Synthesis and Anti-inflammatory Activity of Antioxidants, 4-Alkylthio-*o*-anisidine Derivatives

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In order to search for anti-inflammatory agents against autoimmune diseases, we synthesized 4-alkylthio-*o*-anisidine derivatives possessing antioxidant activity, and tested them for anti-inflammatory activity against the Arthus reaction in mice. Experimental inflammations, including the Arthus reaction, concanavalin A, phorbol ester and pyrophosphate-induced edemas in rats were inhibited by 4-propylthio-*o*-anisidine, which inhibited autoxidation of rat brain homogenate and suppressed the lipopolysaccharide-induced increase in the plasma malondialdehyde level in mice. An antioxidant may be an effective agent in immune complex type inflammation where active oxygen species play an important role.

Keywords 4-alkylthio-*o*-anisidine; 4-propylthio-*o*-anisidine; anti-inflammatory; antioxidant; Arthus reaction

The immune mechanisms involved in the pathogenesis of autoimmunity were first formalized by the now familiar Gell and Coombs classification into types I through IV. Immune complexes with the type III mechanism are well established as mediators of immune injury, particularly in relation to glomerulonephritis, systemic lupus erythematosis and rheumatoid arthritis. The complex of antigen plus antibody induces the activation of a complement system, the infiltration of polymorphonuclear leukocytes, and the release of lysosomal enzymes and active oxygen species (AOS), and thereby initiates inflammatory processes.[1] Some antirheumatic agents, such as salicylate and penicillamine, are present at sites of inflammation at concentrations capable of interfering with AOS production or scavenging AOS.[2]

In this study, we synthesized 4-alkylthio-*o*-anisidine derivatives possessing antioxidant activity and evaluated their anti-inflammatory effect on the Authus reaction, which is the most typical of inflammatory responses resulting from the type III immune mechanism.

Chemistry The key compounds, 4-alkylthio-*o*-anisidines (**3**), were synthesized using methods A or B shown in Chart 1. The reaction of 5-chloro-2-nitroanisole (**1**) with alkanethiol in the presence of sodium hydride (NaH) in dimethylformamide (DMF) produced 5-alkylthio-2-nitroanisoles (**2**), which were treated with sodium dithionite ($Na_2S_2O_4$) to give **3** (method A). Alkylation of 4-mercapto-2-methoxybenzoic acid (**4**) with alkyl bromide in the presence of Et_3N produced 4-alkylthio-2-methoxybenzoic acids (**5**). Conversion of **5** into **3** was carried out by Curtius rearrangement (diphenylphosphoryl azide (DPPA), Et_3N, MeCN, reflux, 1 h) followed by alkaline hydrolysis (method B). The physical and analytical data of **3** are listed in Table I. The derivatives of **3C** were synthesized by the pathways shown in Chart 2, which include *N*-alkylation, *N*-acylation and *S*-oxidation. The *N*-monoalkyl derivatives (**12**) were synthesized by acylation of **3C** with appropriate acyl chloride and subsequent reduction with lithium aluminum hydride ($LiAlH_4$) for **12B**—D, or by methylation of acetamide (**7**) with methyl iodide in the presence of NaH followed by alkaline hydrolysis for **12A**. The sulfoxides (**9**, **11**, **15**) were synthesized by oxidation of the corresponding *N*-acyl derivatives (**8**, **7**, **6A**) with sodium metaperiodate ($NaIO_4$). The sulfide (**7**) was oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) to afford the sulfone (**13**) which was in turn hydrolyzed to give the aminosulfone (**14**). *N*,*N*-Dimethyl-4-propylthio-*o*-anisidine (**19**) was prepared from *N*,*N*-dimethyl-*o*-anisidine (**17**) by a modification of the procedure reported by Monkovic *et al.*[3] as shown in Chart 3. Oxidation of **19** with $NaIO_4$ afforded the sulfoxide (**20**). The physical and analytical data of the derivatives are listed in Table II.

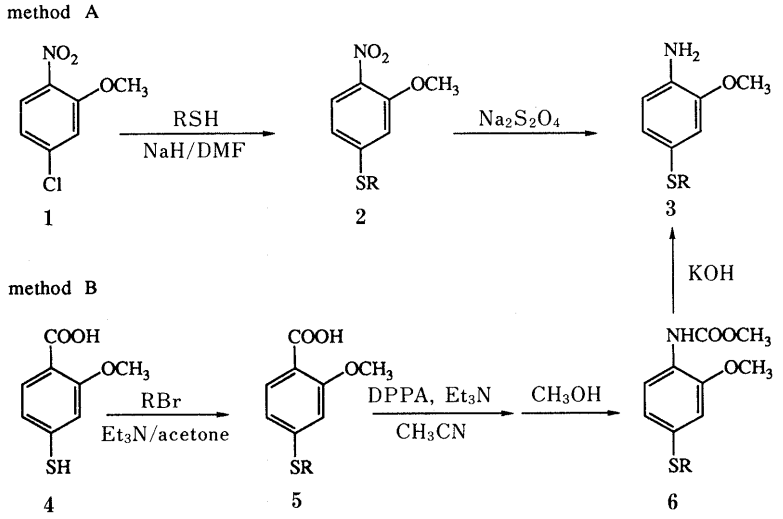
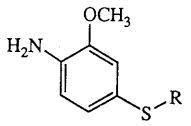


