

2-Oxo-1,3-dioxoles as Specific Substrates for Measurement of Arylesterase Activity

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Various 4-arylthiomethyl-2-oxo-1,3-dioxole derivatives IIIa–o were synthesized. Their hydrolysis rates by arylesterase (EC 3.1.1.2) and cholinesterase (EC 3.1.1.8) in human serum were evaluated. Some of them were not hydrolyzed by cholinesterase, but were hydrolyzed easily by arylesterase.

Among the substrates, sodium 4-((5-methyl-2-oxo-1,3-dioxol-4-yl)methylthio)benzenesulfonate (IIIg) was selected for its substrate reactivity toward arylesterase and its good water solubility. In addition, neither aliesterase (EC 3.1.1.1), acetyesterase (EC 3.1.1.6) nor cholesterol esterase (EC 3.1.1.13) hydrolyzed the compound. IIIg is thus concluded to be a specific substrate for arylesterase.

Our assay system for serum arylesterase using IIIg can be readily applied to an automatic analyzer in the diagnosis of liver cirrhosis.

Keywords human serum; arylesterase activity; specific substrate; cirrhosis diagnosis; 4-arylthiomethyl-2-oxo-1,3-dioxole

Augustinsson *et al.* studied the types of esterase present in vertebrate plasma, and found three to be present: aliesterase (carboxylic-ester hydrolase, EC 3.1.1.1, AliE), arylesterase (aryl-ester hydrolase, EC 3.1.1.2, ArE), and cholinesterase (acyl-choline acyl-hydrolase, EC 3.1.1.8, ChE). ArE and ChE are present in mammalian plasma.¹⁾ Serum ArE activity, as determined by phenyl acetate^{2,3)} or β -naphthyl acetate⁴⁾ as a substrate, has been reported to decrease in patients with liver disease, particularly cirrhosis. However, these substrates for the assay of ArE activity are hydrolyzed by ChE as well as ArE.^{5,6)} Their lack of specificity makes necessary the separation of ArE from ChE by electrophoresis,⁷⁾ chromatography or other troublesome methods prior to accurate measurement of ArE activity.

In the studies of enzymatic hydrolysis of a series of ampicillin prodrugs, it was suggested that lenampicillin, (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester of ampicillin,⁸⁾ is hydrolyzed to ampicillin by ArE, and ArE recognizes the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group as a substrate.

5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB; Ellman's reagent) reacts rapidly with a thiol and the resulting product (5-thio-2-nitrobenzoic acid, TNB) is yellow in color (Fig. 1).⁹⁾ If a thiol is released following the hydrolysis of an ArE-specific substrate, it should be possible to readily assay ArE activity by colorimetric determination using an automatic analyzer in the diagnosis of cirrhosis. Therefore, we combined a benzenethiol with the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group, regarded as the recognition domain of ArE.

The present study is conducted to examine the synthesis, properties, structures and reactivity toward the ArE relationship of several (2-oxo-1,3-dioxol-4-yl)methyl sulfide derivatives, and to choose substrate for the determination of serum ArE activity.

Chemistry 4-Halomethyl-2-oxo-1,3-dioxole derivatives⁸⁾ I were allowed to react with thiols II in the presence of triethylamine in dichloromethane at room temperature to afford sulfides III (Fig. 1). Sulfides IIIa–f and IIIj–o were isolated upon silica-gel column chromatography. Sulfides IIIg and IIIi were obtained by salting-out techniques with NaCl. Sulfide IIIh was obtained upon ion-exchange column chromatography using a Na-form resin.

Enzymatic Hydrolysis As human plasma contains ArE and ChE, the hydrolysis rates of sulfides IIIa–o by these were measured (Table I). When the 5-substituent (R) was fixed on a methyl group, the hydrolysis rates of substrate III by ArE varied with the phenyl substituent (X), being fast when X was an electron withdrawing group. The relationship between the rate by ArE (R_{ArE}) and Hammett's σ -value of X (*m*- and *p*-position) is as follows:

$$\log R_{ArE} = 0.255\sigma - 0.491 \quad (n=8, r=0.752).$$

Substrates IIIa–o were scarcely hydrolyzed by ChE.

