml)

The compounds were given orally 0.5 h before the carrageenan injection. Each data point represents the mean ± S.E. $**p<0.01$, as compared with the control group. The small numbers indicate % inhibition.

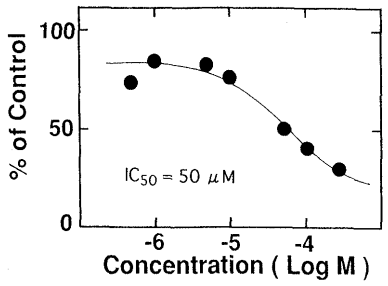


Fig. 3. Concentration-Dependent Effect of Oxime 2 on COX-1 Activity *in Vitro*

preincubated with 5 μl of dimethyl sulfoxide solution of a test compound for 30 min at 24 °C. Next, 5 μl of 2 mM [1-^{14}C]arachidonic acid (5×10^4 cpm in 5 μl of ethanol) was added to a final concentration of 50 μM, and the mixture was incubated for 1 min at 24 °C. The reaction was stopped by addition of 0.3 ml of a mixture of diethyl ether–methanol–1 M citric acid (30 : 4 : 1, v/v). A 100-μl aliquot of the organic phase was applied to a silica-gel thin layer chromatography plate, which was then developed in diethyl ether–petroleum ether–glacial acetic acid (85 : 15 : 0.1, v/v) at −20 °C for 50 min. The distribution of radioactivity on the plate was detected by a BAS2000 imaging analyzer (Fujix), and the conversion rate of arachidonic acid to the oxygenated products was calculated. The percent inhibition of COX activity was calculated by comparing the values of the test compound and the blank test.

Table 4. COX-1 and COX-2 Inhibitory Activity Data[a)]

Compd.	COX-1 activity (% inhibition)	COX-2 activity (% inhibition)
1	13	15
Ac-**1**	—	21
BF-**1**	—	27
FF-**1**	—	NT
BA-**1**	—	NT
FA-**1**	—	NT
BG-**1**	16	NT
2	53	—
Ac-**2**	27	—
BF-**2**	19	—
BG-**2**	42	—
3	39	—
Ac-**3**	11	—
BF-**3**	—	—
4	—	—
Ac-**4**	—	—
BF-**4**	13	—
BA-**4**	—	NT
5	—	—
Ac-**5**	—	—
BF-**5**	—	—
6	—	—
Ac-**6**	—	—
Aspirin	51	NT
Indomethacin	86	NT

a) The structures and abbreviations of the compounds are shown in Table 3. The concentration of test compounds used in screening of the COX-1 and COX-2 inhibition was 50 μM, and the assays were conducted as explained in the experimental section. The percent inhibition is shown as follows: 10% or less inhibition (−) and not tested (NT).

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References and Note

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5) All amino acid residues mentioned in this paper are of the L-configuration.