Chart 1

TABLE I. Physical Data for 4-Alkylthio-*o*-anisidines



Compd. No.[a]	Substituents R	Yield (%)	(Method)	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
3A	Me	78	(B)[b]	161—163	EtOH	$C_8H_{12}ClNOS$	46.71	5.88	6.81	46.93	5.82	6.86
3B	Et	42	(A)[c]	166—168	EtOH–AcOEt	$C_9H_{14}ClNOS$	49.20	6.42	6.38	49.35	6.38	6.52
3C	Pr	35	(A), 71 (B)	165—166	EtOH–AcOEt	$C_{10}H_{16}ClNOS$	51.38	6.90	5.99	51.65	6.85	5.95
3D	iso-Pr	39	(A)	158—159	EtOH–AcOEt	$C_{10}H_{16}ClNOS$	51.38	6.90	5.99	51.17	6.75	6.01
3E	Allyl	54	(A)	144—145	EtOH–AcOEt	$C_{10}H_{14}ClNOS$	51.83	6.09	6.04	51.84	6.07	5.99
3F	Bu	53	(A)	104—105	EtOH–AcOEt	$C_{11}H_{18}ClNOS$	53.32	7.32	5.65	53.12	7.29	5.62
3G	iso-Bu	54	(A)	94—96	EtOH–AcOEt	$C_{11}H_{18}ClNOS$	53.32	7.32	5.65	53.31	7.10	5.41
3H	Pent	57	(A)	148—150	EtOH–AcOEt	$C_{12}H_{20}ClNOS$	55.05	7.70	5.35	55.20	7.71	5.23
3I	Oct	54	(A)	146—147	EtOH–AcOEt	$C_{15}H_{26}ClNOS$	59.48	8.32	4.62	59.64	8.59	4.33
3J	Dec	45	(A)	142—144	EtOH–AcOEt	$C_{17}H_{30}ClNOS$	61.51	9.11	4.22	61.77	9.20	4.28
3K	Dodec	43	(A)	142—144	EtOH–AcOEt	$C_{19}H_{34}ClNOS$	63.39	9.52	3.89	63.66	9.57	3.97
3L	Benzyl	56	(A)	155—157	EtOH–AcOEt	$C_{14}H_{16}ClNOS$	59.67	5.72	4.94	59.87	5.74	5.17

a) All the compounds are HCl salt. *b*) Yield from **4**. *c*) Yield from **1**.

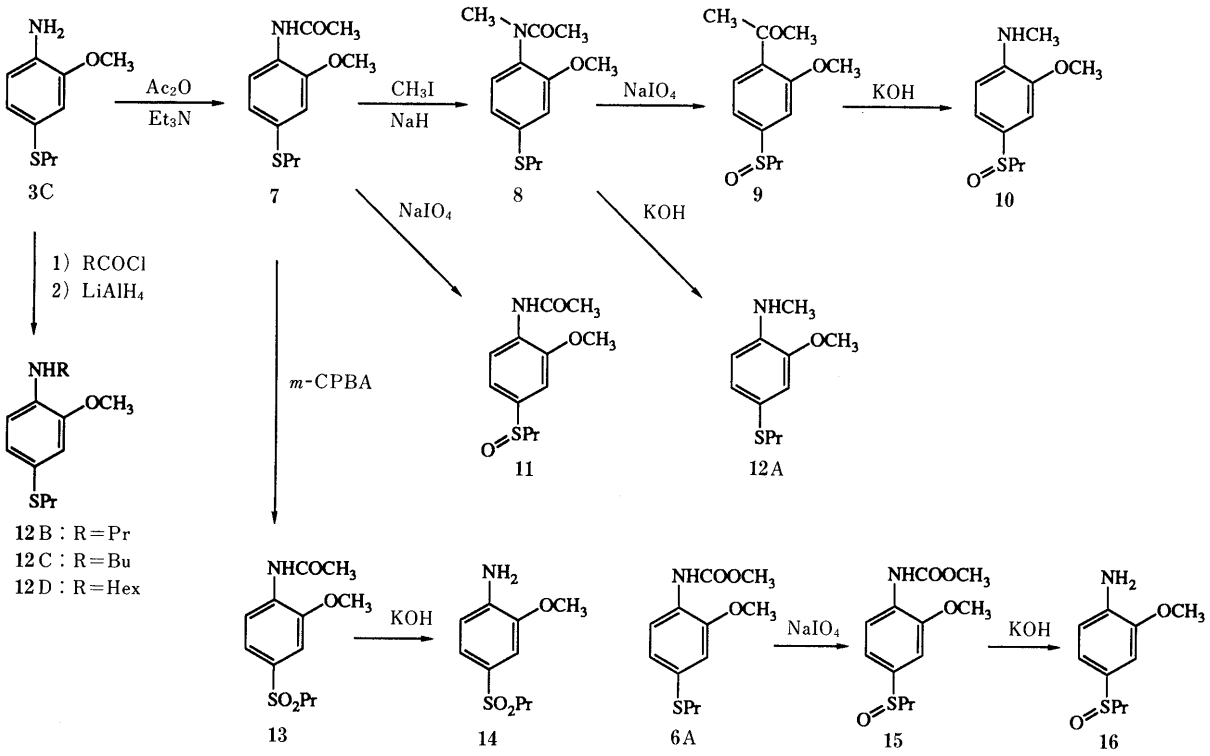


Chart 2

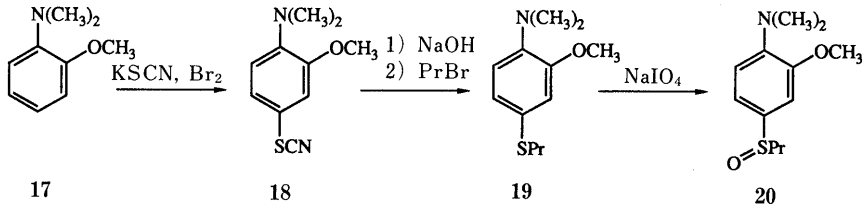
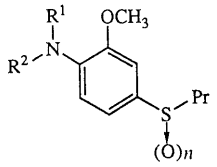