Among the substrates examined for enzymatic hydrolysis (Table I), sodium 4-((5-methyl-2-oxo-1,3-dioxol-4-yl)methylthio)benzenesulfonate (IIIg) was further studied for clinical application described below, because it was easy to handle owing to its high solubility in water (about 110 mg/ml). Other compounds except for IIIh and IIIi were

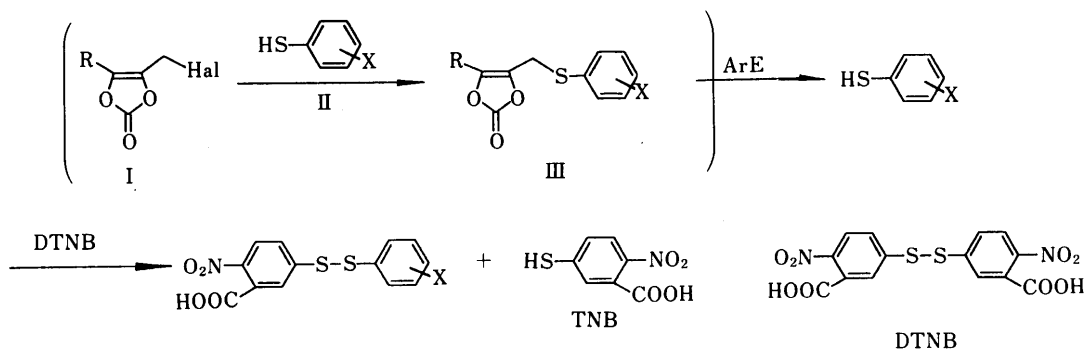


Fig. 1. The Synthetic Method and Principle of the Assay System

TABLE I. Hydrolysis Rates of Compound IIIa—o by ArE and ChE

Compound			Formula	Analysis (%)			σ -Value of X	Hydrolysis rate ^{a)}	
No.	R	X		Calcd	(Found)			ArE	ChE
IIIa	CH ₃	H	C ₁₁ H ₁₀ O ₃ S	59.44	4.54		0.00	0.385	<10 ⁻³
IIIb	CH ₃	<i>p</i> -F	C ₁₁ H ₉ FO ₃ S	54.99	3.78)	0.06	0.387	<10 ⁻³
IIIc	CH ₃	<i>p</i> -Cl	C ₁₁ H ₉ ClO ₃ S	51.47	3.53)	0.23	0.423	<10 ⁻³
IIId	CH ₃	<i>p</i> -NO ₂	C ₁₁ H ₉ NO ₃ S	49.44	3.39	5.24	0.78	0.437	<10 ⁻³
IIIe	CH ₃	<i>p</i> -CH ₃	C ₁₂ H ₁₂ O ₃ S	61.00	5.12	5.25	-0.17	0.223	<10 ⁻³
IIIf	CH ₃	<i>p</i> -OCH ₃	C ₁₂ H ₁₂ O ₄ S	60.93	5.00)	-0.27	0.247	<10 ⁻³
IIIg	CH ₃	<i>p</i> -SO ₃ Na	C ₁₁ H ₉ NaO ₆ S ₂	57.13	4.80)	0.09	0.368	<10 ⁻³
IIIh	CH ₃	<i>m</i> -SO ₃ Na	C ₁₁ H ₉ NaO ₆ S ₂	57.01	4.84)	0.05	0.335	<10 ⁻³
IIIi	CH ₃	<i>o</i> -SO ₃ Na	C ₁₁ H ₉ NaO ₆ S ₂	39.64	3.02)		0.115	<10 ⁻³
IIIj	CH ₃	<i>p</i> -Cl, <i>m</i> -CO ₂ H	C ₁₂ H ₉ ClO ₅ S	39.64	3.02)		0.111	<10 ⁻³
IIIk	H	H	C ₁₀ H ₈ O ₃ S	47.93	3.02)		0.280	<10 ⁻³
IIIl	H	<i>p</i> -F	C ₁₀ H ₇ FO ₃ S	48.09	2.84)		0.258	<10 ⁻³
IIIIm	H	<i>p</i> -NO ₂	C ₁₀ H ₇ NO ₃ S	53.09	3.12)		0.349	<10 ⁻³
IIIIn	C ₆ H ₅	H	C ₁₆ H ₁₂ O ₃ S	52.96	2.96	5.53		0.024	<10 ⁻³
IIIo	C ₆ H ₅	<i>p</i> -NO ₂	C ₁₆ H ₁₁ NO ₃ S	47.43	2.79	5.57		0.012	<10 ⁻³
				47.26	2.82				
				67.59	4.25				
				67.79	4.17				
				58.35	3.37	4.25			
				58.36	3.17	4.38			