Chart 3

Pharmacological Methods A. Lipid Peroxidation *in Vitro* Antioxidant activity of the test compounds was measured according to the methods of Shintomi *et al.*[4] and Kubo *et al.*[5] To 1.25 ml of test sample solution in 67 mM phosphate buffer (pH 7.4) was added a 10% homogenate of rat forebrain in the same buffer (0.25 ml). The mixture

TABLE II. Physical Data for 4-Propylthio-*o*-anisidine Derivatives



Compd. No.	Substituents R^1	Substituents R^2	n	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
12A[a]	H	Me	0	99—101	AcOEt	$C_{11}H_{18}ClNOS$	53.32	7.32	5.65	53.51	7.27	5.51
10[a]	H	Me	1	107—108	EtOH–AcOEt	$C_{11}H_{18}ClNO_2S$	50.09	6.88	5.31	50.14	6.79	5.56
12B[a]	H	Pr	0	140—142	EtOH–AcOEt	$C_{13}H_{22}ClNOS$	56.61	8.04	5.08	56.62	8.09	4.84
12C[a]	H	Bu	0	129—130	AcOEt	$C_{14}H_{24}ClNOS$	58.01	8.34	4.83	58.22	8.52	5.07
12D[a]	H	Hex	0	113—115	AcOEt	$C_{16}H_{28}ClNOS$	60.45	8.88	4.41	60.22	8.83	4.25
16[a]	H	H	1	125—128	EtOH–AcOEt	$C_{10}H_{16}ClNO_2S$	48.09	6.46	5.61	48.19	6.47	5.73
14[a]	H	H	2	127—128	EtOH	$C_{10}H_{16}ClNO_3S$	45.20	6.07	5.27	45.41	6.13	5.36
19[a]	Me	Me	0	104—105	EtOH–AcOEt	$C_{12}H_{20}ClNOS$	55.05	7.70	5.35	55.13	7.51	5.54
20[b]	Me	Me	1	Liquid		$C_{12}H_{19}NO_2S$[c]		241.3476			241.3477	
7	H	Acetyl	0	65—66	Hexane	$C_{12}H_{17}NO_2S$	60.22	7.16	5.85	60.34	6.93	6.06
11	H	Acetyl	1	112—113	AcOEt	$C_{12}H_{17}NO_3S$	56.45	6.71	5.49	56.67	6.73	5.57
13	H	Acetyl	2	134—135	EtOH	$C_{12}H_{17}NO_4S$	53.12	6.32	5.16	53.29	6.39	5.42

a) HCl salt. *b*) Free base. *c*) HR-MS analysis.

was then shaken vigorously for 1 h at 37 °C, and deproteinated with the addition of 20% trichloroacetic acid (0.5 ml). To the resulting supernatant was added 1.2% sodium thiobarbiturate (0.5 ml), which was heated for 10 min at 100 °C. After cooling, the optical density at 532 nm was measured.

B. Lipid Peroxidation *in Vivo* Male ICR mice 5 weeks old, purchased from Japan SLC (Hamamatsu, Japan), were injected intraperitoneally with 30 mg/kg of lipopolysaccharide (LPS) (*E. coli*, 055 : B5). Seventeen hours later, the animal were bled from the abdominal vein using ethylenediaminetetraacetate 2K as an anticoagulant. Plasma malondialdehyde (MDA), an index of lipid peroxidation,[6] was measured by colorimetric reaction with thiobarbituric acid. Test compounds (30 mg/kg) were dissolved in saline or suspended in 0.2% Tween 80–saline and administered intraperitoneally 30 min before and 15 h after the injection of LPS.

C. Arthus Reaction The method of Takahashi *et al.*[7] was used. Male Balb/c mice 5 to 7 weeks old, purchased from Charles River Japan (Atsugi, Japan), were immunized intraperitoneally with 4×10^8 of sheep red blood cells (SRBC) suspended in 0.2 ml of saline on day 0 and were boosted according to the same protocol on day 14. The animals' right hind paws were injected subcutaneously with 2×10^8 SRBC suspended in 0.025 ml of saline on day 19. Three hours later, the thickness of both hind paws was measured, and the paw edema was expressed as the difference in thickness between the treated and nontreated paws.

Test compounds (100 or 300 mg/kg) were dissolved in water or suspended in 0.5% carboxymethyl cellulose (CMC) Na, then administered orally 30 min before the subcutaneous injection of SRBC. The control mice were administered water or 0.5% CMC. The drug effects were calculated as millimeter decreases in paw edema in relation to the control mice.

Effect of Compound 3C on Rat Paw Edema Male SD rats weighing 120—150 g, purchased from Charles River Japan, were used. Thirty minutes after the oral administration of the test compounds, the edema was induced by subcutaneous injection into the right hind paw with 0.1 ml of phlogistic agents. Immediately before the induction of the edema, the foot volume was measured by the displacement of water and then at various intervals. Carrageenan (1%), 1% concanavalin A (Con A), 1 μg/ml phorbol myristate acetate (PMA) and 0.2 mmol/ml sodium pyrophosphate (PPi) were used as phlogistic agents.

Results and Discussion

A series of 4-alkylthio-*o*-anisidines were described in a German patent as intermediates for dyes, pharmaceuticals and pesticides;[8] however their antioxidant activity has not yet been reported.

In the present study, we observed the *in vitro* antioxidant activity of 4-alkylthio-*o*-anisidines which inhibited autoxidation of lipids in rat brain homogenate. The IC_{50} values of the test compounds are shown in Table III. All of the listed compounds with different alkylthio moieties had nearly the same potency except methylthio derivative (**3A**), which had about a 10 times lower IC_{50} value than the others.

Their *in vivo* antioxidant activity was further tested in LPS shocked mice. Intraperitoneal injection of LPS into mice induced a prominent elevation in the plasma MDA level from the normal level of 3—5 nmol/ml to the level of 20—100 nmol/ml. The test compounds which inhibited the elevation in MDA level by more than 50% were regarded as positive in this assay system. We could not quantify the potency of the compounds because of large inter-assay variance in the elevated levels of the control animals. In this assay, the derivatives possessing 2 to 8 carbon atoms (**3B**—D, G—I) were positive, while long chain derivatives (**3J**, K) and the methyl derivative (**3A**), which had strong *in vitro* activity, were negative, suggesting that some

pharmacokinetic factors were favorable for the former molecules to exert *in vivo* activity.

To investigate the anti-inflammatory activity of the antioxidants, the Arthus reaction was employed as an AOS-induced inflammation model, and the inhibitory activity of 4-alkylthio-*o*-anisidines (**3A**—L) on this model was tested. In the Arthus reaction, leukocytes produce AOS in response to immune complex-stimulation.[1] The activity of **3A**—L largely depended on the length of the carbon chain attached to the sulfur atom. Only the derivatives possessing three (**3C**, D, E) or four (**3F**, G) carbon atoms had prominent activity, inhibiting paw edema by more than 0.3 mm, which corresponded to about 25% inhibition. The activity of **3C** was compared with that of indomethacin and hydrocortisone acetate (Fig. 1). Orally administered **3C** inhibited paw edema dose dependently. Hydrocortisone acetate showed nearly the same activity as that of **3C**, while a cyclooxygenase inhibitor, indomethacin, was devoid of the activity.

We selected **3C** as a key compound for further chemical modifications to obtain more potent compounds. The anti-inflammatory activity of *N*-methyl derivative (**12A**) was comparable or more potent than **3C**, although propyl (**12B**), butyl (**12C**) and hexyl (**12D**) congeners were inactive. *N,N*-Dimethyl derivative (**19**) also showed activity as potent as that of **12A**. The sulfoxides (**10**, **16**, **20**) had not only antioxidant activity but also anti-inflammatory activity comparable to those of corresponding sulfides. The sulfones (**13**, **14**) had neither antioxidant activity nor anti-inflammatory activity. Anti-inflammatory activity was also

TABLE III. Biological Properties of 4-Alkylthio-*o*-anisidines

Compound	Antioxidant activity		Arthus reaction
	in Vitro IC_{50} (μM)	*in Vivo* activity[a]	Inhibition (mm)
3A	0.49	−	−0.04
3B	16	+	−0.03
3C	5.3	+	0.42
3D	14	+	0.34
3E	6.2	NT	0.38
3F	1.8	NT	0.34
3G	3.8	+	0.47
3H	2.4	+	0.15
3I	3.6	+	0.11
3J	3.9	−	−0.02
3K	7.8	−	0.12
3L	5.6	+	0.11
12A	0.94	+	0.46
10	3.6	NT	0.27
12B	2.3	NT	0.00
12C	2.5	NT	0.10
12D	4.2	NT	0.08
16	27	+	0.52
14	>80	NT	0.12
19	0.87	+	0.40
20	67	NT	0.28
7	>80	+	0.11
11	>80	−	0.52
13	>80	NT	0.14

a) −, negative; +, positive; NT, not tested.

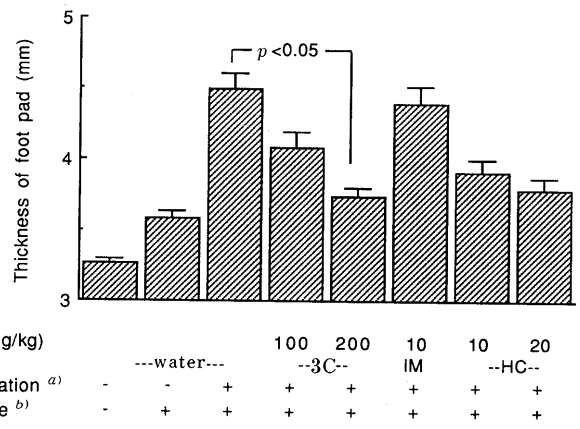


Fig. 1. Effect of **3C** on Arthus Reaction in Mice

Male ICR mice were immunized with SRBC, and were challenged by the injection of SRBC into the hind paw. Four hours later, the thickness of the foot pad was measured. The mice were administered *p.o.* with indomethacin (IM) or **3C** 30 min before the challenge, or with hydrocortisone acetate (HC) 2 h before the challenge. Each value represents mean+S.E.M. of 8 to 13 mice. *a*) −, non-immunized; +, immunized. *b*) −, non-challenged; +, challenged.