a) Hydrolysis rate of each compound was expressed as μmol of compound hydrolyzed/min per U esterase preparation.⁶⁾

insoluble in water.

Substrate Reactivity The substrate reactivity of compound IIIg for several esterases was compared with that of reported substrates, phenyl acetate and β -naphthyl acetate (Table II). Of the 3 substrates tested for enzymatic hydrolysis, IIIg was not hydrolyzed by ChE, but was easily by ArE. In addition, it was not hydrolyzed by AliE, acetylcholinesterase (EC 3.1.1.6, AcE) or cholesterol esterase (EC 3.1.1.13, CE). IIIg was thus concluded to be a specific substrate for ArE. Its hydrolysis by ArE may possibly be correlated with the fact that aliphatic esters such as isopropenyl acetate and vinyl acetate are hydrolyzed by ArE.¹⁰⁾ Indeed, these esters have a double bond in the alcohol moiety nearest to the ester linkage, as shown in Fig. 2, which is essential for ArE hydrolysis.

Phenyl acetate^{2,3)} and β -naphthyl acetate⁴⁾ were hydrolyzed by ChE, AliE, AcE, and CE, as well as by ArE.

Conclusions

For the diagnosis of acute hepatitis, there are excellent biochemical parameters such as glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT), which can be easily measured and are widely used. However, only a few reliable biochemical parameters which indicate liver cirrhosis are presently available. The activity of serum ArE has been shown to decrease in patients with certain liver diseases, particularly cirrhosis.²⁻⁴⁾ In the determination of ArE activity, the main problems related

TABLE II. Substrate Reactivity for Several Types of Esterase

Substrate	Conc. (mM)	Hydrolysis rate ($\mu\text{mol}/\text{min}$ per U)				
		AliE	ArE	AcE	ChE	CE
Compd. IIIg	3.2	<10 ⁻³	0.368	<10 ⁻³	<10 ⁻³	<10 ⁻³
Phenyl acetate	4.0	0.098	1.000	0.001	0.028	0.031
β -Naphthyl acetate	0.49	2.66	0.190	0.354	0.049	1.94

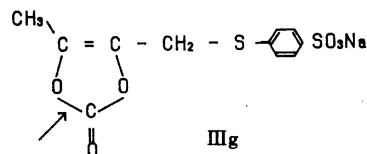
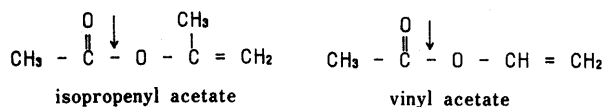


Fig. 2. Aliphatic Esters Hydrolyzed by ArE
Arrows show positions of the ester linkage

to the substrate are: (a) low specificity to ArE⁵⁾ and (b) poor solubility in water.

To solve these problems, IIIg was selected as a substrate for the determination of ArE activity. It was found possible to assay ArE activity without disturbing other esterases by using the new specific substrate for ArE. The assay is easy

to conduct using an automatic analyzer.¹¹⁾ Activity can thus be easily measured, as can other biochemical parameters.

In a preliminary clinical study,¹¹⁾ the activity of ArE, as calculated by this assay, markedly decreased in patients with cirrhosis. Particularly, the activities of severely decompensated cirrhosis patients were lower than those of compensated ones. Therefore, further clinical studies on the positive correlation between serum ArE activity and the severity of chronic liver disease are now being conducted.

Experimental

Enzymatic Hydrolysis ArE was purified from human serum by precipitation with ammonium sulfate and chromatography on Affi-Gel Blue (Bio-Rad Laboratories, U.S.A.) and on DEAE-Toyopearl (Tosoh, Japan) at pH 7.4.⁶⁾ The purified preparation had a specific activity of 34.6 μmol phenyl acetate hydrolyzed/min per mg protein by the method of Junge and Klees,¹²⁾ and was confirmed to be free from ChE by electrophoresis. ChE, AliE and CE were obtained from Boehringer Mannheim GmbH (FRG). AcE was obtained from Sigma chemical company (U.S.A.). The hydrolysis rates of sulfides IIIa—o were assayed using DTNB as the coloring reagent by modification^{6,11,13)} of Ellman's method⁹⁾ according to the principle shown in Fig. 1. The summary of the procedure was as follows: 100 μl of 10 U/ml esterase preparation was added to 100 μl of the color reagent (3.2 mM DTNB solution), and 800 μl of substrate solution (0.4 mM, pH 6.5) was added and reacted at 25°C. An increase in absorbance at 412 nm/min was measured, and the hydrolysis rate ($\mu\text{mol}/\text{min}$ per U) was calculated. The methods used for other substrates were; phenyl acetate using the method of Junge and Klees,¹²⁾ and β -naphthyl acetate according to Burlina and Galzigna.¹⁴⁾

Chemistry Melting points were determined on a Yamato capillary melting point apparatus, Model MP-21, and were uncorrected. Proton nuclear magnetic resonance (¹H-NMR) spectra were determined at 60 MHz on a Hitachi R-24B spectrometer using tetramethylsilane as an internal reference. Infrared (IR) spectra were recorded with a Hitachi IR 270-50 infrared spectrophotometer. Compounds IIIa—o were analyzed for C, H, and N, and the analytical values were within $\pm 0.4\%$ of the theoretical values.

5-Methyl-4-phenylthiomethyl-1,3-dioxol-2-one (IIIa) Benzenethiol (1.5 g, 13.6 mmol) was added to a solution of 4-chloromethyl-5-methyl-1,3-dioxol-2-one (2.0 g, 13.5 mmol) in 40 ml of dichloromethane. Then, triethylamine (1.4 g, 13.8 mmol) was added slowly, and the mixture was stirred at room temperature for 30 min. The reaction mixture was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate. The insoluble materials were filtered off, and the filtrate was evaporated *in vacuo*. The residue was chromatographed on a column of Silica gel 60 (230—400 mesh, E. Merck) with chloroform-*n*-hexane (1:2) as an eluent under medium pressure. The desired fraction was evaporated *in vacuo*, and the residue was subjected to bulb-to-bulb distillation (about 180°C/0.1 mmHg) to give IIIa (2.3 g, 76%) as a colorless liquid, bp 185°C (0.1 mmHg). ¹H-NMR (CDCl₃) δ : 1.7 (s, 3H, CH₃), 3.7 (s, 2H, CH₂), 7.2—7.7 (m, 5H, aromatic protons). IR (CDCl₃): 1819 (C=O), 1733 (C=C) cm⁻¹.

4-(4-Fluorophenyl)thiomethyl-5-methyl-1,3-dioxol-2-one (IIIb) By a procedure similar to that described for IIIa, 4-fluorobenzenethiol (1.8 g, 14.0 mmol) and 4-chloromethyl-5-methyl-1,3-dioxol-2-one (2.0 g, 13.5 mmol) gave IIIb (1.8 g, 55%) as pale yellow crystals after recrystallization from a mixture of ethyl acetate and *n*-hexane, mp 60—62°C. ¹H-NMR (CDCl₃) δ : 1.7 (s, 3H, CH₃), 3.7 (s, 2H, CH₂), 6.9—7.7 (m, 4H, aromatic protons). IR (KBr): 1811 (C=O), 1734 (C=C) cm⁻¹.