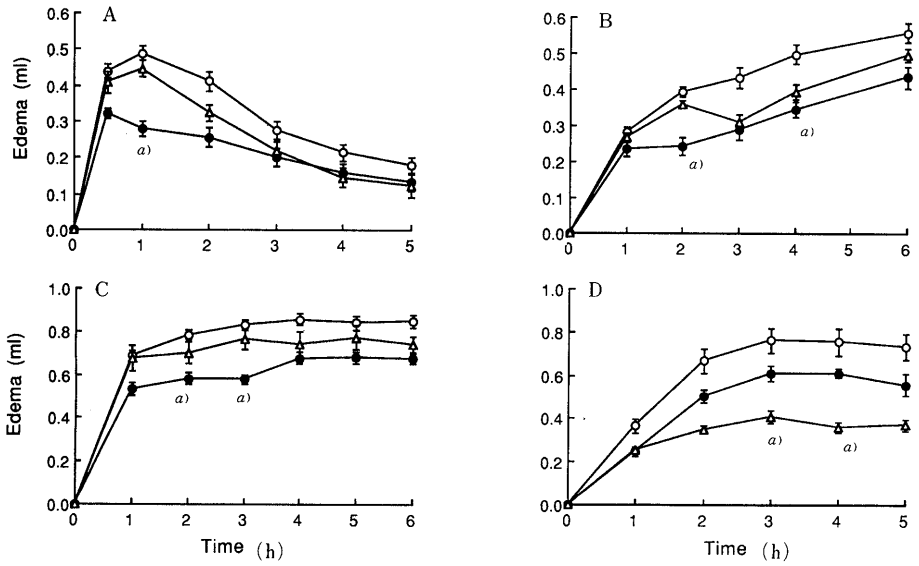


Fig. 2. Effect of **3C** on Paw Edema in Rats Induced by Phorbol Ester (A), Con A (B), Pyrophosphate (C) and Carrageenan (D)

Each point represents mean±S.E.M. of 6 to 13 rats. *a*) $p<0.05$ *versus* the control. ○, control; ●, **3C** 400 mg/kg; △, indomethacin 10 mg/kg.

observed in *N*-acyl derivative (**11**), which did not show antioxidant activity in the *in vitro* assay. It might be hydrolyzed *in vivo* affording **16** which in turn exerted the activity.

The anti-inflammatory activity of **3C** against chemically induced inflammation was tested. Against PMA, Con A and PPi induced edemas, **3C** was a more potent inhibitor than indomethacin (Fig. 2A—C). In contrast, it was a weak inhibitor against carrageenan induced edema, which indomethacin significantly inhibited (Fig. 2D). Prostaglandins are unlikely to play a significant role in Con A or PMA induced edema, since indomethacin does not inhibit these edemas.[9,10] Con A stimulated leukocytes to release AOS and lymphokines, both of which are mediators of inflammation.[11] PMA induces the release of inflammatory mediators including AOS, histamine, PGI_2 and platelet activating factor (PAF).[10] PPi, which is often found in high concentration in the synovial fluid of arthritic patients, stimulates macrophages to produce a superoxide radical.[12]

Participation of AOS and the partial inhibition by **3C** is common to the experimental inflammations induced by Con A, PMA and PPi. It is therefore possible that **3C** exerted its anti-inflammatory activity by inhibiting AOS production in inflammatory cells. Besides AOS, several mediators participate in inflammation, such as lysosomal enzymes in Con A edema and histamine and PAF in PMA edema. It is reasonable that an agent with a single mechanism of action, *i.e.*, antioxidant, cannot inhibit inflammation completely. However, antioxidant may be effective in immune complex type inflammation where AOS plays an important role.

Experimental

Melting points were determined on a Yanako micro melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL FX-100 spectrometer and field desorption-mass spectra (FD-MS) were taken on a Hitachi M-80. Elemental analyses (C, H, N) were performed on a Perkin-Elmer 240C elemental analyzer.

Synthesis of 4-Alkylthio-*o*-anisidines (3A—L) Typical examples are given to illustrate the general procedure for methods A and B.

4-Propylthio-*o*-anisidine (3C) Method A: i) A solution of propanethiol (5.9 ml) in DMF (50 ml) was added dropwise to a stirred solution of NaH (2.6 g) in DMF (100 ml) on an ice bath. After 40 min of stirring, to the mixture was added a solution of 5-chloro-2-nitroanisole (**1**, 10.2 g) in DMF (50 ml) over another 40 min with continued cooling. The reaction mixture was stirred for 2 h at the same temperature, diluted with H_2O and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4 and concentrated. The resulting residue was chromatographed on a silica gel column to give 2-nitro-5-propylthioanisole (**2**) (8.4 g, 68%) as pale yellow needles, mp 43—43.5 °C (EtOH). *Anal.* Calcd for $C_{10}H_{13}NO_3S$: C, 52.85; H, 5.77; O, 6.16. Found: C, 52.75; H, 5.75; N, 6.36. ^{1}H-NMR ($CDCl_3$) δ: 7.85 (1H, d, J=8.5 Hz, arom. H), 6.88 (1H, d, J=2.0 Hz, arom. H), 6.84 (1H, dd, J=8.5, 2.0 Hz, arom. H), 3.96 (3H, s, $OC\underline{H}_3$), 2.98 (2H, t, J=7.2 Hz, S–$C\underline{H}_2$), 1.75 (2H, m, $C\underline{H}_2$–CH_3), 1.07 (3H, t, J=7.2 Hz, CH_2–$C\underline{H}_3$). MS *m/z*: 227 (M^+).

ii) A solution of $Na_2S_2O_4$ (20 g) in H_2O (150 ml) was added to a solution of **2** (7.3 g) in MeOH (300 ml). The mixture was stirred for 30 min at room temperature and warmed up to reflux. Ten more grams of $Na_2S_2O_4$ were added and stirring was continued for 10 more min at reflux. The mixture, on an ice bath was adjusted to pH 1—2 with conc. HCl, followed by the addition of aq. NaOH solution to change it to pH 11—12; it was then extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated to give **3C** (3.3 g, 52%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 6.82—6.95 (2H, m, arom. H), 6.62 (1H, d, J=8.5 Hz, arom. H), 3.85 (3H, s, OCH_3), 2.76 (2H, t, J=7.2 Hz, S–CH_2), 1.60 (2H, m, $C\underline{H}_2$–CH_3), 0.98 (3H, t, J=7.2 Hz, CH_2–$C\underline{H}_3$). MS *m/z*: 197 (M^+). HCl salt: mp 165—166 °C (EtOH–AcOEt). *Anal.* Calcd for $C_{10}H_{16}ClNOS$: C, 51.38; H, 6.90; N, 5.99. Found: C, 51.65; H, 6.85; N, 5.95.