4-(4-Chlorophenyl)thiomethyl-5-methyl-1,3-dioxol-2-one (IIIc) By a procedure similar to that described for IIIa, 4-chlorobenzenethiol (2.0 g, 13.8 mmol) and 4-chloromethyl-5-methyl-1,3-dioxol-2-one (2.0 g, 13.5 mmol) gave IIIc (2.9 g, 83%) as colorless crystals after recrystallization from a mixture of ethyl acetate and *n*-hexane, mp 54—57°C. ¹H-NMR (CDCl₃) δ : 1.8 (s, 3H, CH₃), 3.7 (s, 2H, CH₂), 7.2—7.4 (m, 4H, aromatic protons). IR (KBr): 1814, 1804 (C=O), 1730 (C=C) cm⁻¹.

5-Methyl-4-(4-nitrophenyl)thiomethyl-1,3-dioxol-2-one (IIId) By a procedure similar to that described for IIIa, 4-nitrobenzenethiol (2.1 g, 13.5 mmol) and 4-chloromethyl-5-methyl-1,3-dioxol-2-one (2.0 g, 13.5 mmol) gave IIId (2.4 g, 66%) as yellow crystals after recrystallization from ethyl acetate, mp 80—83°C. ¹H-NMR (CDCl₃) δ : 2.1 (s, 3H, CH₃), 4.0 (s, 2H, CH₂), 7.3—7.6 (m, 2H, aromatic protons), 8.0—8.6 (m, 2H, aromatic protons). IR (KBr): 1846, 1818 (C=O), 1733 (C=C) cm⁻¹.

5-Methyl-4-(4-methylphenyl)thiomethyl-1,3-dioxol-2-one (IIIe) By a procedure similar to that described for IIIa, 4-methylbenzenethiol (1.7 g, 13.7 mmol) and 4-chloromethyl-5-methyl-1,3-dioxol-2-one (2.0 g, 13.5 mmol) gave IIIe (2.2 g, 69%) as yellow crystals after recrystallization from a mixture of ethyl acetate and *n*-hexane, mp 57—59°C. ¹H-NMR (CDCl₃) δ : 1.7 (s, 3H, CH₃), 2.3 (s, 3H, PhCH₃), 3.6 (s, 2H, CH₂), 7.0—7.5 (m, 4H, aromatic protons). IR (KBr): 1808 (C=O), 1734 (C=C) cm⁻¹.

4-(4-Methoxyphenyl)thiomethyl-5-methyl-1,3-dioxol-2-one (IIIf) By a procedure similar to that described for IIIa, 4-methoxybenzenethiol (1.9 g, 13.6 mmol) and 4-chloromethyl-5-methyl-1,3-dioxol-2-one (2.0 g, 13.5 mmol) gave IIIf (2.3 g, 67%) as pale yellow crystals after recrystallization from a mixture of ethyl acetate and *n*-hexane, mp 59—61°C. ¹H-NMR (CDCl₃) δ : 1.6 (s, 3H, CH₃), 3.6 (s, 2H, CH₂), 3.8 (s, 3H, OCH₃), 6.7—7.0 (m, 2H, aromatic protons), 7.2—7.5 (m, 2H, aromatic protons). IR (CDCl₃): 1819 (C=O), 1733 (C=C) cm⁻¹.

Sodium 4-(5-Methyl-2-oxo-1,3-dioxol-4-yl)methylthio)benzenesulfonate (IIIg) To a solution of a mixture of 4-chloromethyl-5-methyl-1,3-dioxol-2-one (0.4 g, 2.7 mmol) and 4-mercaptobenzenesulfonic acid (0.5 g, 2.6 mmol) in 20 ml of dichloromethane was slowly added triethylamine (0.5 g, 4.9 mmol). After being stirred at room temperature for 3 h, the reaction mixture was concentrated *in vacuo*, and the residue was dissolved in water (50 ml) and washed with ethyl acetate. The aqueous layer was chromatographed on an ion-exchange column (20 ml of Dowex 50W-X2, 100—200 mesh, H-form) with water as an eluent. The acidic fraction was collected and concentrated *in vacuo* to a volume of about 15 ml, then saturated with NaCl. The resulting precipitate was collected and recrystallized from ethanol to give IIIg (0.51 g, 58%) as colorless crystals, mp 260°C (dec.). ¹H-NMR (DMSO-*d*₆) δ : 1.9 (s, 3H, CH₃), 4.1 (s, 2H, CH₂), 7.2—7.7 (m, 4H, aromatic protons). IR (KBr): 1815 (C=O), 1732 (C=C) cm⁻¹.

4-Mercaptobenzenesulfonic acid was prepared by modification of the reported method¹⁵⁾ as follows. 4-Aminobenzenesulfonic acid (86.6 g, 0.5 mol) was dissolved in a solution of sodium hydroxide (20 g, 0.5 mol) in 500 ml of water, then 100 ml of conc. hydrochloric acid was added. To the resulting suspension was gradually added a solution of sodium nitrite (34.5 g, 0.5 mol) in 100 ml of water at 1—3°C, and the resulting mixture was poured into a solution of sodium disulfide, prepared by co-melting sulfur (16 g, 0.5 mol) and sodium sulfide nonahydrate (120.1 g, 0.5 mol) at 65°C, in 500 ml of water. After being warmed until the evolution of gas ceased, the mixture was returned to room temperature, and the insoluble materials were filtered off. A solution of aniline (93.4 g) and conc. hydrochloric acid (84 ml) in 200 ml of water was added to the filtrate. The resulting precipitate was collected by filtration, recrystallized from water, and washed with ethanol and *n*-hexane to give dianilinium 4,4'-dithiobis(benzenesulfonate) (58.7 g, 41%) as colorless crystals. Then, this salt (6.0 g, 10.6 mmol) was subjected to an ion-exchange column chromatography (28 ml of Dowex 50W-X2, 100—200 mesh, H-form) with methanol-water (2:1) as an eluent. An acidic fraction was collected and evaporated *in vacuo*. The residue was dissolved in methanol (100 ml) and to the resulting solution were added triphenylphosphine (6.1 g, 23.3 mmol) and about 1 ml of water. After being stirred overnight at room temperature, the mixture was evaporated *in vacuo* and the residue was dissolved in 60 ml of dichloromethane, then the products were extracted with water. The extract was washed with dichloromethane and evaporated *in vacuo*. The residue was dissolved in dioxane, and insoluble materials were filtered off. The filtrate was evaporated *in vacuo* and the resulting syrup was crystallized from benzene to give 4-mercaptobenzenesulfonic acid (1.6 g, 39% yield from dianilinium salt) as pale yellow crystals (mp 99—101°C).

Sodium 3-(5-Methyl-2-oxo-1,3-dioxol-4-yl)methylthio)benzenesulfonate (IIIh) By a procedure similar to that described for 4-mercaptobenzenesulfonic acid, 3-aminobenzenesulfonic acid (17.3 g, 0.1 mol) gave dianilinium 3,3'-dithiobis(benzenesulfonate) (9.8 g, 34%) as colorless crystals, and from this salt (5.0 g, 8.9 mmol) 3-mercaptobenzenesulfonic acid was obtained as a pale yellowish-brown syrup (2.4 g, 70%) and was subjected to the following reaction. To a solution of 3-mercaptobenzenesulfonic acid (2.2 g, 11.6 mmol) and triethylamine (2.4 g, 23.7 mmol) in dichloromethane (20 ml) was added a solution of 4-chloromethyl-5-methyl-1,3-dioxol-2-one (1.9 g, 12.8 mmol) in 4 ml of dichloromethane. After being stirred at room temperature for 2.5 h, the reaction mixture was evaporated *in vacuo*, the residue was dissolved in water (60 ml), and the solution was washed with ethyl acetate. The aqueous layer was chromatographed on an ion-exchange column (30 ml of Dowex 50W-X2, 100—200 mesh, Na-form) with water as an eluent. The desired fraction (about 140 ml) was collected, and thallium acetate (20 g) was added to this fraction. After being allowed to stand overnight at 4°C, the insoluble materials were