Method B: i) 1-Bromopropane (3.0 g) was added to an ice-cooled solution of 4-mercapto-2-methoxybenzoic acid[13] (**4**) (4.0 g) and Et_3N (4.4 g) in acetone (100 ml). The mixture was stirred for 2 h at room temperature, diluted with H_2O, adjusted to pH 3—4 with 1 N HCl and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4 and concentrated to give crude 2-methoxy-4-propylthiobenzoic acid (**5**).

ii) The crude **5** obtained above was dissolved in MeCN (150 ml), and to the solution were added Et_3N (4.4 g) and DPPA (9.0 g). The mixture was stirred for 1 h at reflux, during which time MeOH (75 ml) was added. The reaction mixture was stirred for an additional hour at reflux, diluted with H_2O and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4 and concentrated to give the residue. The residue was subjected to a silica gel column to yield *N*-methoxycarbonyl-4-propylthio-*o*-anisidine (**6**) (4.59 g, 83% from **4**) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 7.99 (1H, d, J=8.5 Hz, arom. H), 7.15 (1H, br s, NH), 6.86—7.03 (2H, m, arom. H), 3.86 (3H, s, $ArOCH_3$), 3.77 (3H, s, $COOCH_3$), 2.84 (2H, t, J=7.0 Hz, SCH_2), 1.44—1.82 (2H, m, $C\underline{H}_2$–CH_3), 1.03 (3H, t, J=7.0 Hz, CH_2–$C\underline{H}_3$). MS *m/z*: 255 (M^+). HRMS Calcd for $C_{12}H_{17}NO_3S$: 255.3312. Found: 255.3310.

iii) A 35% aq. KOH solution (20 ml) was added to a solution of **6** (4.59 g) in MeOH (40 ml). The mixture was stirred for 3.5 h at reflux, diluted with H_2O and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on a silica gel column to yield **3C** (3.0 g, 85%).

Synthesis of 2-Methoxy-4-(propylthio)acetanilide (7) Et_3N (5.5 g) and acetic anhydride (Ac_2O, 3.3 g) were added to a solution of **3C** (5.5 g) in CH_2Cl_2 (100 ml). The mixture was stirred for 9h at room temperature, diluted with sat. aq. $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated to give **7** 6.5 g, 97%) as colorless leaflets (from hexane), mp 65—66 °C. *Anal.* Calcd for $C_{12}H_{17}NO_2S$: C, 60.22; H, 7.16; N, 5.85. Found: C, 60.34; H, 6.93; N, 6.06. ^{1}H-NMR ($CDCl_3$) δ: 8.28 (1H, d, J=8.1 Hz, arom. H), 7.68 (1H, br, NH), 6.85—7.06 (2H, m, arom. H), 3.88 (3H, s, OCH_3), 2.86 (2H, t, J=7.2 Hz, SCH_2), 2.19 (3H, s, $COCH_3$), 1.45—1.80 (2H, m, $C\underline{H}_2$–CH_3), 1.01 (3H, t, J=7.2 Hz, CH_2–$C\underline{H}_3$). MS *m/z*: 239 (M^+).

Synthesis of 2-Methoxy-*N*-methyl-4-(propylthio)acetanilide (8) NaH (1.25 g) was added in portions to a stirred and ice-cooled solution of **7** (5.0 g) in DMF (120 ml). After stirring for 30 min with cooling, to the mixture was added MeI (1.56 mg). The reaction mixture was stirred for an additional 5.5 h at the same temperature, diluted with H_2O and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4 and concentrated to give the crude product. Purification with a silica gel column to yield **8** (4.21 g, 80%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 7.06 (1H, d, J=8.5 Hz, arom. H), 6.81—6.94 (2H, m, arom. H), 3.83 (3H, s, OCH_3), 3.14 (3H, s, NCH_3), 2.94 (2H, t, J=7.3 Hz, SCH_2), 1.53—1.85 (2H, m, $C\underline{H}_2$–CH_3), 1.80 (3H, s, $COCH_3$), 1.06 (3H, t, J=7.2 Hz, CH_2–$C\underline{H}_3$). MS *m/z*: 253 (M^+). HRMS Calcd for $C_{13}H_{19}NO_2S$: 253.3586. Found: 253.3586.

Synthesis of *N*-Alkyl-4-propylthio-*o*-anisidines (12B—D) A typical example is presented to illustrate the general procedure for compound **12B**.