filtered off, and the filtrate was concentrated to a volume of about 14 ml. The precipitated solid was collected by filtration, dissolved in water (120 ml), and concentrated *in vacuo* to afford crystals. This operation was repeated once more to give 2 g of colorless crystals, and they were chromatographed on an ion-exchange column (8 ml of Dowex 50W-X2, 100–200 mesh, Na-form) with water as an eluent. The desired fraction was evaporated *in vacuo*, and the residue was recrystallized from ethanol to give IIIh (1.1 g, 28%) as colorless crystals, mp 190 °C (dec.). ¹H-NMR (DMSO-*d*₆) δ: 1.9 (s, 3H, CH₃), 4.1 (s, 2H, CH₂), 7.2–7.7 (m, 4H, aromatic protons). IR (KBr): 1811, 1795 (C=O), 1736 (C=C) cm⁻¹.

Sodium 2-((5-Methyl-2-oxo-1,3-dioxol-4-yl)methylthio)benzenesulfonate (IIIi) To a solution of 4-chloromethyl-5-methyl-1,3-dioxol-2-one (0.36 g, 2.4 mmol) and triethylammonium 2-mercaptobenzenesulfonate (0.63 g, 2.2 mmol) in 10 ml of dichloromethane was added triethylamine (0.22 g, 2.2 mmol). After being stirred at room temperature for 2 h, the reaction mixture was worked up by a procedure similar to that described for IIIg to give IIIi (0.36 g, 50%) as colorless crystals, mp 110 °C (dec.). ¹H-NMR (DMSO-*d*₆) δ: 1.8 (s, 3H, CH₃), 4.1 (s, 2H, CH₂), 7.1–8.0 (m, 4H, aromatic protons). IR (KBr): 1826 (C=O), 1730 (C=C) cm⁻¹.

Triethylammonium 2-mercaptobenzenesulfonate was prepared by modification of the reported method¹⁵ as follows. 2-Aminobenzenesulfonic acid (17.3 g, 0.1 mol) was dissolved in a solution of sodium hydroxide (4.0 g, 0.1 mol) in 100 ml of water, then 20 ml of conc. hydrochloric acid was added. To the resulting suspension was gradually added a solution of sodium nitrite (6.9 g, 0.1 mol) in 20 ml of water at 1–3 °C, and the resulting mixture was poured into a solution of sodium disulfide, prepared by co-melting sulfur (3.2 g, 0.1 mol) and sodium sulfide nonahydrate (24.0 g, 0.1 mol) at 65 °C, in 100 ml of water. After being warmed until the evolution of gas ceased, the mixture was returned to room temperature and the insoluble materials were filtered off. The filtrate was evaporated *in vacuo*, and the residue was dissolved in 200 ml of methanol. The insoluble materials were filtered off and the filtrate was evaporated *in vacuo*. The residue was dissolved in 120 ml of water, and 30 ml of acetic acid and 20 g of zinc dust were added. After being warmed for 40 min, the insoluble materials were filtered off and the products were extracted with aniline–benzene (1:1). The extract was evaporated *in vacuo* and the resulting residue was stirred in 150 ml of benzene to give powder. The powder was recrystallized from methanol to give anilinium 2-mercaptobenzenesulfonate (5.39 g, 19%) as colorless crystals. Then, this salt (2.0 g, 7.1 mmol) was subjected to ion-exchange column chromatography (30 ml of Dowex 50W-X2, 100–200 mesh, H-form) with methanol–water (4:1) as an eluent. An acidic fraction was collected and evaporated *in vacuo*. The residue was dissolved in 50 ml of isopropanol and evaporated *in vacuo* to give 1.1 g of syrup. This syrup was dissolved in 4 ml of isopropanol following the addition of triethylamine (0.58 g, 5.7 mmol) to give triethylammonium 2-mercaptobenzenesulfonate (0.84 g, 40%) as colorless crystals (mp 89–90 °C).

2-Chloro-5-((5-methyl-2-oxo-1,3-dioxol-4-yl)methylthio)benzoic Acid (IIIj) By a procedure similar to that described for IIIa, 2-chloro-5-mercaptobenzoic acid (5.1 g, 27.0 mmol) and 4-chloromethyl-5-methyl-1,3-dioxol-2-one (4.0 g, 26.9 mmol) gave IIIj (4.8 g, 59%) as colorless crystals after recrystallization from ethanol, mp 162–164 °C. ¹H-NMR (DMSO-*d*₆) δ: 1.9 (s, 3H, CH₃), 4.2 (s, 2H, CH₂), 7.4–7.9 (m, 3H, aromatic protons). IR (KBr): 1814, 1800 (C=O), 1738 (C=C) cm⁻¹.