***N*-Propyl-4-propylthio-*o*-anisidine 12B** i) Propionyl chloride (3.06 g) was added dropwise to a stirred and ice-cooled solution of **3C** (4.10 g), Et_3N (8.96 g) and 4-dimethylaminopyridine (DMAP, 0.40 g) in tetrahydrofran (THF, 250 ml). The mixture was stirred for 3 h at room temperature, then 1.02 g more of propionyl chloride was added. Stirring was continued for an additional hour at room temperature the mixture was diluted with 10% aq. NaOH, stirred for 30 min and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on a silica gel column to give *N*-propionyl-4-propylthio-*o*-anisidine (2.93 g, 75%) as waxy solid. ^{1}H-NMR ($CDCl_3$) δ: 8.31 (1H, d, J=7.9 Hz, arom. H), 7.69 (1H, br s, NH), 6.83—7.02 (2H, m, arom. H), 3.87 (3H, s, OCH_3), 2.86 (2H, t, J=6.7 Hz, SCH_2), 2.42 (2H, q, J=7.6 Hz, $COCH_2$), 1.48—1.77 (2H, m, S–$C\underline{H}_2$–CH_3), 1.24 (3H, t, J=7.6 Hz, $COCH_2C\underline{H}_3$), 1.00 (3H, t, J=7.0, S–CH_2–$C\underline{H}_3$). MS *m/z*: 253 (M^+). HRMS Calcd for $C_{13}H_{19}NO_2S$: 253.3586. Found 253.3584.

ii) The solution of the product obtained above (2.0 g) in THF (20 ml) was added dropwise to a stirred and ice-cooled suspension of $LiAlH_4$, (0.9 g) in THF (50 ml) under a N_2 atmosphere. The mixture was stirred for 2 h at reflux. After cooling, Na_2SO_4 (10 H_2O), was added, then the mixture was stirred for a while, diluted with H_2O and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography to give **12B** (0.53 g, 28%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 6.70—6.90 (3H, m, arom. H), 3.90 (3H, s, OCH_3), 3.0—3.4 (2H, m, NCH_2), 2.90 (2H,

t, J=7.1 Hz, SCH_2), 1.4—2.1 (4H, m, $C\underline{H}_2$–CH_3), 0.8—1.2 (6H, m, CH_2–$C\underline{H}_3$). MS m/z: 239 (M^+).

Treatment of **12B** with methanolic HCl gave its hydrochloride as colorless needles (from EtOH–AcOEt), mp 140—142 °C. *Anal.* Calcd for $C_{13}H_{22}ClNOS$: C, 56.61; H, 8.04; N, 5.08. Found: C, 56.62; H, 8.09; N, 4.84.

Preparations of Sulfoxide and Sulfone Derivatives (9, 11, 13, 15, 20) Typical examples are given to illustrate the general procedures for compounds **11** and **13**.

2-Methoxy-4-(propylsulfinyl)acetanilide (11) $NaIO_4$ (5.6 g), in H_2O (10 ml) was added to a stirred and ice-cooled solution of **7** (3.2 g) in MeOH (250 ml). The reaction mixture was stirred for 8.5 h at room temperature, diluted with H_2O and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated to give the residue. The residue was subjected to a silica gel column to yield **11** (2.5 g, 73%) as colorless plates (from AcOEt), mp 112—113 °C. *Anal.* Calcd for $C_{12}H_{17}NO_3S$: C, 56.45; H, 6.71; N, 5.49. Found: D, 56.77; H, 6.73; N, 5.57. ^{1}H-NMR ($CDCl_3$) δ: 8.50 (1H, d, J=8.2 Hz, arom. H), 7.86 (1H, br, NH), 7.31 (1H, d, J=1.8 Hz, arom. H), 7.04 (1H, dd, J=8.2, 1.8 Hz, arom. H), 3.97 (3H, s, OCH_3), 2.65—2.87 (2H, m, SCH_2), 2.23 (3H, s, $COCH_3$), 1.55—1.99 (2H, m, $C\underline{H}_2$–CH_3), 1.04 (3H, t, J=7.2 Hz, $CH_2C\underline{H}_3$). MS m/z: 255 (M^+).

2-Methoxy-4-(propylsulfonyl)acetanilide (13) *m*-CPBA (13.0 g) was added in portions to a stirred and ice-cooled solution of **7** (6.07 g) in CH_2Cl_2 (150 ml). The reaction mixture was stirred for 80 min with cooling. After adding $CHCl_3$, the solution was washed with sat. aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated to give the crude product, which was washed with ether to give the crystalline solid of **13** (5.26 g, 76%). Recrystallization from EtOH gave pure **13** as colorless plates, mp 134—135 °C. *Anal.* Calcd for $C_{12}H_{17}NO_4S$: C, 53.12; H, 6.32; N, 5.16. Found: C, 53.29; H, 6.39; N, 5.42. ^{1}H-NMR ($CDCl_3$) δ: 8.59 (2H, d, J=8.5 Hz, arom. H). 7.93 (1H, br, NH), 7.51 (1H, dd, J=8.5, 1.8 Hz, arom. H), 7.36 (1H, d, J=1.8 Hz, arom. H), 3.97 (3H, s, OCH_3), 2.92—3.16 (2H, m, SCH_2), 2.25 (3H, s, $COCH_3$), 1.55—1.94 (2H, m, $C\underline{H}_2$–CH_3), 0.99 (3H, t, J=7.3 Hz, CH_2–$C\underline{H}_3$). MS m/z: 271 (M^+).

Hydrolysis of *N*-Acetyl and *N*-Methoxycarbonyl Groups for Preparation of 10, 12A, 14 and 16 A typical example illustrates the general procedure for compound **12A**.