4-Phenylthiomethyl-1,3-dioxol-2-one (IIIk) By a procedure similar to that described for IIIa, benzenethiol (0.7 g, 6.4 mmol) and 4-bromomethyl-1,3-dioxol-2-one⁹ (1.0 g, 5.6 mmol) gave IIIk (0.7 g, 60%) as a colorless oil after bulb-to-bulb distillation (about 170 °C/0.1 mmHg), bp 175 °C

(0.1 mmHg). ¹H-NMR (CDCl₃) δ: 3.8 (d, 2H, CH₂), 6.7 (t, 1H, CH), 7.2–7.7 (m, 5H, aromatic protons). IR (CDCl₃): 1849, 1817 (C=O) cm⁻¹.

4-(4-Fluorophenyl)thiomethyl-1,3-dioxol-2-one (IIIl) By a procedure similar to that described for IIIa, 4-fluorobenzenethiol (0.8 g, 6.2 mmol) and 4-bromomethyl-1,3-dioxol-2-one (1.0 g, 5.6 mmol) gave IIIl (0.52 g, 41%) as pale yellow crystals after recrystallization from a mixture of ethyl acetate and *n*-hexane, mp 57–59 °C. ¹H-NMR (CDCl₃) δ: 3.7 (d, 2H, CH₂), 6.7 (t, 1H, CH), 6.9–7.6 (m, 4H, aromatic protons). IR (CDCl₃): 1849, 1819 (C=O) cm⁻¹.

4-(4-Nitrophenyl)thiomethyl-1,3-dioxol-2-one (IIIm) By a procedure similar to that described for IIIa, 4-nitrobenzenethiol (0.9 g, 5.8 mmol) and 4-bromomethyl-1,3-dioxol-2-one (1.0 g, 5.6 mmol) gave IIIm (0.5 g, 37%) as yellow crystals after recrystallization from a mixture of chloroform and *n*-hexane, mp 109–112 °C. ¹H-NMR (CDCl₃) δ: 4.0 (d, 2H, CH₂), 6.9 (t, 1H, CH), 7.2–7.6 (2H, aromatic protons), 8.0–8.5 (2H, aromatic protons). IR (KBr): 1870, 1838, 1806 (C=O), 1786 (C=C) cm⁻¹.

5-Phenyl-4-phenylthiomethyl-1,3-dioxol-2-one (IIIn) By a procedure similar to that described for IIIa, benzenethiol (0.09 g, 0.82 mmol) and 4-bromomethyl-5-phenyl-1,3-dioxol-2-one⁹ (0.20 g, 0.78 mmol) gave IIIn (0.20 g, 85%) as colorless crystals after recrystallization from a mixture of chloroform and *n*-hexane, mp 87–89 °C. ¹H-NMR (CDCl₃) δ: 4.0 (s, 2H, CH₂), 7.1–7.7 (m, 10H, aromatic protons). IR (KBr): 1818 (C=O) cm⁻¹.

4-(4-Nitrophenylthio)methyl-5-phenyl-1,3-dioxol-2-one (IIIo) By a procedure similar to that described for IIIa, 4-nitrobenzenethiol (0.12 g, 0.77 mmol) and 4-bromomethyl-5-phenyl-1,3-dioxol-2-one⁹ (0.20 g, 0.78 mmol) gave IIIo (0.22 g, 86%) as pale yellow crystals after recrystallization from a mixture of chloroform and *n*-hexane, mp 137–140 °C. ¹H-NMR (CDCl₃) δ: 4.2 (s, 2H, CH₂), 7.2–7.6 (m, 7H, aromatic protons), 7.9–8.2 (m, 2H, aromatic protons). IR (KBr): 1826 (C=O) cm⁻¹.

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