***N*-Methyl-4-propylthio-*o*-anisidine (12A)** 35% aq. KOH solution (150 ml) was added to a solution of **8** (2.1 g) in MeOH (150 ml). The mixture was stirred for 4 d at reflux, diluted with H_2O and extracted with $CHCl_3$. The extract was concentrated and the resulting residue was chromatographed on a silica gel column to give **12A** (1.38 g, 79%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 6.99 (1H, dd, J=7.8, 1.2 Hz, arom. H), 6.85 (1H, d, J=1.2 Hz, arom. H), 6.49 (1H, d, J=7.8 Hz, arom. H), 4.26 (1H, br, NH), 3.84 (3H, s, OCH_3), 2.85 (3H, s, NCH_3), 2.75 (2H, t, J=7.2 Hz, SCH_2), 1.4—1.8 (2H, m, $C\underline{H}_2$–CH_3), 0.98 (3H, t, J=7.1 Hz, CH_2–$C\underline{H}_3$). MS m/z: 211 (M^+).

Treatment of **12A** with methanolic HCl gave its hydrochloride as colorless needles (from AcOEt), mp 99—101 °C. *Anal.* Calcd for $C_{11}H_{18}ClNOS$: C, 53.32; H, 7.32; N, 5.65. Found: C, 53.51; H, 7.27; N, 5.51.

Synthesis of *N*,*N*-Dimethyl-4-propylthio-*o*-anisidine (19) i) A solution of bromine (1.3 ml) in MeOH (100 ml) was added dropwise to a stirred solution of *N*,*N*-dimethyl-*o*-anisidine (**17**, 4.9 g), KSCN (6.3 g) and Et_3N (6.5 g) in MeOH (200 ml). The mixture was stirred for 1.5 h at room temperature, then KSCN (6.3 g) and a methanolic solution (40 ml) containing bromine (1.3 ml) were added. Stirring was continued for an additional 1.5 h at room temperature. The mixture was diluted with water, alkalized with 35% aq. KOH and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4 and concentrated to give crude *N*,*N*-dimethyl-4-thiocyanato-*o*-anisidine (**18**).

ii) Compound **18** obtained above was dissolved in MeOH (200 ml); to this mixture was added NaOH (5.5 g). The mixture was stirred for 30 min at reflux, followed by the addition of propyl bromide (5.0 g). Stirring was continued for 1.5 h at reflux. After evaporation *in vacuo*, the residue was partitioned between H_2O and AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give the crude product. Purification by silica gel column chromatography gave **19** (2.5 g, 34% from **17**) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 6.76—7.01 (3H, m, arom. H), 3.88 (3H, s, OCH_3), 2.84 (2H, t, J=6.8 Hz, SCH_2), 2.77 (6H, s, NCH_3), 1.44—1.85 (2H, m, $C\underline{H}_2$–CH_3), 1.01 (3H, t, J=6.8 Hz, CH_2–$C\underline{H}_3$). MS m/z: 225 (M^+).

Treatment of **19** with methanolic HCl gave its hydrochloride as colorless plates (from EtOH–AcOEt), mp 104—105 °C. *Anal.* Calcd for $C_{12}H_{20}ClNOS$: C, 55.05; H, 7.70; N, 5.35. Found: C, 55.13; H, 7.51; N, 5.54.

References

1) J. C. Fantone and P. A. Ward, *Hum. Pathol.* **16**, 973 (1985); G. Weissmann and H. Korchak, *Inflammation*, **8**, S3 (1984); P. J. Bailey and D. S. Fletcher, "Methods in Enzymology," Vol. 162, ed. by G. D. Sabato, Academic Press, San Diego, 1988, pp. 478—483.
2) B. Halliwell, J. R. Hoult and D. R. Blake, *FASEB J.*, **2**, 2867 (1988).
3) I. Monkovic, D. Willner, M. A. Adam, M. Brown, R. R. Crenshaw, C. E. Fuller, P. E. Juby, G. M. Luke, J. A. Matiskella and T. A. Montzka, *J. Med. Chem.*, **31**, 1548 (1988).
4) K. Shintomi, T. Itakura, K. Yoshimoto, Y. Ogawa, T. Fukushima and Y. Matsuoka, *Nippon Yakurigaku Zasshi*, **87**, 427 (1986).
5) K. Kubo, I. Yoshitake, Y. Kumada, K. Shuto and N. Nakamizo, *Arch. Int. Pharmacodyn. Ther.*, **272**, 283 (1984).
6) K. Sugino, K. Dohi, K. Yamada and T. Kawasaki, *Surgery*, **101**, 746 (1987); J, Lunec, "Modern Methods in Pharmacology," Vol. 5, ed. by J. Y. Chang and A. J. Lewis, Alan R. Liss, Inc., New York, 1989, pp. 59—81.
7) K. Takahashi, K. Endoh, N. Yamada, S. Kadowaki, Y. Arai and K. Sugasawa, *Nippon Yakurigaku Zasshi*, **88**, 245 (1986).
8) C. Metzger, Ger. Patent 2706104 (1978) [*Chem. Abstr.*, **90**, 6083 (1979)].
9) A. J. Lewis, J. Cottney and D. J. Nelson, *Eur. J. Pharmacol.*, **40**, 1 (1976); E. Bramm, L. Binderup and E. Arrigoni-Martelli, *Agents Actions*, **11**, 402 (1981).
10) X. F. Qu, M. Hayashi, K. Yamaki and S. Oh-ishi, *Jpn. J. Pharmacol.*, **52**, 500 (1990).
11) Z. Vuk-Pavlovic and M. S. Rhobach, *Am. Respir. Cell. Mol. Biol.*, **3**, 235 (1990); I. G. Colditz and M. I. Cybulsky, *Inflammation*, **11**, 1 (1987).
12) Y. Oyanagi, *Agents Actions*, **7**, 125 (1977).
13) D. W. Robertson, E. E. Beedle, J. H. Krushinski, G. D. Pollock, H. Wilson, V. L. Wyss and J. S. Hayes, *J. Med. Chem.*, **28**, 717 (1985).